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Ocular Complications of Intravitreal Avastin: a Report from Tobruk Medical Center

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Abstract: A retrospective statistical study was done at the ophthalmology department of Tobruk Medical Center on all the patients who received intravitreal medication with Avastin (anti-VEGF (anti-vascular endothelial growth factor)) in the period between August 1st and December 31st, 2018. It is aimed to report the complications of the intravitreal injection (IVI) and how they were managed. Out of the 56 recorded patients, there were 32 (51.9 %) females, all the patients received multiple intravitreal injections, with a total number of 131 injections. The average age of the patients was 56.5 years (ranged from 40-70 years). The most common complications after intravitreal injection were subconjunctival hemorrhage (19%), discomfort/pain (13.7%), blurring of vision (6 %), leaking at injection site (4.6%), floaters (3%), and increase intraocular pressure (IOP) in (13.7%). Six cases out of the eighteen that had high IOP received Diamox (Acetazolamide) Tab. 250 mg one-two hours before the time of injection which did not prevent the post-injection spike of IOP and that was statistically not significant (*P*=0.09). Thirteen eyes (10 %) developed sudden loss of vision due to sudden increase in IOP immediately after the injection, and all the cases of the high IOP were managed by anterior chamber paracentesis and the vision also improved. Endophthalmitis was recorded in only one case (0.8%), at the third-day post intraocular Avastin injection, the causative microorganism was unknown and treated with intravitreal antibiotics (vancomycin) along with topical and systemic antibiotics and steroids, but the patient did not recover until pars plana vitrectomy was done to him, and the patient recovered his preoperative vision. The patients who had glaucoma or rubeosis iridis suffered significantly (P = 0.01) from an increase in IOP levels (digitally measured) after injection with Avastin, while most other patients who didn't have both pathologies did not suffer from an increase in IOP. Concluded that despite Anti-VEGF has a dramatic effect on the quality of life by improving the central vision, it can cause serious complications that could be prevented by early diagnosis and treatment.

Keywords: Avastin; Complications; Intravitreal Injection; Tobruk.

INTRODUCTION

Sub-retinal neovascularization and pathologic ocular angiogenesis are common causes of progressive, irreversible impairment of central vision, and dramatically affect the quality of life. Anti-vascular endothelial growth factor (anti-VEGF) therapy has improved the quality of life for many patients with age-related macular degeneration, diabetic retinopathy, and other ocular diseases involving neovascularization and edema. In these pathologies, the inhibition of intraocular VEGF is the only therapy that can preserve vision. (Semeraro et al., 2015).

Adverse events following intravitreal anti-VEGF injections have no relation to underlying ocular disease; Common complications of intravitreal injection (IVI) are injection site

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discomfort, subconjunctival hemorrhage, vitreous reflux, transient intraocular pressure (IOP) elevation, and defective vision. The patient may also develop floaters, vitreous or retinal hemorrhage, and retinal detachment. (Frenkel et al., 2010). The most dreaded complications of intravitreal injection are endophthalmitis and loss of vision. (Aiello et al., 2004).

The intravitreal injection can cause an immediate rise in intraocular pressure IOP (spike) that is not merely the result of added volume, but also for the properties of the injected drug. The sudden increased intraocular pressure (IOP), due to various reasons, causes corneal edema and damage to the endothelial cells with optic nerve damage and can lead to significant loss of vision. (Fang *et al.,* 2006). Intraocular pressure spikes after intravitreal injections of Anti-VEGF are common, and in most cases transient, and usually return to baseline in a few minutes. (Qureshi *et al.,* 2016) The increase of the IOP may take longer to normalize in patients with glaucoma and need to be monitored. In patients who develop high IOP spikes, the use of ocular massage, topical IOP reducing agent, oral medication, and anterior chamber tap can be used as means to reduce IOP to prevent optic nerve damage and possibility central retinal artery occlusion. (Uyar, *et al.,* 2019). In patients who have primary open-angle glaucoma or ocular hypertension, the intravitreal injection is not considered a contraindication. It requires closer observation and the use of topical medical therapy. (Frenkel *et al.,* 2010; Aiello et al., 2004).

Post intravitreal injections endophthalmitis is a serious inflammation within the eye due to bacterial or fungal infection, including involvement of the vitreous and/or aqueous humor. It usually causes irreversible damage to retinal photoreceptors that may lead to loss of vision, even with intensive medical and surgical management. The post intraocular injection's endophthalmitis is rare. It accounts for 0.03%-0.072% of all cases of anti-vascular endothelial growth factor (VEGF) injection (Hoevenaars, et al*.,* 2012; Park, et al., 2014). The most common causative organisms are *Coagulase-negative-staphylococci, Staphylococcus aureus, and Streptococci viridians*. The post anti-VEGF injection endophthalmitis, in general, has a moderate visual outcome of 6/60 if managed properly in comparison with other types of endophthalmitis (Dhoot, et al., 2013; Solborg et al., 2013).

Although matters have improved recently with fast diagnosis and better management of endophthalmitis, this disease is still serious and can lead to destructive complications to the eye that may be irreversible, and sometimes cause blindness. (Barry, et al., 2013). In most cases of post intravitreal endophthalmitis, the bacterial endotoxins and other bacterial products appear to cause a direct cellular inflammatory effect. The inflammation is most often in the first 3-4 days after the operation. (Solborg, et al., 2013). The most commonly reported causes of endophthalmitis after surgery are from the patient's conjunctival flora, contamination of sterilized instruments, disposable supplies, prepared solutions, surgical field, or the intraocular lens. (Dancer, et al., 2012; Damasceno et al., 2015).

Aim of the study: This article is intended to highlight important aspects of post-injection complications and how to deal with these complications to avoid the risk of a serious outcome, which may lead to permanent vision loss.

MATERIALS AND METHODS

A retrospective study was done through data collection from the Statistical office in Tobruk Medical Center. Statistical analysis was performed using Microsoft Office Excel program. *P*-value ≤ 0.05 or a level of (95%) was consid-

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ered statistically significant. Unfortunately, the International Classification of Disease (ICD) codes are not used in Tobruk Medical Center. Data were collected from the files of 56 admitted patients (131 eyes) in the period between August 1st and December 31st, 2018; there were 24 male cases (received 63 injections) and 32 female cases (received 68 injections). The recorded data included patient diagnosis, time of the injection, place of operation, clinical features, complications soon after the time of injection, and later at the first post-operative day, and method of management of these complications. All the cases received an intravitreal injection of 2.5 mg/0.1 ml of Avastin with a 27 Gauge needle, cleaning of periocular skin with 10 % povidone-iodine solution, after 60 seconds the eyelids were retracted with a speculum to avoid contamination by lashes, and topical povidone-iodine 5 % was instilled into the conjunctival sac 2 min before the injection. All the IVIs were done by the same surgeon in the OT room in complete aseptic conditions. The site of injection was in the upper temporal area. Routine prophylactic use of IOP-lowering medications with Diamox (Acetazolamide) Tab. 250 mg one-two hours before the time of injection was given to 52 patients. The ocular digital massage measurement of IOP was done after the anti-VEGF injection in all eyes. The fundus was observed by a direct ophthalmoscope to control the central retinal artery (CRA) perfusion. All the patients underwent anterior-chamber paracentesis and Fundus examination after the IVIs injections were administered by the same treating ophthalmologist.

RESULTS

There were 56 patients with an average age of 56.5 years and ranged from (40-70 years). They received 131 injections, 68 (51.9%) of which were females. The indications for the Avastin injections were; exudative age-related macular degeneration (AMD), diabetic macular edema (DME), branch retinal vein occlusion (BRVO), and ocular ischemia (OI) which occurs most of the time in patients with old age (table 1).

Table:(1). The indications for the Avastin injections in the study group

Diagnosis	Avastin indication	No. of total injected eyes (injections)
DME	Cystoid macular edema	125
BRVO	Cystoid macular edema	3
AMD	Wet age-related macular degeneration	2
OI	Rubeosis iridis	1

DME; diabetic macular edema, BRVO; Branch retinal vein occlusion, AMD; Age related maculopathy, OI; Ocular ischemia

In the present study, most of the cases with DME had cystoid macular edema (95.4%). Some of these patients were having other ocular pathologies as shown in table 2.

Table:(2). Patients with other ocular pathologies associated with DME

Total DME cases	DME ass. with rubeosis iridis	DME ass. With COAG	DME ass. With retinal neovascularization
125	9	5	7

DME; Diabetic macular edema, COAG; Chronic open angle glaucoma.

About 93 eyes (71%) developed complications because of the intravitreal injection. There were some transient and easily treatable complications that did not lead to dangerous or permanent insult to the eyes like subconjunctival hemorrhage, eye pain/ discomfort, floaters, and blurred vision. Other rare but serious complications such as endophthalmitis, vitreous hemorrhage, and transient loss of vision due to an acute increase in IOP at the time of injection were also recorded. Serious

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complications, like retinal detachment and central artery occlusion, were not recorded in this study. (table 3).

Table:(3). Complications after intra ocular Avastin injection in the study group (93 out of 131 eyes (injection))

Complication	Time in days of complications after the injections	Number of eyes (%) with complications	Gender
Subconjunctival hemorrhage.	Soon after injection	25 pts. (19%)	15 F 10 M
Discomfort/ pain	First post injection day	18 pts. (13.7%)	10 F 8 M
Blurred vision	First post injection day	8 pts. (6%)	4 F 4 M
Floaters	First post injection day	4 pts. (3 %)	3 F 1 M
Increase IOP	Day of injection	18 pts. (13.7%)	11 F 7 M
Sudden loss of vision	Soon after injection	13pts. (10%)	7 F 6 M
Leak at site of injection	Soon after injection	6 pts. (4.6%)	5 F 1 M
Endophthalmitis	Third day post the injection	1 pts. (0.8%)	1 M

IOP; Intraocular pressure.

There was no statistical significance for the post-Avastin injection complications among gender ($P = 0.46$).

There were eighteen eyes (13.7%) complicated with increase IOP post-Avastin injection (11 females and 7 males), out of them; Six had received Diamox (Acetazolamide) Tablet 250 mg one-two hours before the time of injection.
Cases with glaucoma or rubeosis iridis had no statistically significant difference from those free from it ($P= 0.09$). In both groups, the rise of IOP was relieved with anterior chamber tap.

Thirteen eyes (10%) developed a sudden increase in the IOP on the operating table immediately after the injection (digitally measured IOP showed hard resistance along with corneal edema and decrease vision), which resolved by anterior chamber tap. On the first postoperative day, all of them regained the baseline vision they had before the injection.

The patients who had glaucoma or rubeosis iridis suffered significantly ($P = 0.01$) from increase IOP (digitally measured) after injection with Avastin, while most other patients who didn't have both pathologies didn't suffer from an increase in IOP.

Endophthalmitis was recorded only in one eye (0.8 %) on the third day after the injection. The diagnosis of endophthalmitis was made clinically (patient had severe pain, redness, decreased vision associated with hypopyon, and vitreous opacities detected by ultrasonography). The patient was treated with intravitreal antibiotics (vancomycin) along with topical and systemic antibiotics and steroids, but he did not recover until pars plana vitrectomy was done to him.

DISCUSSION

The Needle Gauge for the intravitreal medication injection is not only important for patient comfort, but also for a safe injection procedure and efficient outcome. The most commonly used needle size ranges from 27 to 30 gauge. Pulido et al., (2007) confirmed that smaller scleral holes and less structural damage occur with decrease needles gauge, independently on the injection technique used, such as the tunneled or the perpendicular technique. Oztas, et al., (2016) reported the location of intravitreal medication should be made through the pars plana, between 3.5 and 4mm from the limbus; posterior to 4 mm can lead to an increased risk of retinal detachment, while a more anterior location increases the risk of traumatic cataract formation. They also recommended avoiding injection in per sclerotomy areas, to prevent vitreous incarceration and a persisting scleral hole.

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Tufan, et al., (2013) confirm the intravitreal injection can be safely performed in 360 degrees through the pars plana. Patients who receive an injection of Avastin may experience less severe side effects related to the preparation procedure. These side effects may include eye pain, subconjunctival hemorrhage, vitreous floaters, inflammation of the eye, and visual disturbances. Other possible complications and side effects of the procedure and administration of Avastin, but not recorded in this study, include but are not limited to retinal detachment, cataract formation, hypotony, permanent damage to the retina or cornea, and bleeding (Hoguet , et al., 2019). The volume change of the vitreous cavity may be the main reason for immediate IOP increasing after Anti-VEGF intravitreal injections. The volume of the vitreous cavity in the human eye is approximately 4 ml, and the volume of Avastin injected into the vitreous is 0.1 ml. Therefore, the increase in fluid volume of the vitreous cavity is 2.5% approximately, which may cause immediate IOP elevation (Song et al., 2016). Transient vision loss is a poorly understood complication of intravitreal injections, (Fang et al., 2006; Uyar, et al., 2019) reported the increased intraocular pressure (IOP) causes corneal edema and damage to the endothelial cells with optic nerve damage and can lead to significant loss of vision. Long-term deformation can cause a significant effect on the endothelial function and it may result in endothelial dysfunction. (Fang et al., 2006) suggested high IOP may affect the function of the endothelial pump and induced corneal edema. It also reduces intraocular blood flow, induces hypoxia and oxidative stress and as a result, could damage the optic nerve. Transient IOP spikes mostly leave the healthy eye without permanent damage to the vascular optic nerve. (Callegan et al*.,* 2002) confirm the IOP has to be rigorously controlled in patients vulnerable to vascular optic nerve damage, which are patients with glaucoma as well as patients predisposed to anterior ischemic optic neuropathy or retinal vein occlusion. It has been reported that 13.7% of patients receiving intravitreal Avastin experienced an IOP rise (digitally hard) after the injection. Hollands et al., (2007) and Song et al. (2016) confirm the patients with a history of glaucoma sustained a rise of IOP with the loss of vision significantly high, suggesting that glaucomatous eyes (higher risk eyes) should be identified before anti-VEGF injections and monitored carefully post-injection for IOP spikes that can cause visual field deterioration. In this study, the 13 cases (10 %) which had a sudden loss of vision were all managed with paracentesis to reduce the high IOP. The vision was improved significantly with no permanent vascular or optic nerve damage. Bertino (2009) showed that a combination of topical anti-glaucoma therapy and performing ocular decompression massages before the procedure significantly reduce IOP. Frenkel, et al., (2011) had reported that prophylactic medication did not prevent post-injection IOP spikes. In the present study; the use of prophylaxis systemic Diamox Tablets to reduce intraocular pressure was statistically not significant (P = 0.09). Patients with and without glaucoma showed a similar rate of IOP normalization.

While the development of targeted molecular therapy to inhibit vascular endothelial growth factor (VEGF) has revolutionized the treatment and visual prognosis of highly prevalent retinal diseases such as diabetic retinopathy and age-related macular degeneration, each intravitreal injection of these agents carries a risk of endophthalmitis which can be visually devastating (Sachdeva et al, 2016). The post-IVI injection endophthalmitis is rare, accounting for 0.03% - 0.072% of all cases of anti-endothelial vascular growth factor (VEGF) injections (Hoevenaars, et al, 2012; Park, et al, 2014). In most cases of post-intravitreal medication, the endophthalmitis is acute, occurring mostly in <28 days after the injection (Hoveenaars et al., 2012 and Shah et al., 2011), it should be suspected in cases of persistent vitritis following treatment, and these cases may require vitrectomy to remove the infected vitreous (Sachdeva, et al, 2016). Similarly, in the present study,

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this serious post-operative endophthalmitis was recorded in one case (0.8%), it occurred in the first 3 days after the injection, not responding to intravitreal antibiotics and improved by vitrectomy. There are several limitations to the present study. First, the most important limitation is that the IOP was measured digitally because of the unavailability of a non-contact tonometer, and fear that a contact tonometer may increase the risk of infection. Second, the central corneal thickness was not measured in this study, and the effect would be investigated in an advanced study. In addition, this study only focused on the short-term effect of the acute rise in IOP, so we recommend investigating the long term effects in future studies.

CONCLUSION

Despite Anti-VEGF having a dramatic effect on the quality of life by improving the central vision in ocular angiogenic disease processes, it can cause different complications, which could be transient like intraocular pressure spikes, or serious like endophthalmitis, that could be prevented by early diagnosis. Apart from glaucoma patients, routine prophylactic use of IOP-lowering medications is essentially ineffective in preventing IOP spikes after intravitreal injection, and routine monitoring of IOP in glaucoma patients receiving intravitreal anti-VEGF therapy is recommended. Also, post-anti-VEGF injection complications are not related to gender.

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REFERENCES

Aiello, L. P., Brucker, A. J., Chang, S., Cunningham, E. T., Jr, D'Amico, D. J., Flynn, H. W., Jr, Grillone, L. R., Hutcherson, S., Liebmann, J. M., O'Brien, T. P., Scott, I. U., Spaide, R. F., Ta, C., & Trese, M. T. (2004). Evolving guidelines for intravitreous injections. *Retina (Philadelphia, Pa.)*, *24*(5 Suppl), S3–S19. https://doi.org/10.1097/00006982-200410001-00002

Barry P, Cordovés L, Gardner S.(2013). ESCRS Guidelines for Prevention and Treatment of Endophthalmitis following Cataract Surgery: Data, Dilemmas and Conclusions. Paper Presented at the European Society of Cataract and Refractive Surgeons. Dublin, Ireland: 2013.https://www.escrs.org/downloads/Endophthalmitis-Guidelines.pdf. Accessed July 25, 2020

Bertino J. S., Jr (2009). Impact of antibiotic resistance in the management of ocular infections: the role of current and future antibiotics. *Clinical ophthalmology (Auckland, N.Z.)*, *3*, 507–521. https://doi.org/10.2147/opth.s5778

Callegan, M. C., Engelbert, M., Parke, D. W., 2nd, Jett, B. D., & Gilmore, M. S. (2002). Bacterial endophthalmitis: epidemiology, therapeutics, and bacterium-host interactions. *Clinical microbiology reviews*, *15*(1), 111–124. https://doi.org/10.1128/cmr.15.1.111-124.2002

Damasceno, N. P., Horowitz, S. A., & Damasceno, E. F. (2016). Leuconostoc as a Cause of Endophthalmitis Post-intravitreal Injection of Ranibizumab. *Ocular immunology and inflammation*, *24*(1), 118–119. https://doi.org/10.3109/09273948.2014.898073.

ISSN: online 2617-2186 print 2617-2178

Dancer, S. J., Stewart, M., Coulombe, C., Gregori, A., & Virdi, M. (2012). Surgical site infections linked to contaminated surgical instruments. *The Journal of hospital infection*, *81*(4), 231–238. https://doi.org/10.1016/j.jhin.2012.04.023

Dhoot SB, Kunjukunju N, Sabates N. (2013).The role of prophylactic antibiotic use in prevention of endophthalmitis following intravitreal injection of anti-vascular endothelial growth factor agents: a meta-analysis. *Open J Ophthalmol*., 3(2):46–49.

Fang, Y. T., Chien, L. N., Ng, Y. Y., Chu, H. F., Chen, W. M., Cheng, C. Y., & Wu, S. C. (2006). Association of hospital and surgeon operation volume with the incidence of postoperative endophthalmitis: Taiwan experience. *Eye (London, England)* , *20*(8), 900–907. https://doi.org/-10.1038/sj. eye.6702045

Frenkel, M.P., Haji, S.A. and Frenkel, R.E. (2010) Effect of Prophylactic Intraocular Pressure-Lowering Medication on Intraocular Pressure Spikes after Intravitreal Injections. *Arch Ophthalmol*, 128, 1523-1527. http://dx.doi.org/10.1001/archophthalmol.2010.297

Hoevenaars, N. E., Gans, D., Missotten, T., van Rooij, J., Lesaffre, E., & van Meurs, J. C. (2012). Suspected bacterial endophthalmitis following intravitreal anti-VEGF injection: case series and literature review. *Ophthalmologica. Journal international d'ophtalmologie. International journal of ophthalmology. Zeitschrift fur Augenheilkunde*, *228*(3), 143–147. https://doi.org/10.1159/000339584

Hoguet, A., Chen, P. P., Junk, A. K., Mruthyunjaya, P., Nouri-Mahdavi, K., Radhakrishnan, S., Takusagawa, H. L., & Chen, T. C. (2019). The Effect of Anti-Vascular Endothelial Growth Factor Agents on Intraocular Pressure and Glaucoma: A Report by the American Academy of Ophthalmology. *Ophthalmology*, *126*(4), 611–622.

Hollands, H., Wong, J., Bruen, R., Campbell, R. J., Sharma, S., & Gale, J. (2007). Short-term intraocular pressure changes after intravitreal injection of bevacizumab. *Canadian journal of ophthalmology. Journal canadien d'ophtalmologie*, *42*(6), 807–811. https://doi.org/10.3129/i07-172

Oztas, Z., Akkin, C., Afrashi, F., & Nalcaci, S. (2016). The short-needle intravitreal injection technique. *International journal of ophthalmology*, *9*(6), 929–930. https://doi.org/10.18240/ijo.2016.06.24

Park, J. C., Ramasamy, B., Shaw, S., Prasad, S., & Ling, R. H. (2014). A prospective and nationwide study investigating endophthalmitis following pars plana vitrectomy: incidence and risk factors. *The British journal of ophthalmology*, *98*(4), 529–533. https://doi.org/10.1136/bjophthalmol-2013-304485

Pulido, J. S., Pulido, C. M., Bakri, S. J., McCannel, C. A., & Cameron, J. D. (2007). The use of 31-gauge needles and syringes for intraocular injections. *Eye (London, England)*, *21*(6), 829–830. https://doi.org/10.1038/sj.eye.6702514

Qureshi, N. A., Mansoor, H., Ahmad, S., Zafar, S., & Asif, M. (2016). Reducing intraocular-pressure spike after intravitreal-bevacizumab injection with ocular decompression using a sterile cotton swab soaked in proparacaine 0.5%: A quasi-experimental study. *Taiwan journal of*

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ophthalmology, *6*(2), 75–78. https://doi.org/10.1016/j.tjo.2015.12.003

Sachdeva, M. M., Moshiri, A., Leder, H. A., & Scott, A. W. (2016). Endophthalmitis following intravitreal injection of anti-VEGF agents: long-term outcomes and the identification of unusual microorganisms. *Journal of ophthalmic inflammation and infection*, *6*(1), 2. https://doi.org/10.1186/s12348-015-0069-5

Semeraro, F., Morescalchi, F., Duse, S., Gambicorti, E., Cancarini, A., & Costagliola, C. (2015). Pharmacokinetic and Pharmacodynamic Properties of Anti-VEGF Drugs After Intravitreal Injection. *Current drug metabolism*, *16*(7), 572–584. https://doi.org/10.2174/1389200216666151001120831

Shah, Chirag P; Garg, Sunir J; Vander, James F; Brown, Gary C; Kaiser, Richard S; Haller, Julia A; and The Post-Injection Endophthalmitis (PIE) Study Team (2011). "Outcomes and risk factors associated with endophthalmitis after intravitreal injection of anti-vascular endothelial growth factor agents." *Ophthalmology*, 118 (10): 2028-2034.

Solborg Bjerrum, S., Kiilgaard, J. F., Mikkelsen, K. L., & la Cour, M. (2013). Outsourced cataract surgery and postoperative endophthalmitis. *Acta ophthalmologica*, *91*(8), 701–708. https://doi.org/10.1111/aos.12279

Song, S., Yu, X. B., & Dai, H. (2016). Effect of prophylactic intraocular pressure-lowering medication (brinzolamide) on intraocular pressure after ranibizumab intravitreal injection: A case-control study. *Indian journal of ophthalmology*, *64*(10), 762–766. https://doi.org/10.4103/0301-4738.195006

Tufan, H.A., Vural, A.H., Gencer, B., Kara, S., Arıkan, S., & Yuksel, E. (2013). Bacterial Contamination of Needles Used for Intravitreal Injections: Comparison between 27-gauge and 30-gauge Needles. *Ocular Immunology and Inflammation, 21*, 366 – 370, DOI: 10.3109/09273948.2013.801988

Uyar, E., Ulas, F., Sahin, S., & Celebi, S. (2019). Major factors affecting intraocular pressure spike after intravitreal ranibizumab injection: Vitreous reflux and its amount. *European Journal of Ophthalmology*, *29*(4), 361–367. https://doi.org/10.1177/1120672119836613

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المضاعفات التي تحدث للعين بعد حقن عقار الافاستين داخل العين: تقرير من مركز طبرق الطبي

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المستخلص: تم إجراء دراسة إحصائية بأثر رجعي على جميع المرضى الذين تلقوا الأدوية بالحقن داخل العين (أفاستين) في قسم طب العيون في مركز طبرق الطبي في المدة بين 01 أغسطس إلي 31 ديسمبر 2018 تهدف إلى تحليل المضاعفات للحقن داخل الجسم الزجاجي للعين وكيفية إدارة هذه المضاعفات. من أصل 56 مريضاً مسجلاً، كان هناك 32 (51.9٪) من الإناث، تلقى جميع المرضى حقنًا متعددة في العين، بإجمالي 131 حقنة. كان متوسط عمر المرضى 56.5 عامًا (تراوحت بين 40 و70 عامًا)، وكانت المضاعفات الأكثر شيوعًا بعد الحقن في الجسم الزجاجي هي: النزف تحت الملتحمة (19٪) ، الانزعاج / الألم (13.7٪)، عدم وضوح الرؤية (6٪)، التسرب عند موقع الحقن (4.6٪) والعوائم (3٪). عانى 18 مريض (13.7٪) من ارتفاع مفاجئ في ضغط العين، من بينهم ست حالات تلقت قرص Diamox (أسيتازولاميد) 250 مجم الخاصة بخفض ضغط العين قبل ساعة الى ساعتين من وقت الحقن، ولكن ذلك لم يمنع ارتفاع ضغط العين ما بعد الحقن وكان غير معتد به إحصائيًا ($P = 0.09$). أما فقدان الرؤية بشكل مفاجئ فقد حدث في 13 عينًا (10٪ من الحالات) بسبب الزيادة المفاجئة في ضغط العين مباشرة بعد عملية الحقن، وفي جميع الحالات تم خفض ضغط العين بواسطة بزل الغرفة الأمامية للعين لغرض تقليل السائل به وقد استجابوا جميعهم لعملية خفض ضغط العين مع التحسن في الرؤية أيضا. تم تسجيل التهاب باطن المقلة في حالة واحدة فقط (0.8 ٪)، في اليوم الثالث بعد حقن أفاستين داخل العين، كانت الكائنات الحية الدقيقة المسببة غير معروفة وعولجت بالمضادات الحيوية داخل الحقنة (فانكومايسين) جنبًا إلى جنب مع المضادات الحيوية الموضعية والجهازية والستيرويدات، لكنه لم يتماثل للشفاء فتم إجراء عملية استئصال السائل الزجاجي له، واسترد المريض رؤيته مثلما كانت قبل الحقن. المرضى الذين يعانون من الزرق (الجلوكوما) أو داء القزحية كانوا يعانون من ارتفاع في ضغط العين ذو دلالة إحصائية ($P = 0.01$) عند مقارنته بالمرضى الذين لا يعانون من هذين المرضين. بالرغم من أن حقن (أفاستين) له تأثير إيجابي كبير على جودة الحياة من خلال تحسين الرؤية، إلا أنه قد يحدث مضاعفات حرجة من الممكن علاجها بدون أن تترك أي أثار جانبية إذا تم تداركها في مرحلة مبكرة وبالطريقة العلاجية المناسبة.

الكلمات المفتاحية : افاستين؛ المضاعفات؛ الحقن داخل الجسم الزجاجي؛ طبرق.

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The Effect of *Nicotiana glauca* Leaf Extract on the Liver and Lung of Female Albino Mice: Physiological and Histopathological Studies

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Abstract: Tobacco *Nicotiana glauca* is a member of the Solanaceae family, found in tropical and subtropical countries especially South America, Cuba, and Brazil, as well as in North Africa, Egypt, and Libya. *N. glauca* is known to be a highly toxic plant. The major alkaloids are anabasine and nicotine. This study was aimed to determine the proper effects of a sublethal dose of the plant on female albino mice, as well as certain histological and physiological parameters. The three sublethal concentrations, 0.25, 0.50, and 2.8 mg/kg of the water extract were tested through oral route on female mice for determination of effects such as body weight, liver and lung weight and histology, blood parameters, and blood chemistry. The results showed that reduced body weight was slightly evident in treated females. Percent decrease of liver weight/body weight and increase lung weight/body weight have reported in 2.8 mg/kg treated female mice compared to control mice. Histology of liver and lung revealed an early sign of cell damage and accumulation of cell necrosis in both the liver and lungs. The blood parameters and blood chemistry did not reveal a significant difference between control and *N. glauca* treated female mice.

Keywords*: Nicotiana glauca*; Female Mice; Physiology; Histopathology.

INTRODUCTION

N. glauca (tree tobacco) is a member of the Solanaceae family, which includes important crops (potato, tomato, eggplant, pepper) (Long et al., 2016).Nicotine crosses the placental barrier and has been shown to have adverse effects on male and female reproduction, fetal weight gain, and development in experimental animals and humans. It was teratogenic in studies in mice, rats, rabbits, and chickens. Developmental abnormalities of the cardiovascular system have been reported in humans (Panter et al., 1999).Pyridine, piperidine, nicotine, and nornicotine were produced from *N. glauca*, however, the major alkaloids are anabasine and less than 1 % concentration of nicotine (Alder et al., 2012).Nicotine is a colorless liquid that turns amber on exposure to light. More recently, nicotine sulfate has been used as a dog-controlling agent (at approximately 285 mg of nicotine/mL). It is also used in animal tranquilizer darts (with a strength of 240 mg of nicotine/mL). However, its use as an insecticide has been drastically reduced (Gupta, 2016).

Anabasine is similar in both structure and effects to nicotine, but it appears to be more potent in humans despite a slower onset of action (Webb and Dalzell, 1997), and the percentage of anabasine is given as 1.3 from the dry plant and 1% from the root (Mitchell and Breyer-Brandwijk, 1962).The toxic effect of anabasine and nicotine was dose-dependent and could cause various physiological effects (Melamed et al., 2004). Clinical signs for nicotine toxicosis include tremors, weakness, and decumbency. Anabasine can also moderately inhibit acetylcholinesterase. Clinical signs include weak-

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ness, ataxia, tremor, and collapse, bulbar palsies, flexure muscle spasm, nausea, vomiting, and diarrhea. Death is usually the result of respiratory compromise, paralysis, blocking of the autonomic ganglia, and acting as a cholinesterase inhibitor (Wink, 2000). Human ingestion of *N. glauca* leaves causes neuromuscular paralysis and death, and this has been attributed to anabasine (Mellick et al., 1999).

Nicotine is rapidly absorbed from the respiratory tract, mouth, and intact skin, but is poorly absorbed from the stomach under acidic conditions since it is a strong base. After absorption, nicotine is rapidly mobilized throughout the body via the blood (Gosselin et al*.,* 1984). In experimental animals and humans, 80-90 % of absorbed nicotine is metabolized primarily in the liver, in addition to the kidney and lung. The remainder is rapidly excreted unchanged in the urine (Goodman and Gilman, 1985). The liver has been reported to be the principal site of detoxification (Schievelbein and Balfour, 1984). For example (Rotenberg, 1982) reported that in a dog, 30 % of the dose excreted in the urine was cotinine and cotinine metabolites. In a rat, 25 % was nicotine, whereas, 15 % of the total dose was exhaled as CO_2 in rats.

The rate of excretion by the kidney was found to depend on the PH of the urine.The designed study was to determine the proper effects of a sublethal dose of the plant on female albino mice as well as certain histological, and physiological parameters.

MATERIALS AND METHODS

Experimental animal: Adult female Swiss albino mice *Mus Musculus* were obtained from the animal house of the faculty of medicine, the University of Benghazi. These animals were reared under laboratory conditions.

The chemicals: The main chemical is the crude water extract of (*N. glauca*) leaves of the plant for which the toxicity is to be studied.

The experimentation: Three sublethal concentrations, 0.25, 0.50, and 2.8 mg/kg of the water extract of *N. glauca* leaves were tested through oral route on female mice for the determination of effects on body and organ weight, blood parameters, blood chemistry, and organ histology (Preece, 1972). Each animal orally received the specified dose via intubation, 0.1 ml or less depending on the bodyweight of the specified concentration using a modified syringe that can be inserted into an animal pharynx with no physical harm. Control animals were dosed with comparable volumes of distilled water alone.

Blood analysis and blood chemistry: For blood collection, the animal was anesthetized with ether and the head was severed using sharp scissors where the seeping blood was collected in vials with or without potassium-EDTA to prevent blood clotting. Between 1 to 1.5 ml of blood volume were collected in this way. The blood was collected for the measurement of Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Hemoglobin (HB), Haematocrit (HCT), Mean Cell Volume (MCV) and Blood Platelets (PLT), and measurement of urea, creatinine, sodium ions (Na^+), potassium ions (k^+), total protein and Alanine transaminase (ALT). Decapitated animals were then dissected for postmortem observation, including treated mice body weight, liver and lung weight, and histology. Organs were transferred to FAA fixative (Formalin-Acetic acid-Alcohol) and kept for weighting and histological studies.

Statistical analysis: The data were subjected to one-way (ANOVA) analysis, and calculation was performed using SPSS (Sokal and Rohalf, 1969) statistical package and Microsoft office excel 2007.

RESULTS

Body weight (BW): Treated female mice's body weight did not reveal a significant difference in percent body weight increase (F =

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2.908, P > 0.101) as compared to the control mice. However, treated females showed a slightly decrease percent body weight gain (6.9400 ± 0.975, 5.6433 ± 2.277 and 5.190 ± 1.4490) for 0.25 mg/kg, 0.5 mg/kg and 2.8 mg/kg respectively compared to control females b. w. means (mean = 8.1267 ± 1.363) (Fig 1).

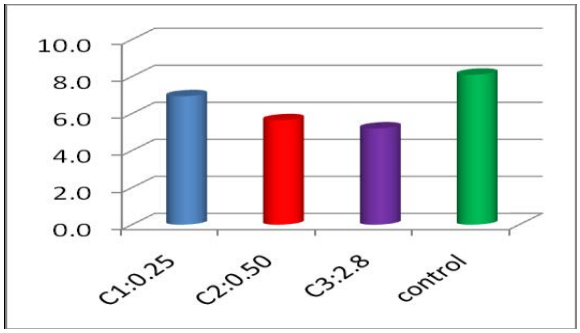

Fig 1. Shows the percent body weight % decrease (BW) of female Swiss albino mice of 0.25 mg/kg , 0.5 mg/kg and 2.8 mg/kg *N. glauca* leaf water extract treated females after four weeks

Organ weight:

Liver weight (LiW/BW): The liver weight of female mice treated with 2.8 mg/kg leaf extract was found to slightly decrease (F = 6.176, P > 0.003) as compared to that of control mice. Both 0.25 and 0.5 mg/kg treated mice haven´t shown any changes as compared to the liver weight of the control. Control females' mean ± SD percent liver weight was 6.3197 ± 0.84 compared to that of the treated females having 6.1600 ± 1.40, 6.4573 ± 0.57, 4.5678 ± 1.38 for 0.25 mg/kg, 0.5 mg/kg and 2.8 mg/kg respectively (Fig 2).

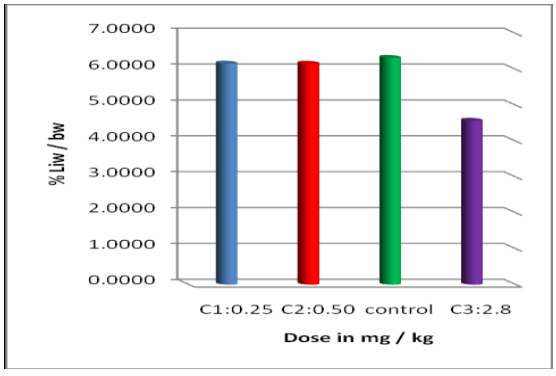

Fig 2. Shows the percent liver weight per body weight of female Swiss albino mice from control and 0.25 mg/kg, 0.5 mg/kg and 2.8 mg/kg *N. glauca* leaf water extract treated females.

Lung weight (LW/BW): The 2.8 mg/kg leaf extract treated female mice lung weight was found slightly increase (F = 8.239, P > 0.001) as compared to the control mice. Whereas the other two doses haven´t shown any changes as compared to the control. The mean ± SD percent lung weight were, 1.2495 ± 0.238 for the 2.8 mg/kg compared to 0.8292 ± 0.221, 0.8744± 0.237, 0.9282± 0.250 for 0.25 mg/kg, 0.5 mg/kg and control respectively (Fig 3).

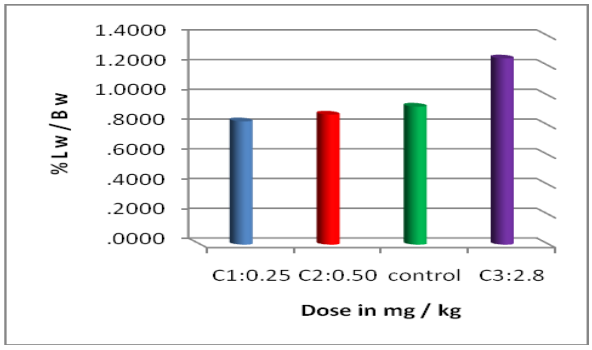

Fig 3. Shows the percent lung weight per body weight of female Swiss albino mice of control and 0.25 mg/kg, 0.5 mg/kg and 2.8 mg/kg *N. glauca* leaf water extract treated females.

Blood parameters: The results revealed that the concentration 2.8 mg/kg had lower values than the control and the other two concentrations 0.25 and 0.5 mg/kg in all parameters except MCV. The result reflects that the higher the concentration the larger the impact (Fig. 4).

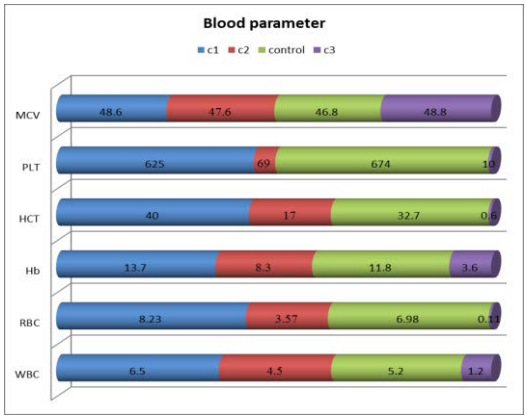

Fig. 4. Showed the blood parameter of female Swiss albino mice from control and 0.25 mg/kg, 0.5 mg/kg and 2.8 mg/kg *N. glauca* leaf water extract.

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Blood chemistry: The blood chemistry of the 2.8 mg/kg leaf extract has shown an elevated value of urea and a relatively high value of creatinine and a low value of Na^+ and k^+ as compared to control and the other concentrations. However, the values of Na^+, k^+, and total protein were not affected by the *N. glauca* treatment. Whereas, ALT reported higher values compared to control and 0.5 and 2.8 mg/kg (Fig. 5).

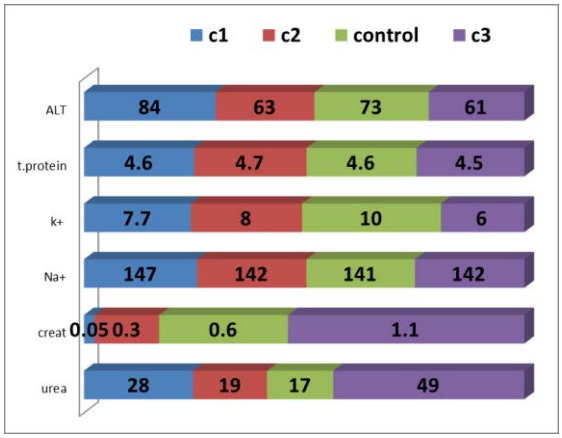

Fig. 5. Shows the blood chemistry of female Swiss albino mice from control and 0.25 mg/kg, 0.5 mg/kg and 2.8 mg/kg *N. glauca* leaf water extract.

Organ Histopathology:

Liver Histopathology:

The central vein of the liver in the control females is surrounded by normal hepatocytes and columns separated by hepatic sinusoids (Fig. 6). Whereas in 0.25 mg/kg treated mice, the central vein is surrounded by hepatocytes, but many of them contain vacuoles (hydropic degeneration) (Fig. 7).

In the 0.50 mg/kg treated mice, hepatocytes showed more vacuoles and few necrotic cells (pykontic nuclei) (Fig. 8).

In the 2.8 mg/kg treated mice, large areas of necrosis with disappeared nucleus and loss of cell boundaries were very evident (Fig.9).

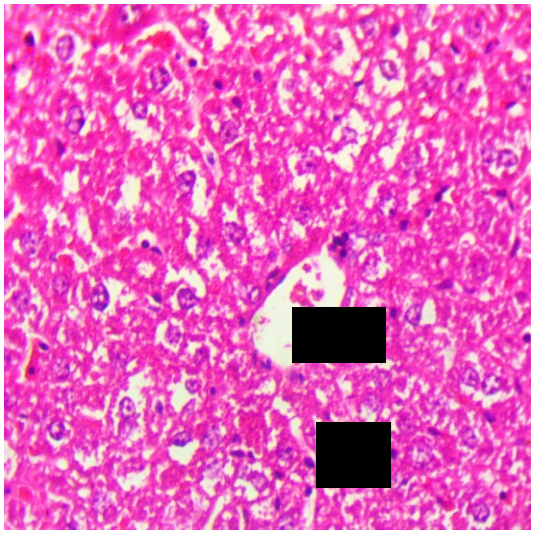

Fig. 6. Shows liver section in control. Central vein is surrounded by normal hepatocytes. (H&E, X 400).

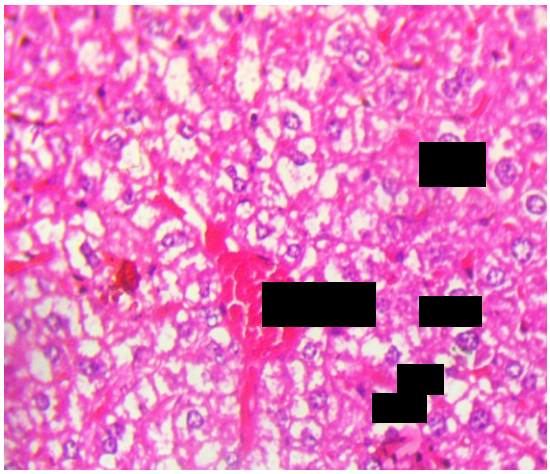

Fig. 7. Shows liver section in 0.25 mg/kg *N. glauca* treated mice. Central vein is surrounded by hepatocytes, many of them contain vacuoles. (H&E, X 400).

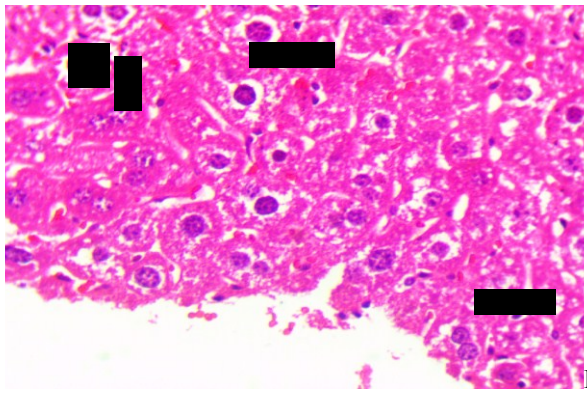

Fig. 8. Shows liver section in 0.50 mg/kg *N. glauca* treated mice. Hepatocytes with more vacuoles and few necrotic cells. (H&E, X 400).

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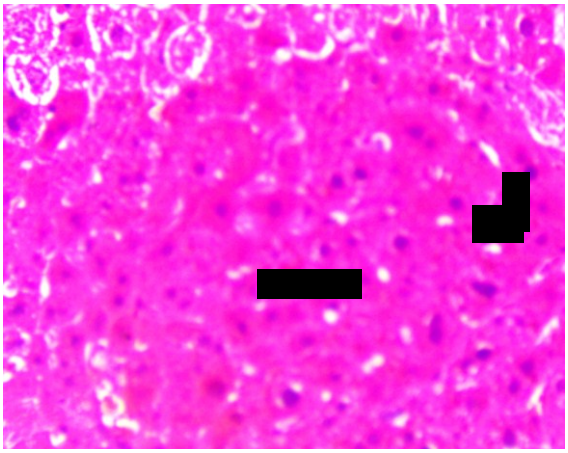

Fig. 9. Shows liver section 2.8 mg/kg *N. glauca* treated mice. Large area of necrosis. (H&E, X400).

Lung Histopathology: The terminal bronchioles of lung tissues are composed of air (alveolar) sacs with a space known as alveolus. Alveoli are separated by alveolar wall which is composed of fibrous tissues and epithelial cells (pneumocytes) and blood vessels, which were observed in the lung section of control female mice (Fig. 10).

In the 0.25 mg/kg treated mice, focal interstitial inflammation (Fig. 11), and in the 0.50 mg/kg treated mice, interstitial inflammation and cellular hyperplasia of the alveolar wall were observed (Fig.12).In the 2.8 mg/kg treated mice, dilation of air space (emphysema) was observed (Fig.13).Emphysema occurs due to elastic damage (resulting from the release of elastase enzyme by inflammatory cells).

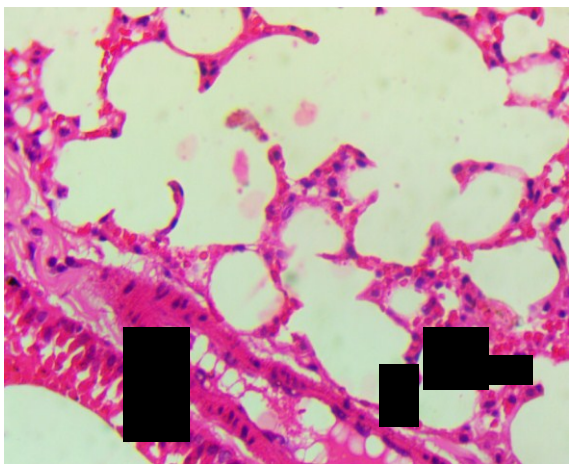

Fig. 10. Shows lung section in control, the normal alveolar with a space known as alveolus and blood vessels. (H&E, X400).

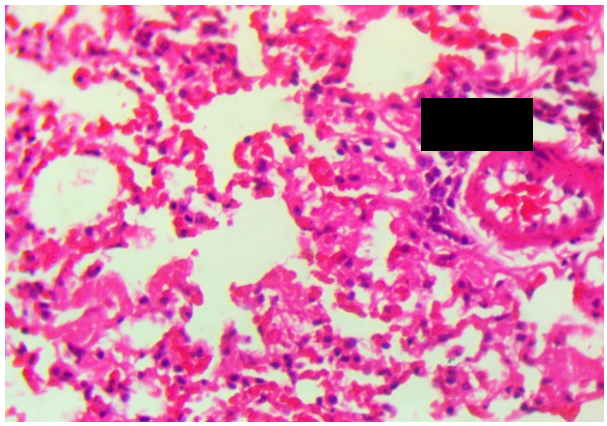

Fig. 11. Shows lung section in 0.25 mg/kg *N. glauca* treated mice, focal interstitial inflammation (H&E, X400).

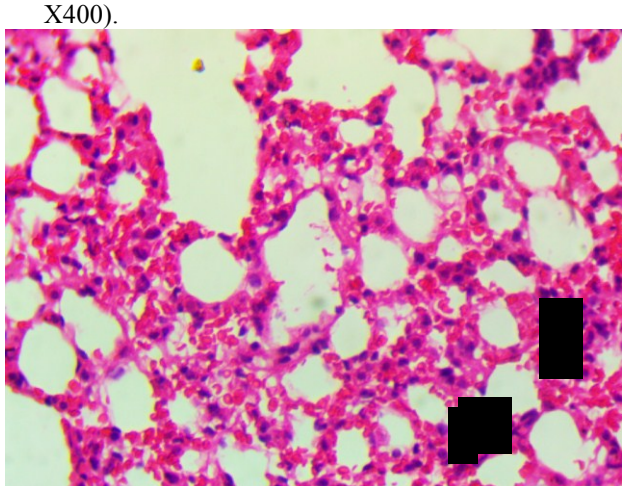

Fig. 12. Shows lung section in 0.50 mg/kg *N. glauca* treated mice, focal interstitial inflammation and cellular hyperplasia of alveolar wall (H&E, X400).

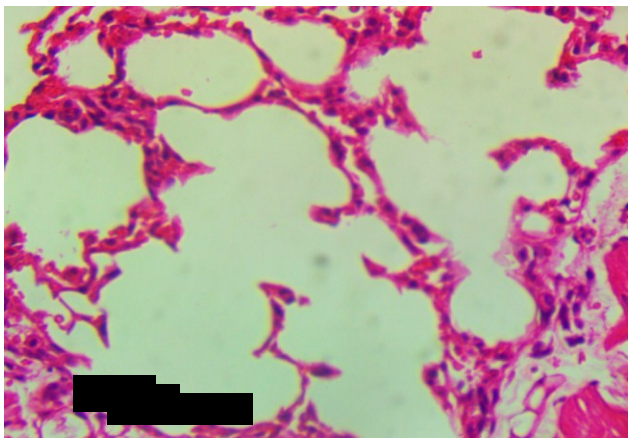

Fig. 13. Shows lung section 2.8 mg/kg *N. glauca* treated mice, emphysema (H&E, X400).

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DISCUSSION

Anabasine, nicotine, and other few glycosides are produced by the tree tobacco *Nicotiana glauca*. Poisoning by anabasine is characterized by nicotine-like toxicity. The clinical effects of nicotine alkaloid toxicity are the result of the stimulation actions at ganglionic sites, motor endplates, and smooth muscle (Schep et al., 2009). Nicotine alkaloids initially stimulate the ganglia of the sympathetic and parasympathetic nervous systems by direct cholinomimetic action on the ganglia. This is quickly followed by prolonged ganglionic blockade due to persistent depolarization. A similar action occurs at the neuromuscular junction causing total paralysis of skeletal muscles and subsequent respiratory failure. Death occurs from respiratory failure due to peripheral blockage of the muscles of respiration (Manoguerra and Freeman, 1982). The results of female mice body weight observed in this study confirm the previously reported cases, where, the percentage change of *N. glauca* treated mice body weight was significantly or numerically lower than that of control. This reduction in the weight of mice following treatment can be attributed to depression, diarrhea, weakness, and vomiting, as these were among the evident signs of symptoms observed on the treated mice. These results have also been confirmed by the findings of (Wilson and DeEds, 1936 and Rowell et al., 1983).

The effects of *N. glauca* water leaf extract on blood parameters and blood chemistry have shown relatively elevated values of WBC, RBC, Hb, HCT, PLT, and MCV for blood parameters and Urea, Creatinine, Na^+, k^+, Total protein and ALT for blood chemistry, in comparison with the standard range. However, no such changes in these values were detected between the lower *N. glauca* doses treated and non-treated female mice in the experimental animals in this study. This finding could mean that *N. glauca* extract has not induced direct toxicological effects on blood parameters and blood chemistry when used at low doses. However, (Saneck et al., 1994) have not reported significant alterations in clinical chemical values for the vitreous (blood urea nitrogen, creatinine, chloride, sodium, potassium, calcium, magnesium, and phosphorus).
Lack of previous studies concerning this part of toxicity, however, made it difficult to give any further elaboration for now.

Histological sections from the liver and lung of *N. glauca* treated female mice showed inflammation and necrosis, particularly in the liver and lung. No other severe pathological effects could be detected in either of the tissues examined, where, hepatocytes showed more vacuoles (hydropic degeneration) and necrotic cells in the treated mice liver and interstitial inflammation, cellular hyperplasia of alveolar wall, and emphysema that occurs due to elastic damage resulting from the release of elastase enzyme by inflammatory cells in the treated mice lung. These findings were confirmed by (Saneck et al., 1994) who found that lesions in mules had induced severe, diffuse swelling of renal proximal tubular epithelial cells. Whereas, centrilobular hepatocytes had undergone a mild fatty change and necrosis, in scattered foci. Pulmonary alveoli contained moderate quantities of proteinaceous fluid and rare red blood cells. Myocardial cells had undergone diffuse, mild swelling.

Damage to the structural integrity of the liver is reflected by an increase in the liver hepatospecific enzymes in alkaline phosphate (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in the serum because they are cytoplasmic in location and are released into circulation after cellular damage (Janbaz and Gilani, 1995).
The liver represents the primary site of metabolism of nicotine, although the lung and kidney also contribute to metabolism.

On average, 30 % of the nicotine that enters the liver exits unchanged, and 70 % leaves the liver in the form of metabolites. Events that change the hepatic flow, such as eating, posture, and

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exercise, reduce hepatic blood flow, and reduce the rate of nicotine metabolism (Benowitz and Jacob, 1997).

Renal clearance of unmetabolized nicotine depends on urinary pH and can account for 2-35 % of nicotine excretion. Nicotine is reabsorbed from the proximal tubules, where the pH is high.

Anabasine, the plant's main active ingredient, can cause severe systemic intoxication due to its nicotinic receptor agonist action, with respiratory muscle paralysis being the main effect (Ntelios *et al*., 2013).

Nicotine is absorbed via intact skin, oral mucosa, the GI tract, and the respiratory system. It is detoxified mainly in the liver, and also the kidneys and lungs. The major metabolic product of nicotine is cotinine. Cotinine, along with a nonmetabolized form of nicotine, is excreted in the urine. Elimination is complete in 16 h; however, acidic urine increases urinary excretion. Nicotine is also excreted in the milk of lactating women (Gupta, 2016).

CONCLUSION

Prolonged exposure of female Swiss albino mice to concentrations of the water extract (0.25, 0.50, and 2.8 mg/kg) has evidently resulted in bodyweight reduction, liver and lung congestion, and enlargement.
Histopathological examination revealed an early sign of inflammation and cell necrosis of the liver and lung, as well as the accumulation of leucocytes in both.

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REFERENCES

Alder, L. S., Seifert M. G., Wink. And Morse, G. E. (2012). Reliance on pollinators predicts defensive chemistry across tobacco species. *Ecol. Lett.,* 15: 1140-1148.

Benowitz, N. L. and Jacob, P. III. (1997). Individual differences in nicotine kinetics and metabolism in humans [Review]. *Natl. Inst. Drug Abuse. Res. Monogr.,* 173: 48-64.

Goodman, L. S. and Gilman, A. (1985). The Pharmacological Basis of Therapeutics. 7th Edition, *MacMillan Publishing Co., New York*.

Gosseline, R. E., Smith, R. H. and Hodge, H. C. (1984). Nicotine *In :Clini. Cal. Toxicology of Commerical products* Fifth Edition, Williams and wikins, Baltimor, Maryland, *Williams andwikins, Baltimor, Maryland,* pp. III-311-314.

Gupta, P. K. (2016). Toxic effects of cardiac poisons. in Fundamentals of toxicology. 21.4.

Janbaz, K. H. and Gilani, A. H. (1995). Evaluation of the protective potential of *Artemisia maritime* extraction acetaminophen- and CCl4-induced liver damage. *J. Ethnopharmacol.,* 47: 43-47.

Long, N., Ren, X., Xiang, Z., Wan, W. and Dong, Y. (2016). Sequencing and characterization of leaf transcriptomes of six diploid *Nicotiana* species. *J. Biol. Res.,* (Thessalon). 23: 6.

Manoguerra, A. S. and Freeman, D. (1982). Acute poisoning from the ingestion of Nicotianaglauca. *J. Toxicol. Clin. Toxicol.* 19: 861-864.

Melamed, H. T., Markman, S., Aieli, A., Dist, M., Wink, M. and Tzhaki, I. (2004).

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Limited ability of Palestine sunbirds *Nectarinia osea* to cope with pyridine alkaloids in necter of tree tobacco *Nicotiana glauca*. *Func. Ecol*., 18: 844-850.

Mellick, L. B., Makowski, T., Mellick, G. A. and Borger, R. (1999). Neuromuscular blockade after ingestion of tree tobacco (*Nicotiana glauca*). *Ann. Emerg. Med.,* 34: 101-104.

Mitchell, J. W. and Breyer-Brandwijk, E. (1962) Medicinal and poisonous plants of southern and Eastern Africa-2nd ed. and S livingston; 986.

Ntelios, D., Kargakis, M., Topalis, T., Drouzas, A. and Potolidis, E. (2013). Acute respiratory failure due to *Nicotiana glauca* ingestion. *Hippokartia.,* 17 (2): 183-184.

Panter, K. E., James, L. F. and Gardner, D. R. (1999). Lupines, poison hemlock and *Nicotiana* ssp.: toxicity and teratologenicity in livestock. *J. Nat. Toxins*., 8 (1):117-134.

Preece, A. H. (1972). Amanual for Histologic Technicians, 4th ed. (227-231). Little, Brown and company. Boston.

Rotenberg, K. S. (1982). The pharmacokinetics of nicotine. *Pharm. Int.,* 3: 91-93.

Rowell, P. P., Hurst, H. E., Marlowe, C. and Bennett, B. D. (1983). Oral administration of nicotine: Its uptake and distribution after Chronic administration to mice. *J. Pharmacol*. *Methods,* 9: 249-261.

Saneck, R., Gupta, R. C. and Kadel, W. L. (1994). Lethal nicotine intoxication in a group of mules. *J. Vet. Diagn. Invest.,*6: 503-504.

Schep, L. J., Slaughter, R. J. and Beasley, D. M. (2009). Nicotinic plant poisoning. *Clin. Toxicol. (Phila.),* 47: 771-781.

Schievelbein, H. and Balfour, D. J. K. (1984). Nicotine, reabsorption and fate. In: Nicotine and the smoking Habit*, international Encylopedia of pharmacology and Therapeutics, Section 114, Pergamon Press, New, York.,* pp. 1-12.

Sokal, R. R. and Rodlf, F. F.(1969) Biometry, the principle of statistics in biological research. W. H. freeman and company, san fransisco, USA.

Webb, M. and Dalzell, S. (1997) *Nicotiana glauca* toxicity. *Emergency*. *Med.,* 9: 25-28.

Wilson, R., & DeEds, E. (1936). Chronic nicotine toxicity. I. Feeding of nicotine sulfate, tannate, and bentonite. *J. Int. Hyg. Toxicol, 18*, 553-564.

Wink, M. (2000). Interference of alkaloids with neuroreceptors andionchannels. *Bioactive Natural Products*, Vol. 11 (ed. Atta-Ur-Rahman), pp. 3–129. Elsevier Science, Amsterdam.

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تأثير مستخلص أوراق نبات التبغ على كبد ورئة إناث الفئران السويسرية البيضاء: دراسة فسيولوجية ونسيجية مرضية

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المستخلص: نبات التبغ البري *Nicotiana glauca* هو عضو من العائلة الباذنجانية ويوجد في المناطق الاستوائية وشبه الاستوائية خصوصاً امريكا الجنوبية، كوبا والبرازيل، كما يوجد في شمال أفريقيا، مصر وليبيا. يعد نبات التبغ البري عالي السمية. المكونات السامة الرئيسية شبه القلوية لنبات التبغ البري هي الأنبسين والنيكوتين. تهدف الدراسة إلى تحديد التأثيرات المختلفة للجرعات غير القاتلة لنبات التبغ البري على إناث الفأر السويسري الأبيض من الناحية النسيجية والفسيولوجية. لدراسة التأثيرات المطلوبة تم استخدام ثلاثة تركيزات من المستخلص المائي لنبات التبغ البري 0.25، 0.50 و2.8 مليجرام/كيلوجرام التي أعطيت لإناث الفأر السويسري الأبيض من خلال التجريع الفموي والعوامل التي تمت دراستها هي وزن الجسم وتأثير المستخلص على تركيب ووزن كل من الكبد والرئة وعوامل الدم وعوامل الدم الحيوية. أظهرت النتائج انخفاضاً بسيطاً في وزن إناث الفئران المعالجة مقارنة بأوزان فئران السيطرة بينما حدثت تغيرات في التركيب النسيجي للكبد والرئة حيث أظهرت النتائج انخفاضاً في وزن الكبد وزيادة في وزن الرئة وذلك في إناث الفئران المعالجة بالتركيز 2.8 مليجرام/ كيلوجرام مقارنة بمجموعة السيطرة. أما الشرائح المجهرية المعدة من الفئران المعاملة بمستخلص أوراق التبغ البري أظهرت علامات مبكرة لموت الخلايا وتراكماً لنخر الخلايا في الكبد والرئة مقارنة بمجموعة السيطرة. عوامل الدم وعوامل الدم الحيوية لم تظهر أي اختلافات تذكر بين مجموعات السيطرة والمعالجة.

الكلمات المفتاحية: التبغ البري، إناث الفأر، فسيولوجي، علم الأنسجة مرضية.

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Role of Diffusion Weighted Imaging in Enhancing MR Imaging in Recent Ischemic Stroke Patients

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Abstract: Stroke is a common cause of admission to hospitals, and imaging in acute stroke is necessary to differentiate ischemic from haemorrhagic stroke and to exclude other diagnoses. We aimed to evaluate the role of diffusion-weighted magnetic resonance imaging (DW MRI) in the diagnosis of recent cerebral ischemic infarction in a consecutive series of patients with symptoms of acute stroke and its feasibility as first-line imaging for those patients. We report our results with DWI and apparent diffusion coefficient (ADC) mapping comparing the sensitivity of DWI with that of conventional T2 weighted and fluid-attenuated inversion recovery (FLAIR) MRI. A Prospective audit of 87 patients with clinically suspected recent stroke referred for imaging over a consecutive 20-week period was done. The data collected included patient age, time from onset of symptoms, and clinical presentation. We performed DWI echo planar, FLAIR, turbo spin-echo T2-weighted MRI, and ADC maps were generated. Conventional MR images were assessed before DW images. DWI was considered positive for the diagnosis of new arterial stroke whenever hyperintensities with reduced ADC values were observed, and the site of infarct detected on the images was included in patients' data. The results were 47 patients had a final diagnosis of recent ischemic cerebral infarct. With DWI, 98% of the ischemic lesions were detected, whereas with FLAIR, only 70% were detected, and with T2-weighted images, 66% of lesions were found. There was a significant difference between the results of ischemic infarcts' detection on DWI and T2-w/FLAIR in relation to time from onset (*P* value = .012). In our audit, we were able to image 68% (60 of 87) of the referred suspected stroke patients with DW MRI within 48 hours and 39 patients (45%) within 24 hours of the onset of symptoms. DW MRI showed high sensitivity and superiority over conventional T2 and FLAIR imaging for the detection of acute ischemic lesions in stroke patients; it also proved quite feasible as a first-line of neuroimaging.

Keywords: Diffusion Weighted Imaging; Conventional MRI; T2-Weighted; FLAIR; Stroke.

INTRODUCTION

"Stroke is characterized by a rapidly developing neurological deficit that can lead to considerable morbidity and mortality, and it is a common cause of admission to hospitals" (Tan et al., 2006). Imaging in acute stroke is necessary to differentiate haemorrhagic from ischemic stroke, as well as to identify or exclude alternate etiologies of neurological deficit such as neoplasm or extra-axial hemorrhage (Tsiouris & Qian, 2017). In the early stage of ischemia, computed tomography (CT) is the most commonly used technique to exclude hemorrhagic stroke. However, several studies reported lower sensitivity of CT in the visualization of hyperacute ischaemic infarct in the immediate hours subsequent to onset (Chalela et al., 2007; Tan et al., 2006; Van Everdingen et al., 98). Magnetic resonance imaging (MRI) is generally thought to be better than CT for the diagnosis of acute stroke. According to Chalela et al (2007), who compared CT and MRI in emergency settings, MRI was more ac-

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curate than CT in the diagnosis of acute ischemia and should be the preferred test for accurate diagnosis of patients with suspected acute stroke.

Acute ischemia causes cytotoxic oedema, which makes them visible on T2-weighted (T2-w) images of conventional MRI, however, this is usually not apparent in the first few hours post-ictus (Leiva-Salinas & Wintermark, 2010). Whereas the ischemic lesion becomes hyperintense on diffusion-weighted imaging (DWI) relative to normal tissue within minutes of injury in experimental models and within few hours of stroke onset in patients as the infarct appearance is influenced mainly by the apparent diffusion coefficient (ADC) and T2 relaxation time of the tissue (Rivers et al., 2006). The ADC, a measure of the freedom of water diffusion, is believed to be low because of a shift of water, within hypoxic brain parenchyma, from the extracellular to the intracellular compartment, where water diffusion is relatively restricted (Lansberg et al., 2001).

This low ADC is responsible for the high signal of the acute ischemic infarct on DWI. The ADC maps are generated by mathematically eliminating the T2-w images from the DWI, they can be used to determine whether hyperintensity on DWI represents a true reduction of diffusion, which appears dark on ADC map, or T2 shine-through, a later effect in stroke causing high signal on DWI and ADC map due to T2 hyperintensity. (Allen et al., 2012; Maas & Mukherjee, 2005).

We report our results with DWI, including the ADC mapping. The purpose of this study is to determine the role of DWI in the diagnosis of recent cerebral ischaemic infarction in a consecutive series of patients with symptoms of acute stroke, in order to demonstrate it we compared the sensitivity of DWI with that of T2-w and Fluid-attenuated inversion recovery (FLAIR) sequences. We did not include a time limitation in this study. Moreover, we evaluated the feasibility of DWI as a first line neuroimaging for suspected recent stroke patients.

MATERIALS AND METHODS

A single-center prospective study was carried out in a consecutive series of patients. It took place from Sept 30, 2013, to Feb 25, 2014, at Alteba imaging center in Elbeida city in Libya, the only imaging center in the region with CT and MRI scanners at the time. Over a 5-month period, ninety patients underwent MRI. These patients were referred with complaints of focal neurological deficit, hemiparesis/hemiplegia and other clinical findings consistent with stroke. The time of stroke onset was determined to be the time at which the patient was last known to be symptom-free.

All patients were studied on a 1.5 Tesla MR Scanner (Intera 11, R10.6 Philips, Netherlands). The conventional scanning protocol of the MR examination included axial 6mm thick T1 weighted (TR/TE- 550/15) spin-echo sequence, an axial 6mm thick TSE /T2 weighted sequence (550/110) and an axial 6mm thick FLAIR sequence (TR/ effective TE- 800/120, TI- 2000 msec). For DWI, a single shot spin-echo/echoplanar imaging sequence was obtained. Acquisition parameters included 10,000/92 TR/TE, single excitation, 25 cm FOV, 112X112 matrix, and a 5mm section thickness with a 1mm interslice gap. Gradient strength corresponding to b values (diffusion sensitivities) of 0 and 1000 second/mm2 were used. Twenty-Two axial images covering the entire brain were obtained. Apparent diffusion coefficient (ADC) maps were also calculated from T2-weighted and diffusion-weighted echoplanar images.

The conventional MR sequences and DWI images were reviewed in conjunction with relevant clinical history. Signal intensity abnormalities noted on T2-w, FLAIR, and DWI images were compared. Initial DWI was considered positive for the diagnosis of new arterial stroke whenever hyperintensities with reduced ADC values were observed. Old lesions were defined

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as lesions seen on FLAIR without corresponding DWI abnormality or as a DWI hyperintensity without a corresponding reduction in ADC ("T2 shine through"). We classified infarcts using the classification from Allen et al. (2010) according to the time from onset of symptoms into hyperacute (from 0 to 24 hours (h)), acute (24h to 7 days) and subacute (7 days to 3 weeks).

For statistical data analysis we used the SPSS software package, a one-way ANOVA test was performed to determine if the study results were statistically significant i.e. *P* value less than 0.05, then the cross tabulation method was performed to determine exactly which of the MRI sequences (DWI, FLAIR, and T2-w) had a significant difference.

RESULTS

Altogether 90 consecutive patients over a period of 20 weeks initially fulfilled the criteria for inclusion into the audit. However, three patients had not undergone DWI in their MRI examinations. We therefore reviewed the results of 87 patients (54 women, 33 men; median age 65 years; range, 29 to 93 years) with symptoms and signs of a recent stroke. Before the current ischemic event, 16 patients (18%) had suffered from previous ischemic events. The mean time between the onset of symptoms and the MR investigations was 63 hours (range, 3 hours to 14 days). 39 out of 87 patients (45%) were imaged in the hyperacute stage (<24 hours after onset), 39 patients (45%) imaged in acute stage between 24 hours and 7 days after onset (54% of them imaged between 24 and 48h), nine patients (10%) were imaged between 7 and 14 days after onset of symptoms.

A diagnosis of recent ischemic infarct was made in 47 (54%) patients. Their demographic data and presenting clinical symptoms and infarct sites are shown in Table 1. Other diagnoses were old ischaemia in 12 (14%) patients, haemorrhage in 6 patients, neoplasm in 3 patients, encephalitis, demyelination in 2 patients, and normal in 17 (20%) patients.

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Table:(1). Demographic Data, Clinical Symptoms, and Infarct Location on MRI Scan

Patient	Sex	Age	Complaint	Paresis	Lesion location	Side	Artery
1	F	90	HA+HP	a, l	C+SC	L	PCA
2	F	70	HA+HP	a, l	C+SC	R	PCA
3	F	60	HP	a, l	C+SC	R	WS ACA-MCA
4	F	67	Vertigo, ataxia		Cerebellar	R	PICA
5	M	48	HP+PHA	a, l	C+SC	L	MCA
6	F	47	Coma		Posterior circulation		Basilar
7	F	80	HP	a, l	C+SC, Thalamus	R	PCA
8	M	60	HPL+ PHA	a, l, f, s	C+SC+ BG	R	MCA
9	F	88	HP	a, l	C+SC	R	WS MCA-PCA
10	M	70	HP	a, s	C+SC	L	ACA
11	F	93	HP	a, l, f, s	BG	R	LSA
12	F	67	HP+PHA	F	C+SC	L	MCA
13	F	85	HP+PHA	a, l, f, s	C+SC+ CN	R	MCA
14	M	64	PHA	…	Corona radiate	R	WS LSA-MCA
15	F	70	HP	a, l, f, s	C+SC	R	MCA
16	M	60	HP	a, l	Cerebellar, Pons, midbrain	L	SCA, PCA
17	M	65	HA	…	C+SC	R	WS ACA-MCA-PCA
18	M	82	HP	a, l, f	C+SC, Thalamus	L	PCA
19	F	70	HPL+ PHA	a, l, f, s	C+SC	L	MCA
20	F	70	HPL+PHA	a, l, f, s	C+SC	R	MCA
21	M	93	Confusion	…	Cerebellar	L	PICA
22	F	80	HP+PHA	a, l, f	Corona radiate	R	Deep MCA
23	F	90	PHA+HP	a, l ,f, s	C+SC+ BG	R	MCA
24	F	62	HP+HA	a, l, s	C+SC	L/R	PCA
25	M	62	HP	a, l, f	C+SC	R	MCA
26	M	70	HP	a, l, f	C+SC	L	MCA
27	F	65	HP	a, f	Corona radiate	L	Deep MCA
28	M	70	Confusion	…	Cerebellar	L	PICA
29	F	60	HP+PHA	a, f	C+SC	L	WS MCA--PCA
30	M	65	HP	a, l	BG	R	LSA
31	M	44	HP	a, l	Lacunar	R	TGA
32	F	65	HP	a, f	Lacunar	L	WS ACA-MCA
33	M	75	HP	a, l	Lacunar	R	AchA
34	F	85	HPL+ PHA	a, l, f, s	C+SC+ BG	L	MCA
35	F	50	HP	a, l	Corona radiate	R	Deep MCA
36	F	80	HP	a, l	C,SC	R	PCA
37	F	80	HP	a, l, s	C+SC	L	MCA
38	M	57	HP	a, l, f	Lacunar	L	AchA
39	M	65	HP+PHA	a, l	Pons	R	BA branch
40	F	65	HPL	a, l	Corona radiate	L	WS ACA-MCA
41	M	55	HP+PHA	a, l, f	Semiovale, Corona	R	WS MCA-MCA
42	M	73	HP+HA	a, l	C+SC	L	PCA
43	F	65	HP	a, l, f, s	BG	R	LSA
44	F	86	HP+PHA	a, l, f	C+SC	R	MCA
45	F	65	HP	a, l ,f	C+SC, Thalamus	L	PCA
46	F	82	Coma	…	Post circulation		Basilar
47	M	53	HP	a, f	C,SC	L	ACA

PHA indicates dysphasia/aphasia. HP, hemiparesis. HPL, hemiparalysis. HA, hemianopia; a, arm; l, leg. f, face; s, hemisensory loss. C, cortical; SC, subcortical; CN, caudate nucleus; BG, basal ganglia, L, left hemisphere; R, right hemisphere; TGA, thalamogeniculate artery; MCA, middle cerebral artery; AChA, anterior choroidal artery; ACA, anterior cerebral artery; LSA , lateral lenticulostriate artery; WS, watershed infarct; PCA, posterior cerebral artery; and BA, basilar artery. *Note.* The abbreviatios are from "Diffusion-Weighted Magnetic Resonance Imaging in Acute Stroke", by K. J. Van Everdingen et al, 1998, *stroke*, 2

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Overall, of the 47 patients (19 men, 28 women; mean age, 69 years; range, 44 to 93 years) with recent ischemic stroke, DWI clearly identified ischemic lesions in 46 patients (98%) as compared with 33 patients (70%) and 31 patients (66%) in whom ischemic lesions were detected on FLAIR and T2-w images respectively, see figure1 for illustration.

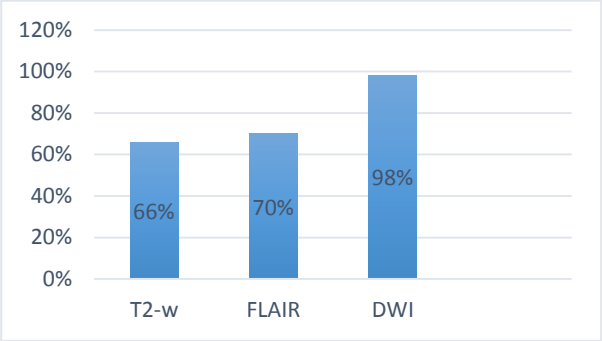

Figure (1). Graph compares the percentage of patients with recent ischemic lesions on DWI and T2-w and FLAIR sequences.

The results for the detection of ischemic lesions with different time onset on T2-w, FLAIR, and DWI scans are summarized in Table 2.

Table:(2). Number (and Percentage) of Ischemic Lesions Detected on T2-w, FLAIR, and DWI Scans.

	All lesions	Time Between Onset and MRI in hour				
		0–6	6–16	16–24	24–168	168-336
T2-w	31/47 (66%)	0/5 (0%)	5/10 (50%)	12/13 (92%)	9/14 (64%)	5/5 (100%)
FLAIR	33/47 (70%)	0/5 (0%)	7/10 (70%)	12/13 (92%)	9/14 (64%)	5/5 (100%)
DWI	46/47 (98%)	5/5 (100%)	10/10 (100%)	13/13 (100%)	14/14 (100%)	4/5 (98%)

Values are number (percentage) of ischemic lesions detected. Lesions are grouped according to the time between the onset of symptoms and the MRI investigation

The one-way ANOVA test and cross-tabulation method analysis of the results in relation to time from onset revealed that there is a statically significant difference between DWI and T2-w/FLAIR in the detection of ischemic infarcts (*P* value = .012). See tables 3, 4.

Table:(3). ANOVA test correlation of ischemic infarct detection versus Time between onset and MRI in hours

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	14.413	3	4.804	4.099	.o12
Within Groups	50.395	43	1.172		
Total	64.809	46			

Table:(4). Ischemic infarct found on DWI, T2 and FLAIR versus Time between onset and MRI in hours Cross tabulation

Count of ischemic infarct found on	Time between onset and MRI in hours					Total
	0-6	6-16	16-24	24-168	168-336	
DWI	5	3	1	5		14
DWI+FLAIR+T2-w		5	12	9	4	30
DWI+FLAIR		2				2
FLAIR+T2-w					1	1
Total	5	10	13	14	5	47

T2-w and FLAIR failed to diagnose recent ischemic infarcts (falsely negative) in 16 and 14 patients respectively. Ischemic lesions were absent on T2-w and FLAIR in all stroke patients imaged within 6 hours from onset of symptoms and were only detected on DWI, see example in figure 2. Nine patients imaged after six-hour onset, who were positive for infarct on DWI, had false-negative findings on both T2-w and FLAIR (four patients imaged in 6-24 h, five patients imaged in 24-168 h from onset of symptoms), all of them had had small or lacunar infarcts (See figures 3, 4, 5, 6). They were missed due to: 1) - nonspecific periventricular white matter hyperintensities in two patients. 2) - adjacent chronic infarct in three patients. 3) - multiple different age lacunar infarcts in three patients. 4-very small (one patient). Only in retrospect after viewing the DWI/ADC images some of the missed acute lacunar ischemic lesions could be seen on T2 and FLAIR.

T2-w and FLAIR results were only different in two patients imaged at 12 h and 15 h from onset. FLAIR and DWI clearly detected the ischemic lesions that were not seen on T2-w in these two cases (patients 38 and 39 in table 1), one had pontine infarct, and the other had posterior limb internal capsule infarct.

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DWI missed recent ischemic damage (i.e. was falsely negative) in only one patient, who was examined 7 days after stroke onset and had a lacunar infarct (patient 32). The lesion appeared hyperintense on T2-w and FLAIR images, less conspicuous on DWI with a normal signal on ADC map.

No false-positive lesions were found with DWI when combined with T2-w.

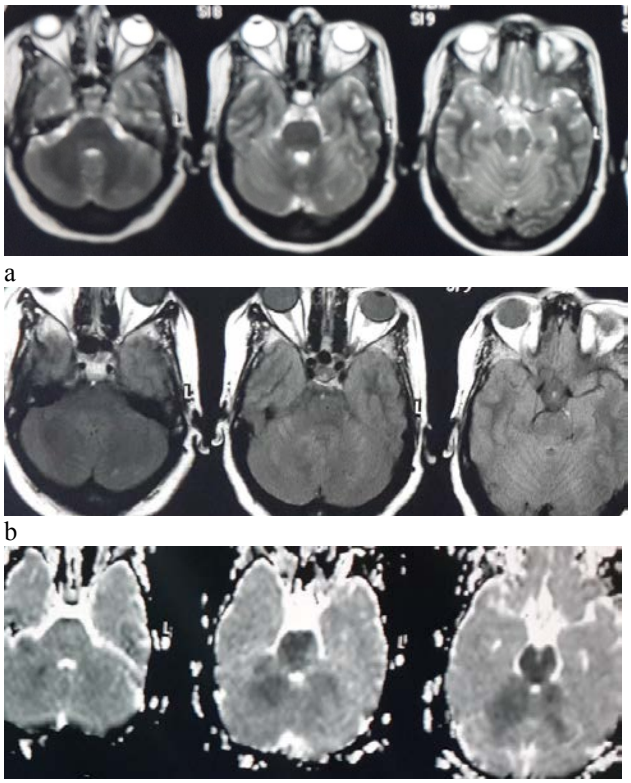

Figure (2). Forty–seven–year-old woman with an acute coma state, starting less than 6 hours before MRI examination (patient 6). On the early T2-w (a) and FLAIR (b) images, no ischemic lesion was visible, the early DWI (not shown) shows hyperintensity in the posterior circulation territory, which can be appreciated as a hypointensity on the ADC trace map (c). In retrospect, loss of signal void of basilar artery (Hyperintense vessel sign) can be appreciated on FLAIR (b) which is indicative of slow blood flow distal to the site of acute arterial obstruction.

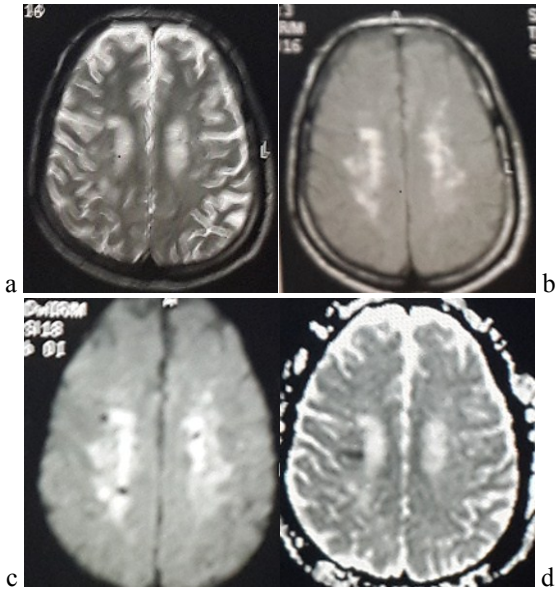

Figure (3). Sixty-four-year-old man with an acute slurred speech 7 hours before MRI examination (patient 14). The right parietal deep WS-infarct was not recognized on T2-w (a) and FLAIR (b) images because of numerous T2 hyperintense deep white matter lesions representing small vessel ischaemic changes. DWI scan (c) shows the same hyperintensity as FLAIR, one of which can be appreciated as a hypointensity on the ADC trace map (image d) representing acute infarct.

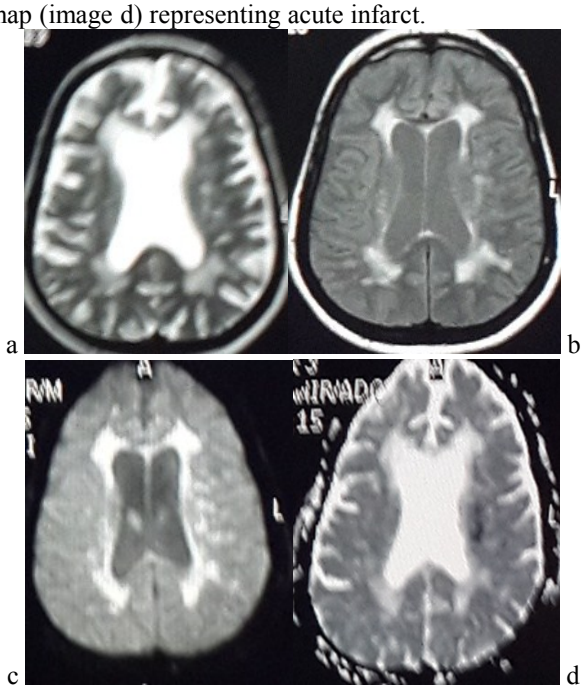

Figure (4). Sixty-five-year-old women with an acute mouth deviation 15 hours before MRI examination (patient 27). There are discrete and confluent patchy deep white matter T2 hyperintensties with left parietal acute ischaemic lacunar infarct appreciated as a hypointensity on the ADC trace map (d), on T2-w (a) and FLAIR (b) images, the lesion would have gone undetected.

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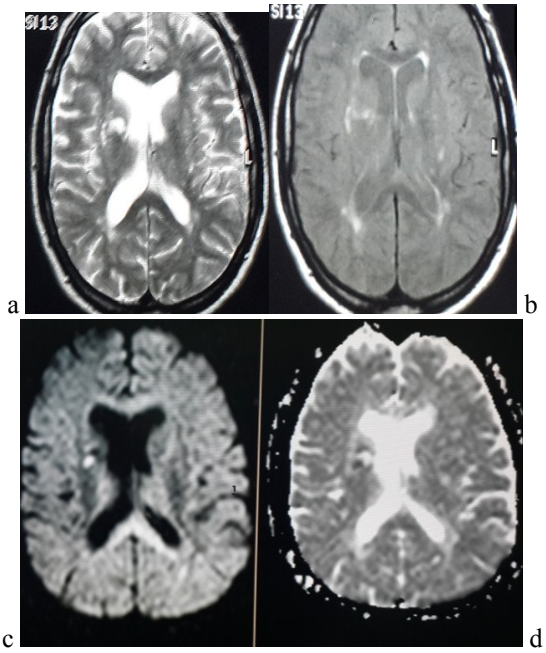

Figure 5. Sixty-five-year-old man with new onset left-sided hemiparesis 22 hours before MRI examination (patient 43). On T2-w (a) and FLAIR (b) images, no recent ischemic lesion was visible, only old lacunar ischaemic infarction at right basal ganglia. DWI scan (c) shows a right-sided hyperintensity in the lentiform nucleus, which can be appreciated as a hypointensity on the ADC trace map (d) indicating a new lacunar infarct.

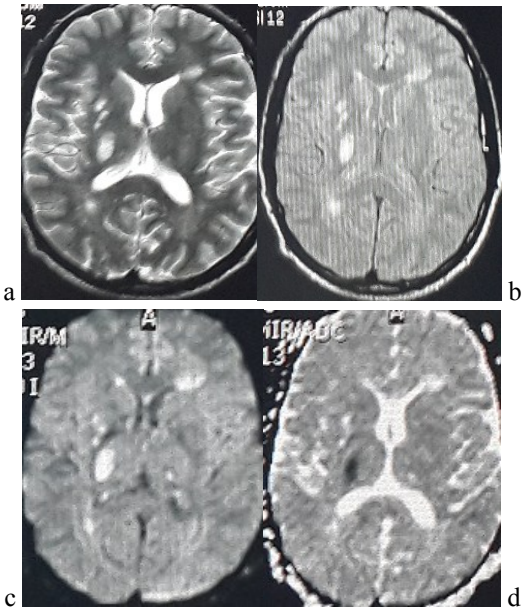

Figure 6. 44-year-old man with acute left-sided hemiparesis around 72 hours before an MRI examination (patient 31). T2, FLAIR, DWI images (a, b, c respectively) revealed multiple lacunar hyperintense lesions at the right thalamus and right lentiform nucleus and left frontoparietal periventricular white matter. The ADC map (d) depicted the right thalamic infarct to be the most recent one as it appeared hypointense.

DWI detected ischemic infarcts with haemorrhagic transformation in two patients (imaged after 24 hours from onset), T2 and FLAIR depicted high signal around the hemorrhage, which can be caused by oedema, surrounding a primary intra-axial hemorrhage. DWI showed a high signal at the margin of the haemorrhage with corresponding low signal on ADC map indicating ischemia with haemorrhagic transformation rather than primary haemorrhage.

DISCUSSION

This study was designed to assess the sensitivity of DW MRI for the detection of recent ischemic infarct over conventional MRI and its accessibility as the first line of imaging. Our sample was representative of a range of patients with a clinical suspicion of recent stroke who are likely to present for imaging at different times from onset of symptoms (a few hours to 2 weeks). We considered the design of no time limit in our study to be clinically relevant, as patients with stroke-like symptoms will be referred for imaging as the initial step in management irrespective of time from onset. We evaluated T2-w images first, FLAIR second and DWI/ADC last because typically DWI is added to conventional MRI rather than done separately.

Our results confirm that DWI is the most useful imaging sequence in the diagnosis of recent ischemic stroke. All infarcts except one were detected on DWI, whereas with T2-w, FLAIR imaging, 34%, and 30% of lesions respectively were not detected. The sensitivity of DWI for hyperacute (0-24h from onset) and acute (24h-7 days) ischemic strokes was 100%. Our findings are in agreement with several other previously reported studies regarding the diagnostic yield of DWI, which reported high sensitivity of DWI ranging from 91 to 100 % (Fiebach et al., 2002; Gonzales et al., 1999; Lansberg et al., 2000).

Early DWI detected ischemic lesions in all positive stroke patients imaged within 6 h from onset of ischemic symptoms before any chang-

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es were evident on conventional MRI. This supports previous reports that a mismatch between positive DWI and negative FLAIR suggests that the stroke occurred at least less than 6 h beforehand (Huisa et al., 2012; Leiva-Salinas & Wintermark, 2010).

The inclusion of ADC maps increased the confidence in the interpretation of DWI with the characteristic appearance of acute ischemic lesions as bright on DWI sequences and dark on ADC maps. DWI/ADC sequences were able to not only confirm the diagnosis of suspected ischemic infarct but also determine the age of the infarct, which is of clinical importance to the patient. The relative ADC remained low in all acute infarcts up to several days from onset. It progressively increased to be pseudonormalized on ADC map at around day seven, which resulted in one patient being missed on DWI. The ADC value elevated after day 7 resulting in high signal on both DWI and ADC map. In a serial study by Lansberg et al. (2001) of 27 patients with acute strokes, a low ADC value indicated, with good sensitivity (88%), and specificity (90%), that a lesion was less than 10 days old.

In general, acute ischemic lesions with characteristic wedge-shaped cortical and subcortical high signal change confined to a vascular territory, with or without an associated mass effect, are well appreciated on conventional T2-w and FLAIR MR Images. However, this is not the case for small or lacunar lesions, especially with preexisting white matter changes and/or chronic ischemic infarct. On T2, they have similar signal intensity making the delineation of the more recent ischemic damage difficult and easily missed by the observer (Augustin et al., 2000). "Diffusion imaging aids in the identification of areas of acute ischemic injury against the backdrop of diffuse nonspecific T2-w signal abnormalities that are often seen in elderly patients, or when infarcts of different ages coexist" (Maas & Mukherjee, 2005).

In our study, the combined use of DWI, conventional T2-w, and FLAIR sequences improved the diagnostic information in 30 patients (64%; $P = .012$) of all MR examinations. DWI was superior to conventional imaging in 14 patients (30%). In nine of them, DWI discriminated new lacunar infarcts from small vessel ischemic white matter changes, older lacunar infarcts, or an adjacent chronic infarct. The signal characteristics of acute infarcts on DWI (bright on DWI, low on ADC map) made them easily separable despite small size from older hyperintensities, thus greatly contributing to the detection of acute lacunar infarcts that would have been missed on T2-w and FLAIR images.

Our experience confirms the findings reported by Choi et al. (2000) in their study about DWI in vascular dementia patients, which concluded that new foci of altered signal intensity were only distinguishable from prior injuries with DWI and not conventional MRI.

We are aware of only one study in the literature that compared DWI and conventional MRI in a consecutive series of patients with suspected recent cerebral ischemia and an interval between onset of clinical symptoms and MR imaging of 1 to 14 days (Augustin et al., 2000). The results of our study were similar to their results. In Augustin et al. (2000) study, 62% of ischemic lesions among 53 patients were identified or suspected on conventional T2-w images (66% in our study), DWI (same as in our study) was falsely negative in only one patient, who was examined 8 days after stroke and had small subcortical lesions. Overall, in Augustin et al. (2000) study DWI provided information not accessible with T2-weighted imaging in 15 patients (28%) (comparable to 14 patients (30%) in our study), and was more likely to make a diagnostic contribution in the first week of stroke and in patients with small lesions or preexisting ischemic cerebral damage than conventional MR imaging, which is in keeping with the findings of our study.

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Furthermore, our findings showed that the DWI was useful in differentiation between primary intracerebral haemorrhage and ischaemic infarction with haemorrhagic transformation, as the latter will show high signal around the haemorrhage on DWI with corresponding low signal on ADC map due to reduced diffusion differentiating it from high signal caused by oedema surrounding primary haemorrhage.

There are studies that reported a false negative rate exists with early DWI scans of posterior circulation ischemic infarcts imaged within 24 hours mainly brain stem lacunar infarcts (Oppenheim et al., 2000; Chelala et al., 2007). The difference between this and our result of no false-negative early DWI findings can be explained by the difference in studies' design. First, the sample size of these studies was large (e.g., 356 patients in Chelala et al. (2007), study) so a larger number of brain stem infarcts were encountered. Second, they did follow up MRI of all initial negative DWI cases, which detected the ischemic lesions that were absent on the initial DWI. Similarly, Chelala et al. (2007) reported high sensitivity and specificity of DWI for acute ischemic infarcts detection, 83%, and 96% respectively.

In our audit, we were able to image 68% (60 of 87) of the suspected recent stroke patients with DW MRI within 48 hours and 39 patients (45%) within 24 hours of onset of symptoms; this indicates that MRI is a feasible and an increasingly preferred imaging tool for clinicians.

A limitation of our study is that we were only able to image about 15% (13 of 87 patients) within 6 hours of onset of symptoms. Many factors we think contributed to the lower number of patients imaged under 6 hours span including delay in patients' presentation to the hospital, especially elderly patients with a previous stroke, and delays in referrals to imaging. The entire process from recognition of symptoms to imaging needs to be improved to achieve best practice standards according to Guidelines for the Early Management of Patients with Acute Ischemic Stroke (Jauch et al., 2013; Powers et al., 2019).

CONCLUSION

In conclusion, this analysis suggests high sensitivity of DWI in the diagnosis of acute & subacute cerebral ischemic infarction, DWI proved superior to FLAIR and T2-w imaging in early detection of ischemic lesions in the first six hours from stroke onset and in cases of acute lacunar infarcts with pre-existing ischemic brain insult. The analysis also suggests that DW MRI is both feasible and accessible as a first-line neuroimaging in clinically suspected recent stroke patients.

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REFERENCES

Allen, L. M., Hasso, A. N., Handwerker, J., & Farid, H. (2012). Sequence-specific MR Imaging Findings That Are Useful in Dating Ischemic Stroke. *RadioGraphics,* 32(5), 1285-1297. DOI: https://doi.org/10.1148/rg.325115760

Augustin, M., Bammer, R., Simbrunner, J., Stollberger, R., Hartung, H. P., & Fazekas, F. (2000). Diffusion-weighted imaging of patients with subacute cerebral ischemia: comparison with conventional and contrast-enhanced MR imaging. *American Journal of Neuroradiology,* 21(9), 1596-1602. Retrieved from http://www.ajnr.org/content/21/9/1596.long

Chalela, J. A., Kidwell, C. S., Nentwich, L. M., Luby, M., Butman, J. A., Demchuk, A.

ISSN: online 2617-2186 print 2617-2178

M., . . . Warach, S. (2007). Magnetic resonance imaging and computed tomography in emergency assessment of patients with suspected acute stroke: a prospective comparison. *Lancet (London, England)*, *369*(9558), 293–298. doi:10.1016/S0140-6736(07)60151-2

Choi, S. H., Na, D. L., Chung, C. S., Lee, K. H., Na, D. G., Adair, J. C. (2000). Diffusion-weighted MRI in vascular dementia, *Neurology*, 54(1), 83-89. DOI: 10.1212/WNL.54.1.83

Fiebach, J. B., Schellinger, P. D., Jansen, O., Meyer, M., Wilde, P., Bender, J., . . . Sartor, K. (2002). CT and diffusion-weighted MR imaging in randomized order: diffusion-weighted imaging results in higher accuracy and lower interrater variability in the diagnosis of hyperacute ischemic stroke. *Stroke*, 33(9), 2206–2210. DOI: https://doi.org/10.1161/01.STR.0000026864.20339.CB

Gonzalez, R. G., Schaefer, P. W., Buonanno, F. S., Schwamm, L. H., Budzik, R. F., Rordorf, G., . . . Koroshetz, W. J. (1999). Diffusion-weighted MR imaging: diagnostic accuracy in patients imaged within 6 hours of stroke symptom onset. *Radiology*, 210(1), 155–62. DOI: https://doi.org/10.1148/radiology.210.1.r99ja02155

Huisa, B. N., Liebeskind, D. S., Raman, R., Hao, Q., Meyer, B. C., Meyer, D. M., … University of California, Los Angeles Stroke Investigators (2013). Diffusion-weighted imaging-fluid attenuated inversion recovery mismatch in nocturnal stroke patients with unknown time of onset. *J Stroke Cerebrovasc Dis*, 22(7), 972–977. doi:10.1016/j.jstrokecerebrovasdis.2012.01.004

Jauch, E. C., Saver, J. L., AdamsJr, H. P., Bruno, A., Connors, J. J., Demaerschalk, B. M., . . . Yonas, H. (2013). Guidelines for the Early Management of Patients With Acute Ischemic Stroke A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke,* 44(3), 870–947. DOI: https://doi.org/10.1161/STR.0b013e318284056a

Lansberg, M. G., Norbash, A. M., Marks M. P., Tong D. C., Moseley, M. E., & Albers, G. W. (2000). Advantages of Adding Diffusion-Weighted Magnetic Resonance Imaging to Conventional Magnetic Resonance Imaging for Evaluating Acute Stroke. *Archives of neurology,* 57(9), 1311–1316. doi:10.1001/archneur.57.9.1311

Lansberg, M. G., Thijs V. N., O'Brien, M. W., Ali, J. O., de Crespigny, A. J., Tong D. C., . . . Albers, G. W. (2001). Evolution of Apparent Diffusion Coefficient, Diffusion-weighted, and T2-weighted Signal Intensity of Acute Stroke. *American Journal of Neuroradiology,* 22(4), 637-644. Retrieved from http://www.ajnr.org/content/22/4/637/tab-article-info

Leiva-Salinas, C., & Wintermark, M. (2010). Imaging of acute ischemic stroke. *Neuroimaging clinics of North America*, 20(4), 455–468. doi:10.1016/j.nic.2010.07.002

Maas, L. C., & Mukherjee, P. (2005). Diffusion MRI: Overview and clinical applications in neuroradiology. *Applied Radiology*. Retrieved from https://appliedradiology.com/articles/diffusion-mri-overview-and-clinical-applications-in-neuroradiology

ISSN: online 2617-2186 print 2617-2178

Oppenheim, C., Stanescu, R., Dormont, D., Crozier, S., Marro, B., Samson, Y., . . . Marsault, C. (2000). False-negative diffusion-weighted MR findings in acute ischemic stroke. *American Journal of Neuroradiology, 21*(8), 1434–1440. Retrieved from http://www.ajnr.org/content/21/8/1434.long

Powers, W. J., Rabinstein, A. A., Ackerson, T., Adeoye, O. M., Bambakidis, N. C., Becker, K., . . . Tirschwell, D. L. (2019). Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke,* 50(12), 344–418. DOI: https://doi.org/10.1161/STR.0000000000000211

Rivers, C. S., Wardlaw, J. M., Armitage, P. A., Bastin, M. E., Carpenter, T. K., Cvoro, V., . . . Dennis, M. S. (2006). Persistent Infarct Hyperintensity on Diffusion-Weighted Imaging Late After Stroke Indicates Heterogeneous, Delayed, Infarct Evolution. *Stroke*, 37(6), 1418–1423. DOI: https://doi.org/10.1161/01.STR.0000221294.90068.c4

Tan, P. L., King, D., Durkin, C. J., Meagher, T. M., & Briley, D. (2006). Diffusion weighted magnetic resonance imaging for acute stroke: practical and popular. *Postgraduate medical journal*, *82*(966), 289–292. doi:10.1136/pgmj.2005.038299

Tsiouris, A. J., Qian, J. (2017). Advanced Imaging in Acute Ischemic Stroke. Retrieved from https://www.reliasmedia.com/articles/141858-advanced-imaging-in-acute-ischemic-stroke

Van Everdingen, K. J., van der Grond, J., Kappelle, L. J., Ramos, L. M. P., & Mali W. P. T. M. (1998). Diffusion-Weighted Magnetic Resonance Imaging in Acute Stroke. *Stroke,* 29(9), 1783–1790. DOI: https://doi.org/10.1161/01.STR.29.9.1783

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دور التصوير الموّزن بمعامل انتشار في تعزيز صور الرنين المغناطيسي في مرضى السكتة الدماغية حديثا

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المستخلص: السكتة الدماغية من الأسباب الشائعة لدخول المستشفيات، والتصوير في السكتة الحادة ضروري للتمييز بين السكتة الإقفارية والنزفية واستبعاد التشخيصات الأخرى. هدف الدراسة تقييم دور التصوير بالرنين المغناطيسي الموّزن بمعامل الانتشار (DW MRI) في تشخيص الاحتشاء الدماغي الإقفاري الحديث في سلسلة متتالية من المرضى الذين يعانون من أعراض السكتة الدماغية الحادة وجدواه كتصوير أولي لهؤلاء المرضى. نقوم بالإبلاغ عن نتائجنا باستخدام DWI وخرائط رسم معامل الانتشار الواضح (ADC) التي تقارن حساسية DWI بحساسية T2 الموزونة (T2-w) وانعكاسات استرداد السوائل (FLAIR) التقليدية. الأساليب: المراجعة المستقبلية لـ 87 مريضًا يعانون من سكتة دماغية مشتبه بها سريريًا ومُحالة للتصوير على مدار فترة 20 أسبوعًا متتالية. شملت البيانات التي تم جمعها كلا من عمر المريض، الوقت من ظهور الأعراض، والعرض السريري. تم إجراء DWI echo planer، FLAIR، MRI T2-w، وخرائط ADC لهؤلاء المرضى. تم تقييم صور التصوير بالرنين المغناطيسي التقليدية قبل صور DWI. اعتبرت DWI إيجابية لتشخيص السكتة الدماغية الشريانية الجديدة كلما لوحظت إشارة عالية مع انخفاض في قيمة ADC. تم تضمين موقع الاحتشاء المكتشف من الصور في بيانات المريض. حيث كان 47 مريضا لديهم التشخيص النهائي للاحتشاء الدماغي. وتم الكشف عن 98 ٪ من الآحتشاءات الدماغية بواسطة DWI، في حين أن مع FLAIR تم اكتشاف 70 ٪، ومع T2-w تم العثور على 66 ٪ فقط من الآحتشاءات. تبين أن هناك فرق مهم إحصائيا ما بين قدرة DWI من جهة و T2-w/FLAIR من جهة أخرى في الكشف عن الآحتشاءات الدماغية بالنسبة للوقت من ظهور الأعراض ($P = .012$). تم تصوير 68 ٪ من المرضى المشتبه بهم (60 من 87 مريضا) بالرنين المغناطيسي DWI في غضون 48 ساعة و39 مريضاً (45 ٪) في غضون 24 ساعة من ظهور الأعراض. حيث تبين أن لدى DW MRI حساسية عالية وخصوصية لتشخيص احتشاء الدماغ الحاد إضافة الى كونه ممكن تمامًا كخط تصوير أولي لهؤلاء المرضى.

الكلمات المفتاحية: التصوير الموّزن بمعامل الانتشار، التصوير بالرنين المغناطيسي التقليدي، T2 الموزونة، FLAIR، السكتة الدماغية.

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Development, Validation, and Application of a Method Based on Reverse-Phase HPLC for the Simultaneous Determination of Six Organochlorine Pesticides in Surface and Groundwater Samples Collected from Northeast Libya

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Abstract: The contamination by organochlorine pesticides (OCPs) of the selected sites in the eastern region of Libya was investigated to estimate the current status of pollution in surface and groundwater sources. This study was conducted to determine the concentrations of some OCPs residue in surface and groundwater samples collected from thirty different sites around Al-Jabal Al-Akhdar northeast of Libya during the summer, autumn, and winter seasons of 2016. A simple and rapid method based on the liquid-liquid extraction method followed by an HPLC with reversed-phase was developed to determine six OCPs in water samples simultaneously. An HPLC instrument was supplied with a C_{18} column (250 mm × 4 mm; 4.6 µm particle size) and a UV detector at 238 nm. The mobile phase was composed of 0.1% propanol in water and acetonitrile (25/75, v/v). The method was validated using reference standards of these six OPCs at different concentration levels and shows good linearity in the concentrations between 5.0 and 25 µg mL^{-1}. The LOD and LOQ ranged from 0.71 to 2.24 µg mL^{-1} and 2.16 to 6.79 µg mL^{-1}, respectively. Relative standard deviation (%RSD) ranged from 0.026 to 0.673 %. The only OCP residue found in the area of study was 2, 4-D, with a variation of its residue levels during the seasons. The overall results showed that surface water was more polluted with 2, 4-D than groundwater, which was detected in the wide range of concentration of 0.037 to 0.385 µg mL^{-1}, 0.003 to 0.047 µg mL^{-1} and 0.012 to 0.039 µg mL^{-1} during summer, autumn, and winter, respectively.

Keywords: HPLC; Validation; Residues; Surface Water; Groundwater.

INTRODUCTION

Pesticides play a vital role in agricultural production to prevent or decrease damage by pests. And as a result can enhance the yield and the quality of production even as far as cosmetic demand, which is regularly vital to consumers (Cooper & Dobson, 2007; Oerke & Dehne, 2004). The nutritional value of food and its safety can be enhanced and improved by pesticides (Cooper & Dobson, 2007; Smilanick, 2008). Data from several studies suggest that Organochlorines pesticides (OCPs) are among the pesticides that are used for agricultural purposes all over the world. Because of their widespread uses, they are detected in various environmental matrices, such as soil, water, and air (Azad et al., 2012; Mariyono, 2008; Sankararamakrishnan et al., 2005). OCPs are characterized as low hydrophilicity and more lipophilicity substances. And therefore, have potential bioaccumulation in the food chain which poses a significant threat to human health and the environment globally (Afful et al., 2010).

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Furthermore, several studies have reported that the persistence of organochlorine pesticides in soil and water of cultivated land has the opportunity to drain into groundwater in different levels of quantity depending on the nature and chemical characterizations of these pesticides (Jamal, 2011). In addition, results of water and sediment samples from Lake Manyas in Turkey showed that these samples were still contaminated by organochlorine pesticides and their residues, despite the bans on their production and usage over a long time (Erkmen et al., 2013).

Furthermore, the determination of persistent pesticides is of particular importance to the environment in the area of toxicology. Thus, various analytical methods such as GC-ECD (Ismail et al., 2014; Saha et al., 2012)1; (Hashmi & Menon, 2015), GC-MS (El-Saeid et al., 2011), gas chromatography–nitrogen-phosphorus detection (Dores et al., 2008) HPLC (Ara et al., 2014) have been reported, and many of these methods involve a solid-phase extraction and/or liquid-liquid extraction procedures prior to the detection by analytical techniques (El-Osmani et al., 2014; Shamsipur et al., 2016); (Leong et al., 2007); (Shakerkhatibi et al., 2014).

Extensive research has shown that OCPs are commonly analysed and detected using Gas chromatography techniques with different detectors (Tata Rao et al., 2014); (Ize-Iyamu et al., 2007). Recently, Azad *et al.* (2012) analysed six OCPs in four sites at four seasons using a liquid-liquid extraction technique and then gas chromatograph equipped with an electron capture detector (GC-ECD). Also they used Soxhlet extraction for fish and sediment samples followed by clean up and then gas chromatography. They found DDE as a predominant residue in all analysed samples, and the lowest levels of OCPs were related to Heptachlor and Chlordane, which none of them were found in water samples (Azad et al., 2012).

Also, Lari *et al.* (2014) evaluated the OCPs and organophosphates pesticides as a potential pollutants and risks to human health. They utilized liquid-liquid extraction followed by the GC-MS technique for evaluating these pesticides in surface water and groundwater. The surface water was found to be more contaminated than groundwater, with a higher number and concentration of pesticides (Lari et al., 2014a). In the same vein, Jayashree and Vasudevan (2007) analyzed some OCPs, chosen to know the level of their contamination in the groundwater of the Thiruvallur district, Tamil Nadu, India. The samples were found highly contaminated with DDT, HCH, endosulfan, and their derivatives. Their study showed that groundwater samples were highly contaminated with organochlorine residues (Jayashree & Vasudevan, 2007). Recently, researchers have shown an increased interest in using GC equipped with ECD and a pulsed flame photometric detector to analyze OCPs in water and sediment samples. In some cases, the residues were further confirmed by Gas Chromatograph-Mass Spectrometer-Quadrupole on Electron Ionization (EI) mode (Akoto et al., 2016; Maurya & Kumar, 2013); (Hellar-Kihampa, 2011).

Surveys, such as that conducted by El Bouraie *et al.* (2011) have shown that the contamination of organochlorine pesticides (OCPs) along El Rahway drain, they have determined the concentrations of eighteen OCPs in surface and groundwater samples collected from six different sites during the rainy and dry seasons. The samples were extracted by the liquid-liquid extraction method. Then, determined qualitatively using GC/ECD (Gas chromatograph equipped with an electron capture detector). The commonly found OCP residues included heptachlor, p,p'-DDE, p,p'-DDD, and endrin, and the overall results showed that surface water was more polluted with OCPs than groundwater (El Bouraie et al., 2011).

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Previous research has suggested that using solid-phase extraction (SPE) coupled with dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry (GC–MS) provides higher extraction efficiency and a larger preconcentration factor for the determination of pesticides residues (Shamsipur et al., 2016). In addition, solid-phase microextraction (SPME) followed by gas chromatography (GC) techniques have been applied for the analysis of chlorinated pesticides in water, with good reproducibility, low detection limits, and wide linear ranges (Boussahel et al., 2002); (Dores et al., 2008).
Okoya *et al*. (2013) investigated levels of OCP residues in drinking water sources using liquid /liquid extraction and supercritical fluid extraction (SFE), and this study concluded that most of the water samples contaminated with OCPs associated with agricultural activities in their areas (Okoya et al., 2013). However, using Static liquid-phase microextraction technique for analysis of OCPs from aqueous samples showed to be quick, inexpensive, and used very little solvent with subsequent analysis by gas chromatography–electron-capture detection (Zhao & Lee, 2001).

On the other hand, HPLC with a UV/visible detector was utilized by Aiken *et al.* They found the concentrations of organochlorine and organophosphorus pesticide residues were significantly higher in the sediment samples when compared to water samples (Akan et al., 2014). As well as Ara *et al*. (2014) who condected a study to analyse pesticides including OCPs which was carried out with the HPLC technique. Pesticides were found in twelve samples, while Organochlorine was absent (Ara et al., 2014).

The objectives of this study involve the development and validation of the HPLC method to determine the residue of six organochlorine pesticides in surface and groundwater and their compatibility with the recognized maximum limits and the absence of a database on the level of pollution of pesticides in the water sources and then directed consumers to look at the dangers.

MATERIALS AND METHODS

Sampling site, collection, and storage: The region of the study is located between Wadi Derna [1] (E 22.619346° N 32.727701°) to Al-abyarIII [30] (E 20.522419° N 32.257841°) in the northeastern region of Libya. 9 samples of surface water and 21 groundwater samples were collected in summer, autumn, and winter season 2016. The sources [Codes 1-30] are shown in Figure (1), and a device of Global Positioning System (GPS) was used to locate positions during sampling. Samples were collected by desterilized pre-cleaned stainless metal containers and then saved in clean ice-covered dark glass bottles and immediately transferred to the laboratory. The samples were performed according to the recommendations by D.T.E Hunt and A.L. Wilson (APHA, 1995; Hunt & Wilson, 1986). They were properly labeled and kept at -20 ˚C before analysis (Uddin et al., 2007).

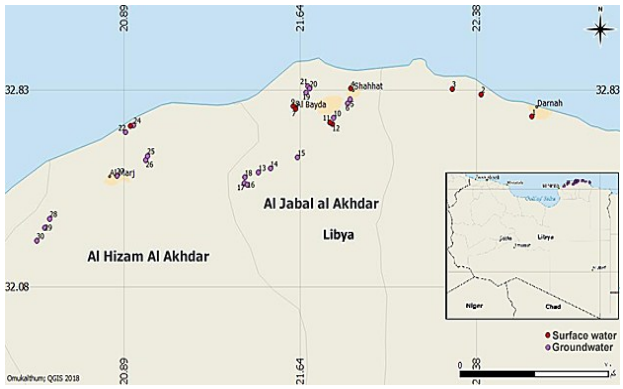

Figure: (1). Study locations that located between Wadi Derna[1] (E 22.619346° N 32.727701°) to Albayar [30] (E 20.522419° N 32.257841°).

Pesticide Standards and chemicals: The individual six organochlorine pesticides (2, 4-D, p,p′-DDE, p,p′-DDT, p,p′-DDD, Dicofol, and Heptachlor) reference standards with a certified purity of 99 % were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). HPLC

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grade acetonitrile and dichloromethane were obtained from Fisher Scientific (UK). Propan-1-ol was obtained from VWR (UK), water was obtained in-house by Water Distillation Unit. All other reagents and chemicals and solvents used were of analytical grade purchased from Sigma-Aldrich (Germany) included: anhydrous sodium sulfate, acetone, Uracil, and Sodium chloride.

Apparatus and chromatographic conditions

High-performance liquid chromatography was performed with an integrated Spectra system Liquid Chromatograph (Thermo electron corporation, USA) fitted with an in-line degasser, 120-place autoinjector, and single-channel, tunable UV absorbance detector.

Data analysis was carried out using Thermo Electron Corporation software (ChromQuest). The flow rate was 1.0 mL min^{-1}, with an injection volume of 20 mL. Three replicate injections of each calibration standard were performed. The analysis was performed using a reversed-phase stationary phase (ACE C_{18}, 250 mm **x** 4.6 mm i.d., particle size:3 μm; PerkinElmer (Shelton, USA)), and samples were isocratically eluted with a 0.1% Propan-1-ol in water and Acetonitrile (25/75, v/v) at wavelength 238 nm.

Preparation of standard sample for HPLC

Calibration standards: 5 mg of each standard were weighed accurately into a 50 mL volumetric flask and diluted to volume with acetonitrile to give a mixed stock solution. The analytical curve was prepared with a standard in final concentrations of 5-25 μg mL^{-1}, originally acquired from a 100 μg $mL^{-1}$of mixed six OCPs stock solution. All samples were prepared in triplicate.

Samples preparation for HPLC

Extraction of water samples: The method prescribed by the Water Environment Federation (WEF) was used, with some modifications, for the extraction of OCP residues from the water samples (APHA, 1998). A liquid-liquid extraction (LLE) method with dichloromethane was used for extracting OCP residues from the water sample. Water samples were filtered with Whatman No. 1 filter paper to remove debris, and then a 200 mL of filtrate water sample was transferred into a 500 mL glass-separating funnel. Then, 20 g of NaCl was added to produce a salt-out effect. It was thoroughly mixed by inverting the separating funnel three to four times. The sample was extracted thrice with 40 mL dichloromethane (20:10:10), shaken for 3–4 min each time with periodic venting. The combined organic phase was dried by passing it through anhydrous Na_2SO_4 and then in a vacuum rotary evaporator (Heidolph). The dried sample was reconstituted in 1 mL of Acetonitrile, and finally, the aliquot was analyzed by HPLC. These samples were analyzed in triplicate (Lari et al., 2014b).

Analytical method validation

System suitability : A mixture solution of six OCPs with a concentration of 100 μg mL^{-1} was injected three times to evaluate the analytical response. The mean and relative standard deviation (RSD %) were calculated. Theoretical plate number and tailing factor were also observed.

Linearity: The analytical curve was obtained with different concentrations of the mixture standard solution (5– 25 μg mL^{-1}). The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis.

***Precision*:** The precision of the assay was determined by repeatability which was evaluated by assaying samples at the same concentration during the same day. Five sample solutions were prepared.

Recovery method (Accuracy): The accuracy was determined by the recovery of known amounts of OCPs standard added to the samples at the beginning of the process. Thus, the recovery experiment was performed by spiked 1 mg of each pesticide standard individually to

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200 mL of water and extracted then dried. Then 10 mL of Acetonitrile was added to reach a concentration of 100 µg $mL^{-1.}$ The percentage recoveries were calculated using the following equation Percentage of recovery = [C_E/C_M × 100], where C_E is the practical concentration obtained from a standard solution of 100 µg mL^{-1} of each compound in acetonitrile, and C_M is the spiked concentration after extraction (Chowdhury et al., 2012).

Statistical analysis*:* Statistical analyses were carried out by analysis of variance (ANOVA) using SPSS 25 software. Differences in pesticides were analyzed by the t-test between the surface and groundwater samples. The statistical significance tests were carried at the 0.05 confidence level ($p < 0.05$). Data between regions and seasons, also interaction was tested using two-way ANOVA and Mean values were analyzed by the Tukey HSD (Honest Significant Difference) test at ($p < 0.05$) levels (Nie et al., 1970).

RESULTS

Optimization of chromatographic conditions

An RP-HPLC method for the simultaneous determination of six OCPs was developed, and excellent separation was only achieved with the C_{18} column length 250 mm.

To optimize the HPLC parameters, isocratic elution with several mobile phase compositions was evaluated to elute all pesticides in the sample in a reasonable amount of time. The separation was increased with more amount of organic solvent in the mobile phase than water. Although run lengths were longer at higher proportions of acetonitrile in the mobile phase, low or no resolution was observed when a higher proportion of water was used. A composition of acetonitrile and water in the mobile phase was varied, and increasing the amount of acetonitrile in the mobile phase was producing the best possible separation within the desired elution time. Flow rates of the mobile phase also varied between 0.5 and 1.0 mL min^{-1}, of which 1.0 mL min^{-1} gave an acceptable resolution (*Rs*>2) and better symmetry (<1.2). Depending on the results of various trials, a mobile phase consisting of acetonitrile: 1% Propan-1-ol in water (75:25 v/v) at ambient temperature was used for this study. Figure 6 shows the separation of all six compounds in reasonable run time, whereby the elution order was 2, 4-D, Dicofol, DDD, Heptachlor, DDT, and DDE.

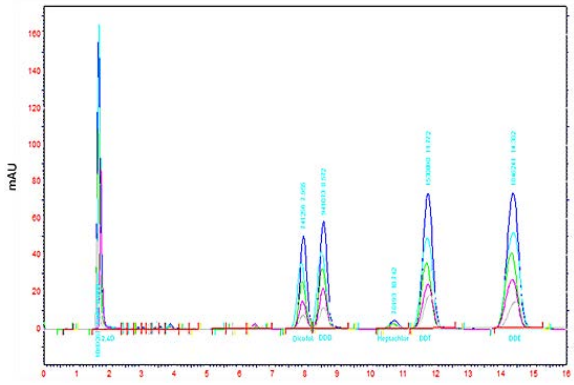

Figure1: (2). Simultaneous separation of 2, 4-D, Dicofol, DDD, Heptachlor, DDT and DDE. Conditions: Column: C_{18}, mobile phase: acetonitrile: 1% Propan-1-ol in water (75:25 v/v), ambient temperature, flow: 1, 0 mL min^{-1}, UV: 238 nm, injection: 20 µl.

Method validation: The good linearity of the calibration curve was validated by the high value of correlation coefficients of the regression graph**.** Calibration standards were prepared and demonstrated a linear response (R^2 =0.99-0.99) over a 5 -25 µg mL^{-1}.

The precision of the proposed method was also determined by mixing six OCPs at 5-25 µg mL^{-1} of each OCP. And was evaluated in terms of repeatability and expressed as the relative standard deviation (RSD,%). The result of precision ranged from 0.026 to 1.374%, which indicates good repeatability. The recovery of the method can be defined as accuracy. It was calculated of the known concentration level by comparing the measured concentration with spiked concentration as the reported method (Yusiasih et al., 2017) with a number replicate of three. The average result of the recoveries of all pesticides obtained ranged from 84.69 to

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98.16%. These values were considered as an acceptable and good result since 70 – 130% recoveries were approved (Demoliner et al., 2010).

The results showed in Table (1) from method validation of 2,4-D, Dicofol, DDD, Heptachlor, DDT, and DDE: Capacity factor (k') ranged from 0.10 (2,4-D) to 8.38 (DDE), Resolution (R) were from 1.13 (Dicofol) to 19.68 (2,4-D), Symmetry factor (As) were from 0.95 (Heptachlor) to 1.08 (Dicofol), Selectivity (ά) were ranged from 41.6 (2,4-D) to 1.31 (DDD) and theoretical plate (N) were ranged from 289 (1156) (2,4-D) to 14200.69 (56802.77) (DDE), as well as a slight peak tailing (As ~1.2-0.9) being observed in each case. The limits of detection and quantification were determined to be 1.73 and 5.25 µg mL^{-1} for 2,4-D, 1.73 and 5.26 µg mL^{-1} for Dicofol, 2.24 and 6.79 µg mL^{-1} for DDD, 1.15 and 3.50 µg mL^{-1} for Heptachlor, 0.71, and 2.16 µg mL^{-1} for DDT, 2.04, and 6.16 µg mL^{-1} for DDE, respectively.

Table (1). Summary of validation data for the quantification of OCPs including 2,4-dichlorophenoxyacetic acid , heptachlor, p, p′-DDE, p, p′-DDD, p, p′-DDT and Dicofol in the presence of common adulterants using Brownlee BIO C_{18} column (250 mm x 4.6 mm id, particle size 5 µm); mobile phase: 0.1% Propan-1-ol in water and Acetonitrile (25:75); detector wavelength: 238 nm.

Names	2,4-D	Dicofol	DDD	Heptachlor	DDT	DDE
t_R (min) (t_0 = 1.533 min[a])	1.69	7.90	8.55	10.73	11.76	14.38
Capacity factor (k′)	0.10	4.16	4.58	6.00	6.67	8.38
Resolution (R)	19.68	1.13	4.03	1.73	5.90	-
Symmetry factor (A_s)	1.05	1.08	1.01	0.95	0.96	1.07
Selectivity (ά)	41.6	1.10	1.31	1.11	1.25	-
LOD [b](µg mL^{-1})	1.73	1.73	2.24	1.15	0.71	2.04
LOQ [c](µg mL^{-1})	5.25	5.26	6.79	3.50	2.16	6.16

DISCUSSION

In thirty samples collected during each season, 2, 4-D pesticide was found in most seasons and regions. It is noteworthy that p, p′-DDE, p, p′-DDD, p, p′-DDT, Dicofol, and Heptachlor were not detected in all samples that were collected in summer, autumn, and winter season of surface and groundwater.Monitored pesticides in this study were detected with residue values less and greater than MRL's (0.030 µg mL^{-1}), according to World Health Organization (WHO) and the Libyan National Center for Standardization and Metrology (LNCSM) (WHO, 2004) in all collected samples.

However, Data showed that 2,4-D was detected in surface water with concentration ranged from 0.037µg mL^{-1} (Aldabusia) [3] to 0.385 µg mL^{-1} (Wadi Darna) [1] and detected in groundwater ranged from lower to greater concentration between 0.013µg ml^{-1} (Marawa III) [18] to 0.127 µg mL^{-1} (Wasita I) [19].The histogram shown in Figure 3 indicates that the major residues of 2, 4-D pesticides in surface water were in Wadi Darna [1] (0.38 µg mL^{-1}). The five other regions were detected in moderate concentrations, and these were Karsa [2], Aldabusia, Apouloana Shahhat I [4], Saylun MassaIII [9] and Tolmeita II [23] with concentration (0.38, 0.063, 0.037, 0.084, 0.061, and 0.048 µg mL^{-1}) respectively. Also, the results of this study indicate that three other regions found no present of 2, 4-D in their samples in surface water during the same season.

In groundwater samples, the histogram indicates that 2, 4-D residues were detected in most of the regions, including Shahhat II[4], Shahhat III[5], Massa II[8], Qandula I[13], Qandula II[14], Qandula III[15], Marawa

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II[17] , Wasita I[19], Wasita II [20], Tolmeita I[22], Alabyar I[28], and Alabyar III[30] with concentration (0.065, 0.120, 0.037, 0.051, 0.043, 0.034,0.094, 0.127, 0.090, 0.042, 0.033, and 0.035 µg mL^{-1}) respectively which were exceeded MRL (0.030 µg mL^{-1}) that determined by World Health Organization (WHO) limits, Libyan National Center for Standardization and Metrology (LNCSM). These findings were in agreement with the previous studies (Adeyemi et al., 2011; Hong et al., 1999; Munn & Gruber, 1997; Que Hee & Sutherland, 1981; Tang et al., 2008) which found the large amount of 2, 4-D as a herbicide. This indicates that 2, 4-D is still used in most countries in the world (Chen et al., 2018). Also, it is still used in neighboring countries such as Egypt, according to Mansour *et al*. study (El-Ghit, 2016; Mansour, 2004).

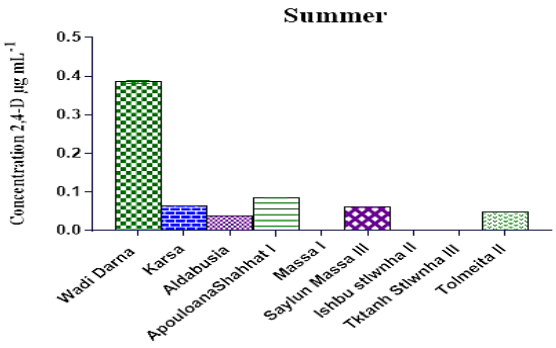

Figure (3). Residues of 2, 4-D pesticides in surface water from different regions of eastern Libya during summer season 2016.

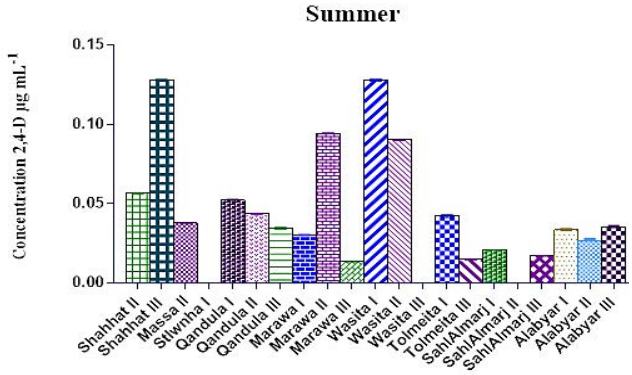

Fig (4). Residues of 2, 4-D pesticides in groundwater from different regions of eastern Libya during summer season 2016.

Histograms shown in Figures (5, 6) indicate 2, 4D residue in the autumn season of water samples collected from nine surface water and twenty-one of groundwater sources in different regions. In the case of surface water samples collected, we found six out of nine samples were contaminated during the autumn season of 2016. 2,4-D was detected with concentrations ranging from 0.003 µg mL^{-1} (Tolmeita II) [23] to 0.047 µg mL^{-1} (Aldabusia) [4] in surface water, which shows that most of the groundwater samples detected had a concentration which ranged between 0.003 µg ml^{-1} (Alabyar III) [30] to 0.036 µg mL^{-1} (Qandula I)[13]. In the autumn season, results from this study showed just 11.11% from surface and groundwater above the (WHO) and (LNCSM), while most samples were lower than the maximum residue limit according to the MRL's level.

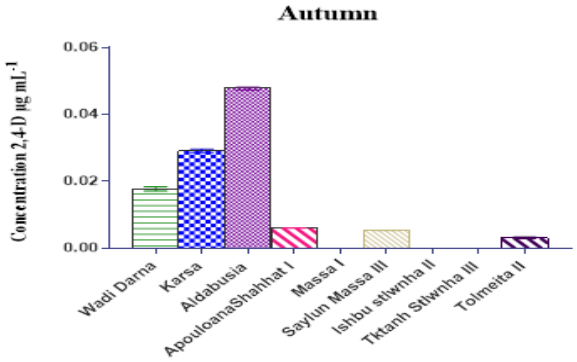

Figure (5). Residues of 2, 4-D pesticides in surface water from different regions of east Libya during the autumn season of 2016.

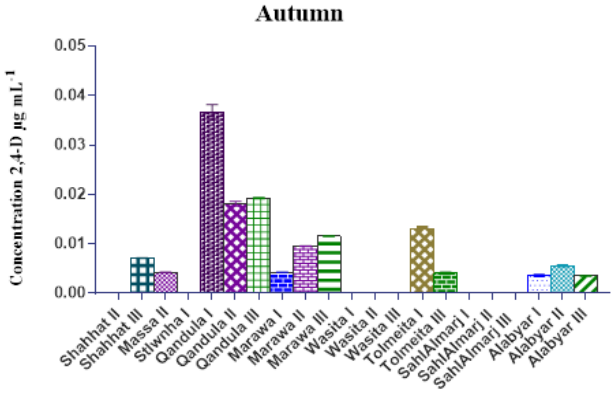

Figure (6). Residues of 2, 4-D pesticides in groundwater from different regions of east Libya during the autumn season of 2016.

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Histograms in Figures (7 and 8) show the result of water samples analysis, which were collected from different regions during the winter season 2016. Dicofol, DDD, DDT, DDE, and Heptachlor were not detected according to the device limit.

Results showed that from all surface water, (88.8%) of all samples were contaminated with 2, 4-D residue. Also, (95.23 %) of the groundwater was contaminated during the winter of 2016. However, most of 2, 4-D concentrations in both surface and groundwater sources were under the limit of WHO and LNCSM excluding Wadi Darna[1], Karsa[2], Aldabusia[3], Apouloana, Shahhat I[4], Shahhat II[4], Qandula III[15], Alabyar II[29], and Alabyar III [30] that were higher than MRL's.

In addition, 2,4-D was found in surface water with a concentration ranging from 0.012 µg mL^{-1} (Saylun Massa III)[9] to 0.039 µg mL^{-1} (Wadi Darna) [1] and detected in groundwater with a concentration ranging from 0.018 µg mL^{-1} (Marawa III)[18] to 0.038 µg mL^{-1} (Shahhat II)[4].

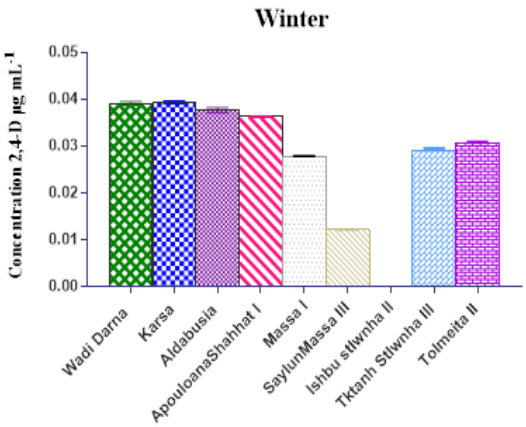

Figure (7). Residues of 2, 4-D pesticides in surface water from different regions of east Libya during the winter season of 2016-2017.

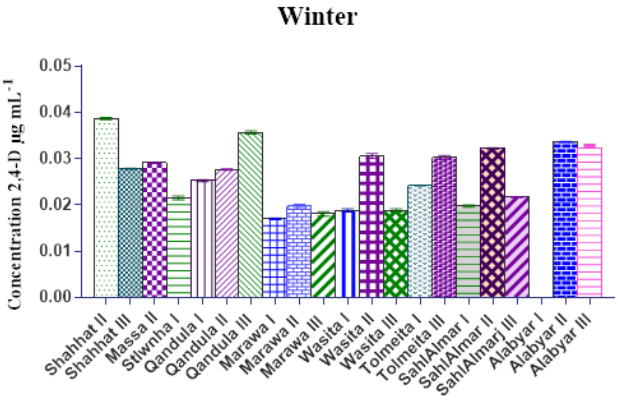

Figure (8). Residues of 2, 4-D pesticides in groundwater from different regions of eastern Libya during the winter season of 2016.

In general, by using an analysis of variance, the 2, 4-D residue in surface water of different regions showed significant differences between Wadi Darna [1] and Karsa[2], Aldabasia[3], Apouloana Shahhat I[4], Saylun, Mass III[9], Tktanh Stlwnha III[12], Tolmeita II[23] in summer, autumn, and winter seasons at ($p < 0.05$). Also, in these seasons, an analysis of variance (ANOVA) for groundwater showed a significant difference between regions at ($p < 0.05$).

In the T-test, a significant difference between surface and groundwater of all analyzed seasons at ($p < 0.05$) was observed in the mean levels of the 2, 4-D residue. Moreover, Analysis of variance (ANOVA) for the concentration of 2, 4-D showed a significant difference in summer, autumn, and winter seasons at ($p < 0.05$).

These results demonstrate that there were certain potential health risks to drinking water in the Wadi Darna [1] region in summer and winter due to the concentration of 2, 4-D residues being higher than MRL's. The high concentration of 2, 4-D in winter from the autumn season in the surface water could be attributed to the agricultural runoff resulting from the extensive agricultural activity around sources of water in

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these regions. The surface water was more polluted than groundwater. This was possibly due to the fact that 2, 4-D can reach the surface by the runoff process due to its low soil absorptivity, high potential of leachability, and high-water solubility. The low biodegradability of 2, 4-D in the water also makes 2, 4-D a major pollutant in the surrounding water sources.

From the results of the analysis of variance of all samples collected from all regions within three seasons (summer, autumn, and winter), The maximum contamination has been found to be in summer followed by winter with a minimum in autumn. The probable reason for the higher residues in summer may have been due to using herbicides frequently (Coady et al., 2013).

Finally, the misuse of these pesticides by the individuals concerned and the lack of, or weak, national control plants are behind the presence of these pesticides in water sources and, as a result, the occurrence of such pesticide residues in drinking water represents an environmental and health hazard. A frequent monitoring program is urgently needed in order to assess health risks associated with such contaminates, especially with chronic exposure or a lifelong intake of contaminated drinking water.

CONCLUSION

A quantitative HPLC–UV method has been developed and validated for the analysis of six OCPs using a liquid-liquid extraction (LLE) method. An isocratic HPLC method has been successfully applied to a routine study of the determination of these six OPCs in water or any other sample prior to extraction. A water and acetonitrile mixture (25:75), at a flow rate of 1.0 ml min^{-1} was found to be an appropriate mobile phase, allowing adequate and rapid separation of six OCPs. This method is simple, accurate, precise, and specific and could separate six OCPs in a mixture solution. Its wide linear dynamic range and low limit of detection and quantitation make this method applicable for water and formulation analyses of OCPs.

Results obtained in this study from samples collected through selected sites in the eastern region of Libya showed only 2, 4-D residue with a high concentration in summer samples than winter samples followed by the autumn season samples. These differences are probably due to climatic variations and agricultural activity between these areas.

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REFERENCES

Adeyemi, D., Anyakora, C., Ukpo, G., Adedayo, A., & Darko, G. (2011). Evaluation of the levels of organochlorine pesticide residues in water samples of Lagos Lagoon using solid phase extraction method. *Journal of Environmental Chemistry and Ecotoxicology, 3*(6), 160-166.

Afful, S., Anim, A., & Serfor-Armah, Y. (2010). Spectrum of organochlorine pesticide residues in fish samples from the Densu Basin. *Res J Environ Earth Sci, 2*(3), 133-138.

Akan, J., Sodipo, O., Mohammed, Z., & Abdulrahman, F. (2014). Determination of organochlorine, organophosphorus and pyrethroid pesticide residues in water and sediment samples by high performance liquid chromatography (HPLC) with UV/visible detector.

ISSN: online 2617-2186 print 2617-2178

Journal of Analytical & Bioanalytical Techniques, 5(6), 1.

Akoto, O., Azuure, A. A., & Adotey, K. (2016). Pesticide residues in water, sediment and fish from Tono Reservoir and their health risk implications. *SpringerPlus, 5*(1), 1849.

APHA. (1995). Standard methods for the examination of water and wastewater. *American Public Health Association, American Water Works association, Water Environment Federation, Washington*(5), 4-12.

APHA. (1998). Standard methods for the examination of water and wastewater. *Water Environment Federation*(2), 9-31.

Ara, A. G., Haque, W., & Hasanuzzaman, M. (2014). Detection of Organochlorine and organophosphorus pesticides residues in water samples of Taragong thana in Rangpur district in Bangladesh. *Research Journal of Environmental and Earth Sciences, 6*(2), 85-89.

Azad, H. N., Kafilzadeh, F., Malekpour, R., & Shiva, A. H. (2012). Determination of Organochlorine Pesticide Residues inWater, Sediments and Fish from Lake Parishan, Iran. *World, 4*(2), 150-154.

Boussahel, R., Bouland, S., Moussaoui, K., Baudu, M., & Montiel, A. (2002). Determination of chlorinated pesticides in water by SPME/GC. *Water research, 36*(7), 1909-1911. http://www.sciencedirect.com/science/article/pii/S0043135401003724

Chen, X., Zhang, H., Wan, Y., Chen, X., & Li, Y. (2018). Determination of 2, 4-Dichlorophenoxyacetic acid (2, 4-D) in rat serum for pharmacokinetic studies with a simple HPLC method. *PLoS ONE, 13*(1), e0191149.

Chowdhury, M. A. Z., Banik, S., Uddin, B., Moniruzzaman, M., Karim, N., & Gan, S. H. (2012). Organophosphorus and carbamate pesticide residues detected in water samples collected from paddy and vegetable fields of the Savar and Dhamrai Upazilas in Bangladesh. *International journal of environmental research and public health, 9*(9), 3318-3329.

Coady, K., Marino, T., Thomas, J., Sosinski, L., Neal, B., & Hammond, L. (2013). An evaluation of 2, 4-dichlorophenoxyacetic acid in the amphibian metamorphosis assay and the fish short-term reproduction assay. *Ecotoxicology and environmental safety, 90*, 143-150.

Cooper, J., & Dobson, H. (2007). The benefits of pesticides to mankind and the environment. *Crop Protection, 26*(9), 1337-1348. http://www.sciencedirect.com/science/article/pii/S026121940700097X

Demoliner, A., Caldas, S. S., Costa, F. P., Gonçalves, F. F., Clementin, R. M., Milani, M. R., & Primel, E. G. (2010). Development and validation of a method using SPE and LC-ESI-MS-MS for the determination of multiple classes of pesticides and metabolites in water samples. *Journal of the Brazilian Chemical Society, 21*(8), 1424-1433.

ISSN: online 2617-2186 print 2617-2178

Dores, E. F., Carbo, L., Ribeiro, M. L., & De-Lamonica-Freire, E. M. (2008). Pesticide levels in ground and surface waters of Primavera do Leste Region, Mato Grosso, Brazil. *Journal of chromatographic science, 46*(7), 585-590.

El-Ghit, H. M. A. (2016). Impact of Post-Emergence Application of Dichlorophenoxy Acetic Acid (2, 4-D) Herbicide on Growth and Development of Three Weeds Associated with Maize Growth. *Int. J. Curr. Microbiol. App. Sci, 5*(2), 794-801.

El-Osmani, R., Net, S., Dumoulin, D., Baroudi, M., Bakkour, H., & Ouddane, B. (2014). Solid phase extraction of organochlorine pesticides residues in groundwater (akkar plain, north Lebanon). *International Journal of Environmental Research, 8*(4), 903-912.

El-Saeid, M., Al-Turki, A., Al-Wable, M., & Abdel-Nasser, G. (2011). Evaluation of pesticide residues in Saudi Arabia ground water. *Research Journal of Environmental Sciences, 5*(2), 171.

El Bouraie, M. M., El Barbary, A., & Yehia, M. (2011). Determination of organochlorine pesticide (OCPs) in shallow observation wells from El-Rahawy contaminated area, Egypt. *Environmental Research, Engineering and Management, 57*(3), 28-38.

Erkmen, B., Yerli, S. V., Erk'akan, F., & Kolankaya, D. (2013). Persistent organochlorine pesticide residues in water and sediment samples from Lake Manyas, Turkey. *Journal of environmental biology, 34*(2), 171.

Hashmi, T. A. S., & Menon, S. K. (2015). Accumulation and distribution of persistent organochlorine pesticides and their contamination of surface water and sediments of the Sabarmati River, India. *Journal of Advances in Environmental Health Research, 3*(1).

Hellar-Kihampa, H. (2011). Pesticide residues in four rivers running through an intensive agricultural area, Kilimanjaro, Tanzania. *Journal of Applied Sciences and Environmental Management, 15*(2).

Hong, H., Chen, W., Xu, L., Wang, X., & Zhang, L. (1999). Distribution and fate of organochlorine pollutants in the Pearl River Estuary. *Marine Pollution Bulletin, 39*(1), 376-382.

Hunt, D. T. E., & Wilson, A. L. (1986). *The chemical analysis of water: general principles and techniques* (Vol. 2). Royal Society of Chemistry.

Ismail, M., Sayed, M., Khan, H. M., & Cooper, W. J. (2014). Analysis of pesticides in water samples and removal of monocrotophos by gamma irradiation. *J Anal Bioanal Tech, 5*(1), 1-10.

Ize-Iyamu, O., Asia, I., & Egwakhide, P. (2007). Concentrations of residues from organochlorine pesticide in water and fish from some rivers in Edo State Nigeria. *International Journal of Physical Sciences, 2*(9), 237-241.

Jamal, H. M. (2011). Occurrence of organochlorine pesticide residues in groundwater and soil from Syrian coastal area. *Agriculture and Biology*

ISSN: online 2617-2186 print 2617-2178

Journal of North America, 2(3), 488-492.

Jayashree, R., & Vasudevan, N. (2007). Organochlorine pesticide residues in ground water of Thiruvallur district, India. *Environmental monitoring and assessment, 128*(1-3), 209. https://link.springer.com/article/10.1007/s10661-006-9306-6

Lari, S. Z., Khan, N. A., Gandhi, K. N., Meshram, T. S., & Thacker, N. P. (2014a). Comparison of pesticide residues in surface water and ground water of agriculture intensive areas. *Journal of Environmental Health Science and Engineering, 12*(1), 1.

Lari, S. Z., Khan, N. A., Gandhi, K. N., Meshram, T. S., & Thacker, N. P. (2014b). Comparison of pesticide residues in surface water and ground water of agriculture intensive areas. *Journal of Environmental Health Science and Engineering, 12*(1), 11.

Leong, K. H., Tan, L. B., & Mustafa, A. M. (2007). Contamination levels of selected organochlorine and organophosphate pesticides in the Selangor River, Malaysia between 2002 and 2003. *Chemosphere, 66*(6), 1153-1159. https://www.ncbi.nlm.nih.gov/pubmed/17027062

Mansour, S. A. (2004). Pesticide exposure—Egyptian scene. *Toxicology, 198*(1-3), 91-115.

Mariyono, J. (2008). Direct and indirect impacts of integrated pest management on pesticide use: a case of rice agriculture in Java, Indonesia. *Pest management science, 64*(10), 1069-1073. http://onlinelibrary.wiley.com/doi/10.1002/ps.1602/epdf?r3_referer=wol&tracking_action=preview_click&show_checkout=1&purchase_referrer=www.google.com.ly&purchase_site_license=LICENSE_DENIED_NO_CUSTOMER

Maurya, A., & Kumar, A. (2013). Organochlorine pesticides in the surface waters from sharda river region, Uttar Pradesh, India. *The SIJ Transactions on Advances in Space Research & Earth Exploration (ASREE), 1*(1), 8-10.

Munn, M. D., & Gruber, S. J. (1997). The relationship between land use and organochlorine compounds in streambed sediment and fish in the Central Columbia Plateau, Washington and Idaho, USA. *Environmental Toxicology and Chemistry, 16*(9), 1877-1887.

Nie, N. H., Bent, D. H., & Hull, C. H. (1970). *SPSS: Statistical package for the social sciences*.

Oerke, E.-C., & Dehne, H.-W. (2004). Safeguarding production—losses in major crops and the role of crop protection. *Crop Protection, 23*(4), 275-285.

Okoya, A. A., Ogunfowokan, A. O., Asubiojo, O. I., & Torto, N. (2013). Organochlorine pesticide residues in sediments and waters from Cocoa producing areas of Ondo State, Southwestern Nigeria. *ISRN Soil Science, 2013*.

ISSN: online 2617-2186 print 2617-2178

Que Hee, S., & Sutherland, R. (1981). The phenoxyalkanoic herbicides. Vol. I. Chemistry, analysis, and environmental pollution. *CRC Pres, Inc, Boca Raton*, 319.

Saha, A., Gajbhiye, V., Gupta, S., & Kumar, R. (2012). Development of multi-residue method for determination of pesticides in river, ground and lake water in Delhi using gas chromatography. *International Journal Agriculture Environment Biotechnology, 5*(3), 199-205.

Sankararamakrishnan, N., Sharma, A. K., & Sanghi, R. (2005). Organochlorine and organophosphorous pesticide residues in ground water and surface waters of Kanpur, Uttar Pradesh, India. *Environment international, 31*(1), 113-120.

Shakerkhatibi, M., Mosaferi, M., Jafarabadi, M. A., Lotfi, E., & Belvasi, M. (2014). Pesticides residue in drinking groundwater resources of rural areas in the northwest of Iran. *Health promotion perspectives, 4*(2), 195.

Shamsipur, M., Yazdanfar, N., & Ghambarian, M. (2016). Combination of solid-phase extraction with dispersive liquid–liquid microextraction followed by GC–MS for determination of pesticide residues from water, milk, honey and fruit juice. *Food chemistry, 204*, 289-297.

Smilanick, J. L. (2008). Postharvest pathogens and disease management. *HortScience, 43*(6), 1932-1933.

Tang, Z., Yang, Z., Shen, Z., Niu, J., & Cai, Y. (2008). Residues of organochlorine pesticides in water and suspended particulate matter from the Yangtze River catchment of Wuhan, China. *Environmental monitoring and assessment, 137*(1), 427-439.

Tata Rao, S., Ch, V., Reddy, A., Veeraiah, K., & Padmavathi, P. (2014). Research article determination of organochlorine pesticides residues in the water of paddy fields of Prakasam district, Andhra Pradesh, India.

Uddin, A., Kumar, P., & Sarma, J. (2007). Early orogenic history of the eastern Himalayas: compositional studies of Paleogene sandstones from Assam, northeast India. *International Geology Review, 49*(9), 798-810.

WHO. (2004). *2,4-D in drinking water. Background document for development of WHO Guidelines for Drinking-Water Quality*.

Yusiasih, R., Nugraha, W., & Hudayya, A. (2017). Determination of carbofuran in a river water sample using LC-MS/MS. IOP Conference Series: Earth and Environmental Science,

Zhao, L., & Lee, H. K. (2001). Application of static liquid-phase microextraction to the analysis of organochlorine pesticides in water. *Journal of Chromatography A, 919*(2), 381-388.

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تطوير وتحقق وتطبيق طريقة على أساس الطور العكسي لجهاز HPLC لغرض التقدير المتزامن لستة أنواع من مبيدات الآفات الكلورية العضوية في عينات المياه السطحية والجوفية المأخوذة من مناطق شمال شرق ليبيا

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المستخلص: تم فحص تلوث المياه بمبيدات الآفات الكلورنينة العضوية في مواقع الدراسة في مناطق شمال شرق ليبيا لتقدير الوضع الحالي للتلوث في مصادر المياه السطحية والجوفية. أجريت هذه الدراسة لتحديد تركيزات بعض متبقيات المبيدات الآفات الكلورنينة في عينات المياه السطحية والجوفية التي تم جمعها من ثلاثون موقعًا مختلفًا في الجبل الأخضر شمال شرق ليبيا خلال فصول الصيف والخريف والشتاء في عام 2016. تم تطوير طريقة بسيطة وسريعة تعتمد على طريقة استخلاص السائل متبوعة باستخدام جهاز الكروماتوافي عالي الأداء .مع استخدام العمود ذو الطور العكسي لتحديد ستة مبيدات كلورينية عضوية في عينات المياه في وقت واحد. استخدم في هذه التجربة جهاز مزودة بعمودC_{18} (250 مم × 4 مم؛ 4.6 ميكروميتر حجم الجسيمات) وكاشف الأشعة فوق البنفسجية بطول موجي 238 نانومتر. يتكون الطور المتحرك من 0.1٪ بروبانول في الماء وأسيتونيتريل (75/25، ح / ح). تم التحقق من صحة هذه الطريقة باستخدام محاليل مرجعية لهذه المبيدات في مستويات تراكيز مختلفة، ويظهر منحي خطي جيد في التركيزات بين 5.0 و25 ميكروغرام لكل مل. حيث تراوحت حدود الكشف وحدود الكمي من 0.71 إلى 2.24 ميكروغرام لكل مل و2.16 إلى 6.79 ميكروغرام لكل مل، على التوالي. وكذلك تراوح الانحراف المعياري النسبي من 0.026 إلى 0.673٪. تبين من النتائج أن متبقيات المبيدات الآفات الكلورنينة الوحيدة الموجودة في منطقة الدراسة هي2,4-D مع اختلاف مستويات متبقياتها خلال الفصول. أظهرت النتائج الإجمالية أن المياه السطحية كانت أكثر تلوثًا بـ 2,4-D عن المياه الجوفية، والتي تم اكتشافها في النطاق الواسع للتركيز من 0.037 إلى 0.385 ميكروغرام لكل مل، 0.003 إلى 0.047 ميكروغرام لكل مل و0.012 إلى 0.039 ميكروغرام لكل مل خلال الصيف، الخريف والشتاء، على التوالي.

الكلمات المفتاحية: التحقق. الجهاز الكروماتوافي عالي الأداء. متبقيات. المياه السطحية. المياه الجوفية.

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The Association Between Gender and Complications of Type 2 Diabetes Mellitus Among Patients in Almarj City-Libya

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Abstract: This study was carried out to provide new data on patients with type 2 diabetes mellitus and any suggestions for the prevention of what is a life-threatening disorder. Four hundred and seventy-three type 2 diabetic mellitus patients from Almarj general hospital were used: (222) females and (251) males for the period from October -2017 to April – 2018.The study focused on the relationship between genders and complications of diabetes mellitus. The study showed no significant differences ($P > 0.05$) between hypertension, heart disease, respiratory dysfunction, nephropathy, urinary tract infection, anemia, hypothyroidism, diabetic ketoacidosis, gastric infection, and gender. On the other hand, a significant ($P \leq 0.05$) relationship was found between hepatopathy and foot complications and gender. Findings showed that males with the risk of the dangers of type 2 diabetes mellitus complications were more than females in Almarj city.

Keywords: Diabetes Mellitus; Complications; Gender Difference.

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder which is characterized by high glucose levels prevalent in the blood for a considerable duration of time. (Behl et al. 2014).

The rate of diabetic patients with major complications has increased, such as stroke, hypertension, amputation, nephropathy, neuropathy, retinopathy, cardiovascular complications, impotence, and skin lesions. However, in the Eastern Mediterranean, the essential health care and facilities for self-care are often inadequate. Also the lacking of basic education services and knowledge on diabetes, as well as its prevention, management, and treatment to people with diabetes and their families, leads to a serious impediment to the provision of the minimum standard of health care. The causes of DM are multi-factorial, which include genetic, physical inactivity, drug toxic agents, obesity, viral infection and location (Adeghate, 2006). Sex-related differences in lifestyle may lead to differences in the risk of developing diabetes mellitus and, in consequence, to differences in the prevalence of this condition in women and men. However, the relationship between a known risk factor for diabetes mellitus – such as obesity – and the development of symptomatic diabetes mellitus may not be straightforward. For example, in many countries of sub-Saharan Africa, women are more likely to be obese or overweight than men, and therefore may be expected to have a higher prevalence of diabetes mellitus. Compared with the corresponding men, women in Cameroon, South Africa, and Uganda were indeed found to have a higher prevalence of diabetes mellitus. However, women in Ghana, Nigeria, Sierra Leone, and rural areas of the United Republic of Tanzania were found to have lower a prevalence of diabetes mellitus than the men in the same study areas. No significant differences between men and women in the prevalence of diabetes mellitus were detected in studies in Guinea, Mali, Su-

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dan, and urban areas of the United Republic of Tanzania (Esayas et al.;2013). This study assesses differences between men and women in the prevalence of type 2 diabetes mellitus and its complications.

This study aimed to determine the effect of diabetes mellitus complications in people of Almarj city\Libya, and investigate the independent association of each of these clinical characteristics with parasympathetic dysfunction.

DATA COLLECTION

This study examined the profile of type 2 diabetic patients in the Almarj clinic/ Libya. We began filling out of data from October -2017 to April – 2018. Data collection was performed through filling the researchers' questionnaire considering the following: gender, age of type 2 diabetic patients and complications: heart disease, hypertension, nephropathy, stroke, foot complications, urinary tract infection, anemia, hypothyroidism, hepatopathy, gastric infection, diabetic ketoacidosis, and respiratory dysfunction.

It is a descriptive statistical study that was carried out on 473 diabetic mellitus patients admitted to Almarj clinic in Almarj city /Libya, females (222), males (251), their age ranged between (29 - 97) years with mean of 67 years.

Data were collected, checked, revised, and entered into a computer. Data were analyzed by SPSS statistical package version 19. Excel computer program was used to tabulate the results and represent it graphically.

RESULTS & DISCUSSION

Over the final few decades, the prevalence of diabetes reached epidemic proportions in western societies and is even higher in developing countries. The World Health Organization has assessed that the worldwide prevalence of diabetes will increase from 2.8 % in 2000 to 4.4 % by 2030 (Ali, A, 2011).

According to this study, a higher incidence of diabetes in males (53.1%) compared to females (46.9%) was reported, which disagrees with a previous study from India (Ramachandran et al., 2008), and agrees with a previous Libyan study (Shambesh et al., 2015) that found that type 2 diabetes was higher in males than females (54.1% and 45.9%) respectively. This discrepancy in results could be due to an inhomogeneous study sample, which could be related to the security situation, particularly in Almarj city

Table: (1). Subject characteristics

Age (years)	Gender				Total	
	Female		Male			
	No.	%	No.	%	No.	%
29-39	3	0.6	4	0.8	7	1.5
40-59	46	9.8	65	13.7	111	23.5
≥60	173	36.5	182	38.5	355	75
Total	222	46.9	251	53	473	100
Age (years) Mean ± SD	67 ± 12					

Table 1 shows that the predominance of diabetes increments with age. Within the age group of 29-39 years, it was evaluated that around 1.5% of subjects had diabetes, whereas within the age group 40-59 years, the value expanded to 23.5 %, and the highest rate of 75 % was found within the age group ≥ 60 years. Amati et al. have explained that older adults are at high risk for the development of type 2 diabetes due to the combined effects of increasing insulin resistance and impaired pancreatic islet function with aging. Age-related insulin resistance appears to be primarily associated with adiposity, sarcopenia, and physical inactivity (Amati et al., 2009).

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Table: (2). shows the distribution of gender with complications of type 2 diabetes mellitus.

Disease		Female		Male		Total	
		No.	%	No.	%	No.	%
Heart disease	non	121	25.6	109	23.0	230	48.6
	exist	101	21.4	142	30.0	243	51.4
Hypertension	non	71	15.0	100	21.1	171	36.2
	exist	151	31.9	151	31.9	302	63.8
Nephropathy	non	190	40.2	219	46.3	409	86.5
	exist	32	6.8	32	6.8	64	13.5
UTI	non	186	39.3	229	48.4	415	87.7
	exist	36	7.6	22	4.7	58	12.3
Anemia	non	206	43.6	225	47.6	431	91.9
	exist	16	3.4	26	5.5	42	8.9
Stroke	non	158	33.4	165	34.9	323	68.3
	exist	64	13.5	86	18.2	150	31.7
Foot complications	non	213	45.0	241	51.0	454	96.0
	exist	9	1.9	10	2.1	19	4.0
Hypothyroidism	non	189	40.0	242	51.2	431	91.1
	exist	33	7.0	9	1.9	42	8.9
Hepatopathy	non	208	44.0	236	49.9	444	93.9
	exist	14	3.0	15	3.2	29	6.1
Respiratory dysfunction	non	167	35.3	193	40.8	360	76.1
	exist	55	11.6	58	12.3	113	23.9
DKA	non	214	45.2	245	51.8	459	97.0
	exist	8	1.7	6	1.3	14	3.0
Gastric infection	non	217	45.9	242	51.2	459	97.0
	exist	5	1.1	9	1.9	14	3.0

This study indicated that heart disease and hypertension were non-significant when it comes to gender, but males (30.0 %) were more likely to have heart disease than females (21.4 %), as in table 2. This finding agrees with those by Aseel, where males were higher than females (18.7 % & 10.8 %) (Ali, 2011). Whereas, hypertension in males was equal to females (31.9 % & 31.9%), as in table 2. Nouh et al. have indicated that a high prevalence of hypertension among diabetic patients was in females compared to males (30.0% & 35.1%) (Nouh et al., 2015). Hypertension is one risk factor for atherosclerosis. The atherosclerotic vessel is more prone to thrombosis and rupture. Vascular problems that happen as a result of diabetes are made worse when blood pressure is raised from other sources such as an unhealthy diet, lack of exercise, or smoking (Ali, 2011).

The present study showed that nephropathy was non-significant for gender, as females (6.8%) were more than males (6.6%), as in table 2. This agrees with a study from Iraq by Aseel that shows females were higher in nephropathy than males (7.2 % & 5.9 %) respectively (Ali, 2011). However, it disagrees with Aho et al., whose findings show that males were higher than females (25.6 % & 17.9 %) respectively (Aho et al., 1998). They have found that high levels of blood glucose increase the risk that a person with diabetes will progress to kidney failure**.**

Our study showed that UTI has no signification with gender, but females were higher than males (7.6 % & 4.7 %), as in table 2 respectively. This result agrees with Shengsheng et al., who have found that females higher than males (12.9 % & 3.9 %) in urinary tract infection among diabetes mellitus in the U.S (Shengsheng et al., 2014). Sridhar et al. have clarified that the urinary tract is the primary site of infection in diabetes. Changes in host defense mechanisms and the presence of diabetic cystopathy and microvascular disease in

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the kidneys may play a part in the higher rate of UTI in diabetic patients (Sridhar et al., 2002). Whereas, Wheat has indicated that the high level of contamination in the urinary tract infection of diabetic women may be determined by the number of microorganisms found within the vagina (Wheat et al., 1980).

We observed that anemia has no signification with gender, as males were more than females (5.5% & 3.4%), as in table 2. Similarly, this agrees with previous study (Kaushik et al., 2018), that showed that males were more than females (68.25 % & 31.74 %) respectively, and this disagrees with another study (Rathod et al., 2016), which showed that females were more than males (18.60 % & 17.54 %) respectively. The development of anemia in diabetes due to different factors such as symptomatic autonomic neuropathy, which can lead to efferent sympathetic denervation of patients taking metformin, have high chances of B12 deficiency leading to clinical symptoms of anemia (Gulati1 et al., 2016).

The results showed no significant relationship between gender and stroke, the males (18.2 %) were higher than females (13.5 %) as in table 2, this agrees with Jehangir et al. They found that male to female ratio of stroke is 2.18:1 (Jehangir et al., 2006), but disagrees with Narayan et al. that have found that stroke diabetes patients were higher in females than males (Narayan et al., 2003). Jehangir et al. have discussed that stroke is a frequent medical problem occurring in patients with hypertension and other risk factors. They conduced that hypertension is the leading risk factor for stroke (Jehangiret al., 2006).

The results showed a significant relationship between gender and foot problems, with males (2.1 %) higher than females (1.9 %) with foot problems, as in table 2. A study by Hussain et al., however, found that females were more than males. Hussain et al. have also explained that the cause of the common diabetic foot complications include neuropathy, infections, vascular disease, and ulcerations (Hussain et al., 2010).

Our study also concluded that there was no significant relationship between gender and hypothyroidism, as females were more than males (7.0 % & 1.9 %). This agrees with the following reference (Sami et al., 2018), which also found that females were more than males (13.8 % & 3.7 %). This sex difference may be due to less consultations, less awareness, and less education regarding women in our study. DM irritates thyroid function by influencing both the hypothalamic control of TSH production and the transformation of T4 to T3 in the peripheral tissue (Ibrahim et al., 2017).

Our study found a significant relationship between gender and hepatopathy, where males (3.2 %) were higher than females (3.0%), as in table 2, and Patel et al. have found that the prevalence rate among males (65.62 %) was higher than for females (34.38 %) (Patel et al., 2018). Another study by Sharavanan et al. in Chennai found the prevalence rate among females (62.2%) was higher than for males (37.8 %) (Sharavanan1 et al., 2015). DM might initiate liver damage by promoting inflammation and fibrosis through an increase in mitochondrial oxidative stress by the activity of leptin, adiponectin, interleulcin-6, and TNF, which are created in chronically inflamed adipose tissue (adiposities) (Marra, and Bertolani, 2009) .

Our study also showed no significant relationship between gender and respiratory function. Males were more than females (12.3 % & 11.6%), as in table 2. This finding is consistent with Qayyum et al., who also found that males were more than females (77.77 % & 22.22 %) (Qayyum et al., 2004), but disagrees with Keerthi et al., that found that the female percentage was higher than that for males (Keerthi et al., 2012). The presence of abundant connective tissue and an extensive microvascular circulation in the lungs increase the possibility that lung tissue may be influenced by the microangiopathy process and non-enzymatic gly-

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cosylation of tissue proteins, this is induced by chronic hyperglycemia, thereby rendering the lung a "target organ" in diabetic patients. An indication of this may be the prevalence of tuberculosis among diabetic patients (Keerthi et al., 2012)**.**

The results showed no significant relationship between gender and DKA. The females were higher than males (1.7 % & 1.3 %) as in table 2. This finding agrees with findings from other studies on the Saudi population, which indicate the higher incidence of DKA among female patients. Yousuf and Chaudhry have reported that a female to male ratio was 2:1 among the Saudi population (Yousuf and Chaudhry, 1994). Al-Rubeaan et al. have reported that a male to female ratio was (1.6:1) (Al-Rubeaan et al., 2011), and also Balasubramanyam et al. have reported that male to female ratio was equal (Balasubramanyam et al., 1999), It shows that the gender difference may not be a significant factor in the prevalence of DKA. However, the blood pH was found significantly lower in females when compared with that of males. This finding may show that ketoacidosis was severe among females when compared to male patients (Balasubramanyam et al., 1999).

We observed that gastric infection has no signification with gender as affected males were more than females (1.9% & 1.1%), as in table 2. This finding agrees with Devrajani et al. that have confirmed that Helicobacter pylori infection were predominant in males (Devrajiani et al., 2010), but disagrees with Kanbay et al. that have reported that Helicobacter pylori infected females were prevalent as compared to males (Kanbay et al.,2005). This predominance may be due to patients with DM being more inclined to infection and severe diseases such as of phagocyte dysfunction and cellular immunity disorders caused by hyperglycemia and diminished vascularization (Keramat et al., 2013).

Table: (3). Distribution of gender with diabetic patients DM T2 with cancer.

Diseases		Female		Male		Total	
		No.	%	No.	%	No.	%
Ovarian	non	221	46.7	251	53.1	472	99.8
cancer	exist	1	0.2	0	0.0	1	0.2
Bladder	non	222	46.9	250	52.9	472	99.8
cancer	exist	0	0.0	1	0.2	1	0.2
Rectal	non	222	46.9	249	52.6	471	99.6
cancer	exist	0	0.0	2	0.4	2	0.4
Breast	non	212	44.8	251	53.1	463	97.9
cancer	exist	10	2.1	0	0.0	10	2.1
Gastric	non	220	46.5	251	53.1	471	99.6
cancer	exist	2	0.4	0	0.0	2	0.4
Colon	non	219	46.3	249	52.6	468	98.9
cancer	exist	3	0.6	2	0.4	5	1.1

The previous table 3 shows the study to know the association between gender and cancer of diabetic patients. The result showed no signification between gender and cancer. T2DM and cancers often share many risk factors such as age, obesity, sedentary lifestyle, smoking, higher intake of saturated fats and refined carbohydrates, and some psychological factors (Giovannucci et al., 2010).

In our study, we found that out of 473 subjects with diabetes, only one woman had ovarian cancer. Additionally, just one diabetic man suffered from bladder cancer. The association between diabetes and ovarian cancer is not clear, but there is ample evidence for biological plausibility. Several studies have assessed the impact of elevated insulin-like growth factor (IGF) (Giovannucci et al., 2010), where IGF-I playing a significant role in the development of bladder cancer, is also supported by studies in animals.

The number of breast cancer incidences of diabetic females was 10 women as in table 3. Hyperinsulinemia with insulin resistance has been postulated to increase the risk of breast cancer (Del Giudice g et al., 1998). Obesity is related to type 2 diabetes and leads to a rise in endogenous estrogen levels.

The total number of diabetic females that had gastric cancer was 2 women, as in table 3. Mi-

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ao et al. found that gender difference was not significantly associated between DM and gastric cancer incidences (Miao et al., 2017). On the other hand, Lin et al. (Lin et al., 1997) found a significant association between DM and the higher risk of gastric cancer. They explained this relationship by stating that hyperglycemia in DM patients may lead to dysregulation of energy balance. Consequently, this could influence intracellular metabolism and impair the immune system and might play a critical role in the progression of gastric cancer (Vigneri et al., 2009). Moreover, women were found to have an increased risk of gastric cancer.The percentage of colon cancer incidences was 1.1 %، 0.6 % in females and 0.4 % in males, and 0.4 % of diabetic females with rectal cancer, as in table 3. Hyperinsulinemia is one of the prime characteristics of T2DM. It might initiate carcinogenesis and also may initiate the proliferation of colonic tumors in vitro and experimental animals (Wu et al., 2004). However, there was no significant difference between men and women in this relationship.

CONCLUSION

It was concluded from this study that men with type 2 diabetes mellitus are more likely to have serious complications compared to women with the same disease.

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REFERENCES

Adeghate, E; Schttner ,P and and Dunn, E. (2006). An update on the etiology and epidemiology of diabetes mellitus, Annual New York Academy of Science, 1084:1-29.

Aho, K ; Harmsen, P; Hateno, S; Maarqyardsen, T;Smirnov, VE and Strasser, T. (1998). On behalf of the participants in the W.H.O collaborative study on control of stroke in the community. Cerebrovascular diseases in the community results of the W.H.O collaborative study, Bulletin of W.H.O, 58: 113-130.

Ali, A. (2011). Diabetic Mellitus and it's complications in Duhok city/ IRAQ, nitro PDF professional.online at nitropdf.com /professional

Al-Rubreaan ,K.A; Saboor, S; Metib, S; et al. (2011).Clinico - laboratory characteristics of diabetic ketoacidosis in adults in a tertiary hospital in Saudi Arabia, European Review for Medical and Pharmacological Sciences, 15: 1202-1206.

Amati, F; Dubé, JJ, Coen, PM; Stefanovic-Racic, M; Toledo, FG; Goodpaster, BH. (2009). Physical inactivity and obesity underlie the insulin resistance of aging. Diabetes Care 32:1547–1549.

Balasubrmanyam A, Zern , Hyman ,DJ and Pavlik, V. (1999).New profiles of diabetic ketoacidosis. Type 1 vs. type 2 diabetes and the effect of ethnicity, Arch Intern Med 159: 2317-2322.

Behl, T; Kaur,N; Kaur, I; Goel, H and Pandey, R.K. (2014). Mediators involved in diabetic nephropathy: an updated review, Asian Journal of Biochemical and Pharmaceutical Research, 2:4: 134-149.

Del Giudice, ME; Fantus, IG; Ezzat, S; McKeown-Eyssen, G; Page, D and Goodwin, PJ. (1998).Insulin and related factors in premenopausal breast cancer risk. Breast Cancer Res Treat , 47:111–120,

Devrajiani B.R., Shah S.Z., Soomro A.A., et al. (2010).Type 2 diabetes mellitus: A risk factor for Helicobacter pylori infection, A hospital based case-control study, Int J Diabetes Dev Ctries, Jan; 30(1):22-6.

ISSN: online 2617-2186 print 2617-2178

Esayas, H.et al. (2013). Differences by sex in the prevalence of diabetes mellitus, impaired fasting glycaemia and impaired glucose tolerance in sub-Saharan Africa, Bull World Health Organ 2013;91:671–682D.

Giovannucci, E; Harlan, DM;Archer ,MC; et al. (2010).Diabetes and cancer: a consensus report,CA Cancer J Clin, 60(4): 207–221.

Gulati1 M and Agrawal ,N. (2016).Study of Prevalence of Anaemia in Patients with Type 2 Diabetes Mellitus, Sch. J. App. Med. Sci., 4(5F):1826-1829, ISSN 2320-6691 .

Hussain, F; Shiekh, M ; Amer; Jamil, H;et al. (2010).Advanced Glycation End-products and Foot Ulceration in Type 2 Diabetic Patients: A Case Control Study, International Journal Of Agriculture & Biology. Issn: 1560–8530; Issn 09–388/Zip/2010/12–1–91–95.

Ibrahim ,M; Elmenshawi, Sultan; S Saleh , A ; et al. (2017).Prevalence of thyroid dysfunction in diabetic patients, Journal of Diabetes, Metabolic Disorders & Control,Volume 4 Issue 2 – 2017.

Jehangir, Kh; Attique-ur-Rehman, Ashfaq, Al and Asif ,Ji. (2006). ,frequency of hypertension in stroke patients presenting at Ayub teaching hospital, J Ayub Med Coll Abbottabad, 18(1).

Kanbay M., Yilmaz U., Arslan H., et al. (2005).The Relationship of ABO Blood Group, Age, Gender, Smoking, and Helicobacter pylori Infection. Dig Dis Sci. July 50(7):1214-7.

Kaushik , D; Parashar, R; Praveen, K and Malik. (2018).Study of anaemia in type 2 diabetes mellitus Kaushik D et al. Int J Res Med Sci, ,May;6(5):1529-1533 pISSN 2320-6071 | eISSN 2320-6012.

Keramat, F; Hashemi ,S; Majlesi ,A; Haddadi-nejad ,S; Esfehani ,A and Poorolajal , J, (2013)The association between diabetes mellitus and Helicobacter pylori infection, Springer, DOI 10.1007/s13410-013-0128-0.

Lin ,SW; Freedman, ND ;Hollenbeck ,AR; Schatzkin, A and Abnet, CC. (1997). Prospective study of self- reported diabetes and risk of upper gastrointestinal cancers. Cancer Epidemiol Biomarkers Prev. 2011; 20; 954-961.Cancer Res , 57: 4667–4672.

Marra, F; Bertolani, C. (2009).Adipokines in the liver disease, Hepatology 50: 957-69.

Miao, Zh; Xu,Hao;;; Ying Xu , Yi; Wang, Z; Ting Zhao ,Ti; Xi Song ,Yong and Mian Xu, Hui.(2017) Diabetes mellitus and the risk of gastric cancer: a meta-analysis of cohort studies, OncotargetOncotarget, Oncotarget, March 22, www.impactjournals.com/oncotarget.

Narayan, KM; Boyle, JP; Thompson, TJ; Sorensen, SW and Williamson, DF. (2003). Lifetime risk for diabetes mellitus in the United States",JAMA 290 (14): 1884–90. doi:.%..10.1001/jama.290.14.1884. PMID 14532317.

Nouh, F; Omar, M and Younis, M. (2015).Prevalence of Hypertension among Diabetic Patients in Benghazi: A Study of Associated Factors, Asian Journal of Medicine and Health, 6(4): 1-11,SSN: 2456-8414,

Patel H et al. (2018).Prevalence of non-alcoholic fatty liver disease in type-2

ISSN: online 2617-2186 print 2617-2178

diabetes mellitus patients, Int J Res Med Sci, Apr;6(4):1322-1326.

Qayyum, A; Shafiq ,M and Farogh,A.(2004).prevalence of pulmonary tuberculosis among diabetics , Biomedica Vol. 20 (Jul. – Dec, 2004).

Ramachandran, A; Mary, S; Yamuna, A et al. (2008). High Prevalence of Diabetes and Cardiovascular Risk Factors Associated With Urbanization in India, Diabetes Care, 31(5):893-898.

Rathod ,GB; Parmar, P; Rathod ,S and Parikh, A.(2016) Prevalence of anemia in patients with Type 2 Diabetes Mellitus at Gandhinagar, Gujarat, India. IAIM, 3(3): 12-16.

Sami, H: Razan, S; Samar, S; Maram, M; et al ,Prevalence of Thyroid Dysfunction among Type2 Diabetic Patients (T2D) in Makkah And Jeddah-KSA, The Egyptian Journal of Hospital Medicine, (2018),Vol. 70 (8), Page 1312-1316.Sampson, M.J;Hughes, D.A; Carrier, M.J; and Davies, I.R. (2002). Status of HbA1c during acute hyperglycemia in type 2 diabetes. Diabetes care, 25, 537-541.

Sandler M.(1990) . Is the lung a "target organ" in diabetes mellitus? Arch int Med., July, Vol. 1501385 – 1388.

Shambesh , M. K; Emahbes, T.M; Kamal1, F.A; Saleh ,Z.E and Shambesh , I.M. (2015).Diabetes Mellitus and Cerebrovascular Accident among North African Population (Libya) Community Based Survey, Sciencedomain international, ISSN: 2278–1005, 10(2): 1-9.

Sharavanan1.T,K.V and Premalatha, E.(2015).prevalence of non-alcoholic fatty liver disease in type 2 diabetes mellitus patients in a rural health care hospital, Sch. J. App. Med. Sci., 3(5A):1834-1837.

Shengsheng, Yu ; Alex, Z. Fu b; Qiu a ,Yi; Samuel, S. En; Shankar, R; Brodovicz, Ki. G, Rajpathak, S and Radican, La.(2014).Disease burden of urinary tract infections among type 2 diabetes mellitus patients in the U.S, Journal of Diabetes and Its Complications , 28 621–626.

Sridhar, CB; Anjana ,S; Mathew: JT. (2002). Acute Infections. RSSDI Text Book of Diabetes Mellitus edited by – Ahuja M.M.S, Tripathy B.B, Sam Moses G.P, Chandalia H. B, Das A.K, Rao. P.V, Hyderabad, India, Chap-34:471-8.

Vigneri, P; Frasca, F; Sciacca, L;Pandini, G and Vigneri, R. (2009) .Diabetes and cancer. Endocr Relat Cancer. 16: 11031123.

Wheat, LJ, Infection and diabetes mellitus, Diabetes Care.(1980).3:187-97.

Wu X; Zhao, H; Do ,KA; et al. (2004). Serum levels of insulin growth factor (IGF-I) and IGF-binding protein predict risk of second primary tumors in patients with head and neck cancer. Clin Cancer Res, 10: 3988–3995.

Yousuf, M and Chaudhry: S. (1994). Diabetic ketoacidosis in Saudi Arabia, Saudi Med J, 15(S): 295-296.

ISSN: online 2617-2186 print 2617-2178

العلاقة بين الجنس والنوع الثاني من مرض السكري ومضاعفاته في مدينة المرج – ليبيا

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المستخلص: الغرض من هذه الدراسة توفير معلومات حديثة عن إمكانية حدوث المضاعفات لمرضى السكري من النوع الثاني وتقديم مقترحات للسيطرة عليها عن طريق منع حدوث مضاعفات خطيرة. شملت الدراسة 473 مريض سكري من النوع الثاني، تم جمع العينات من المرضى المراجعين بقسم الباطنة بمستشفى المرج العام حيث كان العدد 251 ذكراً و222 أنثى للفترة من أكتوبر 2017 إلى إبريل 2018. لقد تمت دراسة علاقة نوع الجنس مع مضاعفات مرض السكري. أظهرت النتائج عدم وجود فرق معنوي ($P > 0.05$) بين النسب المئوية لكلا الجنسين والمضاعفات التالية :- ارتفاع ضغط الدم وأمراض القلب والكلى والجهاز التنفسي والأنيميا والتهاب القناة البولية والتهاب المعوي والأحماض الكيتونية السكرية وقصور الغدة الدرقية وكذالك أظهرت وجود تأثير معنوي لكلا الجنسين ($P \leq 0.05$) مع أمراض الكبد ومشاكل القدم. تم الاستنتاج من خلال هذه الدراسة إن الرجال المصابين بمرض السكري من النوع الثاني هم الأكثر عرضه للإصابة بالمضاعفات الخطيرة مقارنة بالنساء اللاتي تعاني من المرض نفسه.

الكلمات المفتاحية: السكري، المضاعفات، الفروق بين الجنسين.

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Genetic Mutations in the LTR Region of SRLV Viruses in *Capra ibex*

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Abstract: The lentivirus (genus of the retroviruses family) can integrate a significant amount of viral cDNA into the DNA of the host cell and can efficiently infect dividing cells. They are able to spill over from their natural host species to induce new infections and pathologies among hosts of new species. This defines the crossing of species barrier that originates emergent viruses causing emergent diseases. The transmission of lentiviruses was observed between different species (domestic & wild). The small ruminant lentiviruses (SRLV) transmission is accompanied by genetic mutations in the genome of the virus. In this article, we have studied the genetic mutations that accompany the infection and adaptation of SRLV to the new host. Genetic mutations were studied by amplifying and sequencing the Long Terminal Repeat (LTR) region.Blood samples were taken from *Capra ibex* living in the French Alps. Sera were tested using a commercially available ELISA. Peripheral blood mononuclear cells (PBMC) isolated on a Ficoll gradient were cultured in a macrophage differentiation medium to obtain monocyte-derived macrophage (MDM) monolayers for virus isolation. DNAs from non-cultured PBMC were used as templates for the PCR amplification of proviral DNA. PCR products (270 nt) were cloned and sequenced. Sequences were analysed using ClustalW.The alignments of the LTR fragment show three types of nucleotide mutations: replacement, addition, and deletion of nucleotide. Sequence analysis shows that the TATA box and the poly (A) site were highly conserved. The divergence of the LTR region between sequences obtained varied by 0.3 - 5.7 %. These differences were also shown by the phylogenetic tree. It can be seen that proviruses from the *Capra ibex* sequences are a closely related group, quite distinct from the reference sequence.

Keywords: Lentivirus; SRLV; LTR; TATA Box; Sequence.

INTRODUCTION

The lentiviruses – which consist of two major types – are non-oncogenic retroviruses responsible in humans and several species of mammals for slowly progressive diseases: Lentiviruses capable of infecting lymphocytes and some of which can induce immunodeficiency and death of the infected host such as the HIV (Human Immunodeficiency Virus) type 1 and HIV type 2. The second type: Lentiviruses that do not infect lymphocytes and do not induce immunodeficiency but inflammatory or degenerative diseases affecting various organs - after a long latent period- such as the lungs, joints, mammary glands, and the central nervous system such as the small ruminant lentiviruses (SRLV) which cause persistent infections in domestic ruminants and wild small ruminants (Erhouma et al., 2008). The lentiviruses are able to spill over from their natural host species to induce new infections and pathologies among hosts of new species. This defines the crossing of species barrier that originates emergent viruses causing emergent diseases. During these last decades, several emergent lentiviruses were discovered in humans and animals occurring in many parts of the world. The

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presence of many variants in the viral swarm might facilitate *cross-species* infections. HIV-2 is almost certainly a human-adapted variant of the simian immunodeficiency virus (SIV) from sooty mangabeys (Hirsch et al., 1989), and HIV-1 may similarly be derived from a chimpanzee SIV (Corbet et al., 2000). Again, the SRLVs emerged after the *cross-species* transfer of closely related domestic ruminants and wild small ruminants in recent times (Erhouma et al., 2008).

The lentiviral genome is organized from the 5′ to the 3′ end and major protein components are contained in the *gag* gene (group-specific antigen: responsible for encoding the structural proteins), *pol* gene (polymerase: encodes the reverse transcriptase, protease, and integrase) and *env* gene (envelope: encodes the virus envelope glycoprotein). These genes are flanked by two non-coding LTR sequences (Long Terminal Repeat). The LTRs (control center for gene expression) – divided into the U3, R, and U5 regions – flank the proviral DNA and provide the signals required for transcription, integration, and polyadenylation of viral RNA (Jaime et al., 2013; Yang et al., 2015).The SRLVs transmission is accompanied by genetic changes (mutations) in the genome of the virus (Blatti-Cardinaux et al., 2016). The rapid evolution of lentiviruses is linked in part to a high frequency of mutations, these genetic mutations allow the virus to adapt within the new host (Rihn et al., 2017).

In this study, to better understand the mechanisms involved in the natural transmission of SRLV in the new species, we characterized the genetic variation in the LTR sequences of SRLVs which emerged after *cross-species* in *Capra ibex*.

MATERIALS AND METHODS

Blood samples were taken from three *Capra ibex* living in the French Alps. Sera were tested for the presence of specific antibodies against ERLVs, using a commercially available ELISA based on the detection of the recombinant LTR protein. Peripheral blood mononuclear cells (PBMC) isolated on a Ficoll gradient were cultured in a macrophage differentiation medium to obtain monocyte-derived macrophage (MDM) monolayers for virus isolation. Genomic DNA was extracted from $5x10^6$ PBMC cells using the DNeasy blood and tissue kits (Quiagen, Courtaboeuf, french) according to the manufacturer's instructions. DNA concentration and quality were determined spectrophotometrically and stocks were stored at -70°c until use. DNAs from non-cultured PBMC were used as templates for PCR amplification of the proviral genome with primer sets chosen in a conserved region of the LTR region sequences from published CAEV-Co (Saltarelli et al., 1990) genomes. PCR products (270 nt) were cloned and sequenced. Sequences were analysed using ClustalW software (Thompson, 1997). The nature of these variations is shown in phylogenetic trees constructed using the program NEIGHBOR (Perriere & Gouy, 1996) method Neighbor-Joining (NJ) (Saitou & Nei, 1987), with bootstrap values determined over 1000 iterations (Felsenstein, 2002).

RESULTS

The alignments of the LTR fragment (corresponding to the U3 and R regions) show a large discrepancy between these two types of viral sequences (Figure 1). The alignments aligned show three types of nucleotide mutations: replacement, addition, and deletion (one or more) of nucleotide. Replacement of nucleotides: GG by TA at position 5, T by A at position 10, A by G at position 79, A by G at position 88, C by T at position 107, C by T at position 115, AG by CA at position 118, C by T at position 171 and C by T at position 133. The second type of mutation was an addition of nucleotides: A at position 23 (all sequences), T at position 32 (sequence CIa2) and T at position 139 (all sequences). The third type of mutation was a deletion of nucleotides: T at position 22 (sequences CIa2 & CIa4), A at position 64 (all sequences) and C at position 98 (sequence CIa6).

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Examination of the aligned LTR sequences shows that the TATA box (TATAA) and the poly (A) site (AATAAA) in the part R of the LTR were highly conserved in all sequences (Figure 1).

```
              10         20         30         40         50         60
Ref. GGGGGGTTATAAGAAAAGCAAGT-TCACTATG-ACAAAGCAAAATGTAACCGCAAGTGCTGACAGA  64
CIa2 ....TA...A............-A.....G..T.............................-  64
CIa4 ....TA...A............-A.....G..-.............................-  63
CIa5 ....TA...A............CA.....G..-.............................-  64
CIa6 ....TA...A............CA.....G..-.............................-  64
CIa8 ....TA...A............CA.....G..-.............................-  64
CIa9 ....TA...A............CA.....G..-.............................-  64
Cons.GGGG**TTA*AAGAAAAGCAAG+*TCACT*TG ACAAAGCAAAATGTAACCGCAAGTGCTGACAG

                76         86         96        106        116        126
Ref. TGTAACAGCTGACATATCAGCTGATGCTTGCTCATGCTGACACTGTAGCTCTGAGCTGTATATAAG  130
CIa2 ..............C........G..................T.......T..CA...........  130
CIa4 ..........A...C........G..............G....T.......T..CA...........  129
CIa5 ..............C........G..................T.......T..CA...........  130
CIa6 ..............C........G.........-.C.A....T.......T..CA...........  129
CIa8 ..............C........G..................T.......T..CA...........  130
CIa9 ..............C........G..................T.......T..CA...........  130
Cons.TGTAACAGCT*ACA*ATCAGCTG*TGCTTGCTC*T*C*GACA*TGTAGCT*TG**CTGTATATAAG

               142        152        162        172        182        192
Ref. GAGAAGCT-TGCTGCTTGCACTTCAGAGTTCTAGGAGAGTCCCTCCTAGTCTCTCCTCTCCGAGGA  195
CIa2 ........T.................................T.........................  196
CIa4 ........T............................C..T.........................  195
CIa5 ........T.................................T.........................  196
CIa6 ........T.................................T.........................  195
CIa8 ........T.................................T.........................  196
CIa9 ........T.................................T............C............  196
Cons.GAGAAGCT*TGCTGCTTGCACTTCAGAGTTCTAGGAG*GT*CCTCCTAGTCTC*CCTCTCCGAGGA

            208        218        228        238        248        258
Ref. GGTACCGAGACCTCAAAATAAAGGAGTGATTGCCTTACTGCCGAGTGGAGAGTGATTACTGAGCGG  261
CIa2 ....................................T.................................  262
CIa4 ....................................T.................................  261
CIa5 ................C...................T.................................  262
CIa6 ................C...................T.................................  261
CIa8 ................C...................T.................................  262
CIa9 ................C...................T.................................  262
Cons.GGTACCGAGACCTCA+A**AAAGGAGTGATTGC*TTACTGCCGAGTGGAGAGTGATTACTGAGCGG
```

Figure (1): Alignment of nucleotide sequences of the LTR region of the SRLV sequences obtained (*Capra ibex*: CIa2-CIa9) and of SRLV reference strains (CAEV-Co: Ref.). Sequences of the TATA box element (position 124), and the polyadenylation signal (position 214) are within black squares. Sequences of samples with similar scores of mammary lesions are within colored squares. Legend: (·) homology, (−) deletion.

The divergence of the LTR region between sequences obtained (*Capra ibex*: CIa2-CIa9) and of SRLV reference strains (CAEV-Co: Ref.) varied by 4.7 - 5.7%. High divergence was observed between sequences CIa7 and CAEV-Co. The sequence divergence between CAEV-Co, CIa8 and CIa9 varied by 5.3%, 5.0% with that of CIa5 and by 4.7% with that of CIa2. The divergence internal between *Capra ibex* sequences varied by 0.3 - 1.9% (Table 1).

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A phylogenetic tree illustrating the relationships between these different LTR sequences was constructed using the neighbor-joining method. It displays proviruses from the *Capra ibex* sequences (CIa2-CIa9) a closely related group, quite distinct from the reference sequence (CAEV-Co Ref.) (Figure 2).

Table 1: Pairwise nucleotidic genetic distances of the partial LTR region of SRLV reference strain (CAEV-Co) and SRLV strains sequenced in this study.

	Ref	CIa2	CIa4	CIa5	CIa6	CIa8	CIa9
Ref.	-						
CIa2	4.7	-					
CIa4	5.4	1.3	-				
CIa5	5.0	0.9	1.6	-			
CIa6	5.7	1.6	1.9	0.9	-		
CIa8	5.3	1.3	1.9	0.3	1.3	-	
CIa9	5.3	1.3	1.9	0.3	1.3	0.6	-

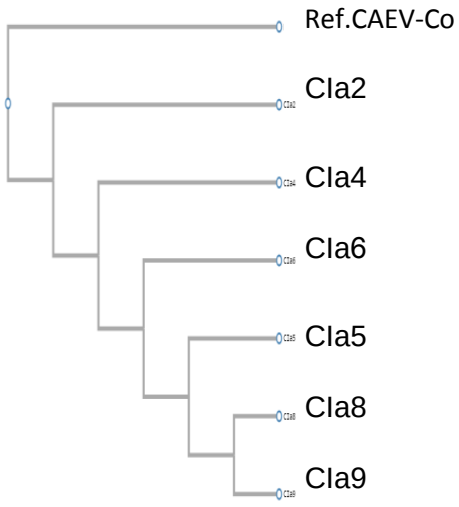

Figure (2): Phylogenetic analysis of the SRLV partial LTR region. Neighbor-joining tree of an approximatively 270 nt fragment from the presently studied sequences. Horizontal lengths are proportional to the estimated genetic distance between the sequences; the bar marker represents a 1% divergence. Bootstrap values derived from 1000 bootstrap replicates are shown on the phylogenetic tree.

DISCUSSION

During these last decades, the emergence of several new pathologies in humans and animals has been observed. Many of these emergences have their origin in the crossing of the species barrier by infectious agents. SRLV infection is widespread in most countries of the world (Mendiola et al., 2019). The objective of this study was to analyze the genetic mutations performed by SRLV during infection of *Capra ibex*. We analyzed approximately 270 nucleotides in the LTR region. The very great homology between the proviral sequences of the LTR region suggests that there has been a passage of SRLV between these species, which is already shown by other studies (Erhouma et al., 2008).

The alignments of the LTR fragment show three types of nucleotide mutations: replacement, addition and the deletion of nucleotide. The sequence analysis shows that the TATA box and the poly (A) site were highly conserved, it is necessary for the virus to maintain these chains of genetic mutations(Mendiola et al., 2019), but the other parties of the LTR may have more mutations (Gayo et al., 2018). Indeed, the regulatory sequences of the genome correspond to non-coding sequences involved in the different stages of the retroviral cycle. The LTR region forms the boundaries between the integrated provirus and the cellular genome. These LTRs are strategic regions of the virus. They include regulatory signals, transcription initiation sites, and signals for integration into the cellular genome.

These terminal regions of the provirus consist of three subdomains U3, R, and U5. The U3 sequence in the 5' LTR is the most terminal sequence of the provirus in contact with the flanking sequences of the host. The terminal nucleotides of this sequence are part of the *att* site required for integration. Again, the stability of the TATA box and poly (A) plays an

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important role in viral transcription. The TATA box is used as a site of transcription initiation. Other sites in this region play an important role in viral replication (Blatti-Cardinaux et al., 2016). These LTR regions are necessary for the integration of the proviral DNA into the genome of the infected cell and are directly involved in the expression of viral genes.

Integration, as the transcription, is a complex and essential step for the expression of viral genes. The ends of the LTRs are cleaved at inverted and repeated (IR) sequences, resulting in the loss of two base pairs at each end of the proviral DNA. The cellular DNA is then cleaved by the viral integrase and then linked to the viral DNA. Finally, viral genes can remain silent for several years or become active in the presence of various cellular stimuli.

CONCLUSION

The most recent studies show that SRLVs have a very wide tropism since they are capable of infecting the cells of several species of small wild ruminants.

The objective of this work was to study the genetic properties of SRLV following the interspecies passage in ibex to determine the genetic modifications that these viruses have mainly accumulated in the LTR region in order to be able to adapt in these new species. The results obtained reveal an accumulation of numerous molecular alterations in the LTR of isolated SRLVs. This allows a clear answer to the initial question posed, that there are many genetic modifications in the LTR region which accompany the infection and adaptation of viruses in the wild ibex.

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REFERENCES

Blatti-Cardinaux, L., Sanjosé, L., Zahno, M.-L., Zanoni, R., Reina, R., & Bertoni, G. (2016). Detailed analysis of the promoter activity of an attenuated lentivirus. *Journal of general virology, 97*(7), 1699-1708.

Corbet, S., Müller-Trutwin, M. C., Versmisse, P., Delarue, S., Ayouba, A., Lewis, J., Brunak, S., Martin, P., Brun-Vezinet, F., & Simon, F. (2000). env sequences of simian immunodeficiency viruses from chimpanzees in Cameroon are strongly related to those of human immunodeficiency virus group N from the same geographic area. *Journal of virology, 74*(1), 529-534.

Erhouma, E., Guiguen, F., Cheblounе, Y., Gauthier, D., Lakhal, L. M., Greenland, T., Mornex, J. F., Leroux, C., & Alogninouwa, T. (2008). Small ruminant lentivirus proviral sequences from wild ibexes in contact with domestic goats. *Journal of general virology, 89*(6), 1478-1484.

Felsenstein, J. (2002). PHYLIP: Phylogeny Inference Package, ver. 3.6 a3. *http://evolution. gs. washington. edu/phylip. html*.

Gayo, E., Cuteri, V., Polledo, L., Rossi, G., García Marín, J. F., & Preziuso, S. (2018). Genetic Characterization and Phylogenetic Analysis of Small Ruminant Lentiviruses Detected in

ISSN: online 2617-2186 print 2617-2178

Spanish Assaf Sheep with Different Mammary Lesions. *Viruses, 10*(6), 315.

Hirsch, V., Olmsted, R., & Murphy-Corb, M. (1989). Pyrcell and Johnson P. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature, 339*, 389-392.

Jaime, M. F. V., Redko, F., Muschietti, L. V., Campos, R. H., Martino, V. S., & Cavallaro, L. V. (2013). In vitro antiviral activity of plant extracts from Asteraceae medicinal plants. *Virology journal, 10*(1), 245.

Mendiola, W. P., Tórtora, J. L., Martínez, H. A., García, M. M., Cuevas-Romero, S., Cerriteño, J. L., & Ramírez, H. (2019). Genotyping based on the LTR region of small ruminant lentiviruses from naturally infected sheep and goats from Mexico. *BioMed Research International, 2019*.

Perriere, G., & Gouy, M. (1996). WWW-query: an on-line retrieval system for biological sequence banks. *Biochimie, 78*(5), 364-369.

Rihn, S. J., Foster, T. L., Busnadiego, I., Aziz, M. A., Hughes, J., Neil, S. J., & Wilson, S. J. (2017). The envelope gene of transmitted HIV-1 resists a late interferon gamma-induced block. *Journal of virology, 91*(7).

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*(4), 406-425.

Saltarelli, M., Querat, G., Konings, D. A., Vigne, R., & Clements, J. E. (1990). Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. *Virology, 179*(1), 347-364.

Thompson, J. (1997). Gibson TJ Plewniak F., Jeanmougin F. and Higgins DG (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*, 4876-4882.

Yang, F.-C., Kuang, W.-D., Li, C., Sun, W.-W., Qu, D., & Wang, J.-H. (2015). Toll-Interacting Protein Suppresses HIV-1 Long-Terminal-Repeat-Driven Gene Expression and Silences the Post-Integrational Transcription of Viral Proviral DNA. *PloS one, 10*(4), e0125563.

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الطفرات الوراثية في الموضع الجيني LTR لفيروسات SRLV في الوعول البرية

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المستخلص: للفيروسات البطيئة Lentivirs (التابعة لعائلة الفيروسات القهقرية Retrovirus) القدرة على إدخال، ودمج الحمض النووي الفيروسي DNA داخل الحمض النووي للخلية العائل والتأثير على الخلايا المنقسمة. كما لهذه الفيروسات القدرة على إحداث الإصابة، ونقل الأمراض الجديدة من عوائلها الطبيعية إلى عوائل أخرى جديدة، وذلك ما يطلق عليه عملية "الانتقال بين الأنواع". وتعتبر هذه العملية الأساس لانتقال الفيروسات، ونشر الأمراض الناتجة عنها. لوحظ انتشار الفيروسات البطيئة من جنس الـ SRLV بين العديد من الأجناس الحيوانية (الأليفة والبرية)، والذي غالبا ما يكون مصحوبا بطفرات وراثية في المادة الوراثية للفيروس (الجينوم)، في هذه الورقة قمنا بدراسة الطفرات الوراثية المصاحبة لإصابة وتأقلم فيروسات الـ SRLV في عوائلها الجديدة. تمت دراسة هذه الطفرات عن طريق مضاعفة الحمض النووي لموضع LTR، وتحليل السلاسل الوراثية المتحصل عليها من هذه العملية. ثم أخذ عينات الدم من الوعول البرية والتي تعيش في جبال الألب الفرنسية، وتحليلها مصليا، بالبحث عن الأجسام المضادة باستخدام تقنية ال ELISA. بعدها تمت زراعة الخلايا الدموية المحيطية (PBMC) المعزولة على الوسط Ficoll، للحصول على الخلايا الليمفاوية البعلمية أحادية النواة (MDM) بغية عزل الفيروس. اختبر الحمض النووي DNA للفيروس المعزول، وتم تحليله باستخدام تقنية الـ PCR باستخدام بادئات من الموضع الجيني المستهدف، للحصول على نسخة مضاعفة من الحمض النووي الفيروسي. خضع الحمض النووي المتحصل عليه (270 قاعدة نيتروجينية) لعملية استنساخ، ومضاعفة (باستخدام تقنية البلازميد) للحصول على السلاسل الوراثية النقية، والتي تم تحليلها فيما بعد باستخدام برنامج ClustalW. بينت دراسة السلاسل الوراثية ثلاثة أنواع من الطفرات الوراثية: استبدال، إضافة وحذف. كما وجد أن موضع box TATA، وموضع عديد الادنين Poly (A) لم تحدث بهما أي طفرات وراثية. معدل الاختلاف بين السلاسل المتحصل عليها كان في ما بين 0.3–5.7%. هذا الاختلاف يمكن ملاحظته على الشجرة الوراثية، حيث بينت أن السلاسل الوراثية للفيروس المعزول من الوعول البرية تكون مجموعة مستقلة، وبعيدة عن الفيروس المرجعي.

الكلمات المفتاحية: الفيروسات البطيئة، SRLV، LTR، صندوق TATA، تسلسل.

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Impact of Salinity Stress on Germination and Growth of Pea *(Pisum sativum* L) Plants

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Abstract: The aim of the present study was to evaluate the effects of salinity stress on germination and growth of pea (*Pisum sativum* L) plants. A laboratory experiment was conducted to evaluate the effect of salinity stress on germination and growth of pea *Pisum sativum* L plant. Seeds of pea were sown in Petri dishes and pots and treated with four different levels of salinity (0, 50, 100, and 150mM NaCl) with completely randomized designs in four replications. Results revealed that seeds of pea were able to germinate at low salinity levels (NaCl 50mM NaCl) without a significant decrease in germination and growth traits, at the same time as a severe decrease in those traits were recorded at higher levels of salinity (100 and 150mM NaCl). The results indicated that seed germination and seedling establishment were inhibited due to the decrease of water potential, which results in the decline in water uptake by seeds, and seed germination was prevented by a high level of salinity stress (150mM NaCl). The results pointed out that germination percentage (GP), mean daily germination (MDG), germination speed (GS), and vigor index (SVI) varied under moderate and high salinity levels. All the studied parameters were reduced with increasing the NaCl level. The max and min GP, MDG, GS, and SVI were observed under control conditions (0mM NaCl) and highest salinity level (150mM NaCl) respectively. The same trend was seen in plant growth traits including: plant height, branch number, leaf number, leaf area, and shoot fresh and dry weight. The results provided important reference information for research on the impact of salinity on germination and growth of pea.

Keywords: Pea (*Pisum sativum* L.); Salinity Stress; Germination; Growth; Seedling Vigor Index.

INTRODUCTION

Salt injure is one of the most important abiotic stress that affects plant productivity worldwide. About 20% of the global land area and over 50% of agricultural irrigated land is salt-affected soils (Cheng et al., 2016). It is also estimated that about 50% of agricultural land will be affected by salt in 2050 (Mahajan and Tuteja, 2005; Yan et al., 2005). Soils become saline when the soil salt concentration reaches about 40mM NaCl (Munns and Tester, 2008). The majority of crops are highly susceptible to saline soil. Crops are typically sown within the top 10-15cm layer of top soils. These layers are more saline than lower layers (Esechie, 1995). Therefore, seeds show irregular germination and poor seedling development. In most crop plants, the yields start decreasing even at fairly low salinity in soil with electrical conductivity of (ECse> 1 dS/m) (Chinnusamy et al. 2005). Saline soils affect the growth of crop plants in two different ways (I) raising the osmotic pressure of soil solution, which results in an additional decrease in the physiological availability of water to the plant and, (II) the accumulation of toxic quantities of various ions within the plant (Hayward and Wadleigh, 1949). Salinity decreases seeds' ability to absorb water and causes a decrease in germination and plant growth, which leads to

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changes in plant metabolic processes (Munns, 1993; Munns, 2002). Seed germination and seedling growth are the most sensitive stages to salt stress (Bhattacharjee, 2008; Hubbard et al., 2012). Salt stress affects seed germination through osmotic stress, ion-specific effects, and oxidative stress (Hayward and Wadleigh, 1949). Salt stress influences the seed germination and plant growth of many plants. Many studies found that seed germination and early establishment of seedlings were inhibited by increasing salinity stress in different crops such as - wheat (Sadak, 2016; Mujeeb-Kazi et al., 2019), cabbage (Sarker et al., 2014; Yan, 2015), maize (Konuşkan et al., 2017), and cowpeas (Abdel-Haleem and El-Shaieny, 2015),

Pea *(Pisum sativum* L.) plant is an important winter season vegetable legumes grown in Libya and the Mediterranean region and used as a source of protein vitamins, minerals, salts, and antioxidants (Nuttonson, 1961; Noreen and Ashraf, 2009). It's economically grown and used for dry grain or fresh fruit (Kaya et al., 2002). Also, the pea plant is grown as a forage crop for farm animals, as a green compost crop for improving soil, and as a cover crop for reducing soil erosion (Wolde and Adamu, 2018).

Pea plants can be grown on a wide range of soil types, from light sandy to heavy clay (McKay et al., 2003). Pea is a legume and has the inherent ability to obtain its nitrogen requirement from the atmosphere by forming a symbiotic relationship with Rhizobium bacteria in the soil (Schatz and Endres, 1999). The pea plant has quite a short growing season and uses less water than many other broadleaf crops (Johnson et al., 2002). However, the plant has been accounted as a salt-sensitive plant as compared with other legumes, such as broad bean, common bean, and soybean (Zahran, 1999; Khan et al, 2015). Nevertheless, the effect of salinity on growth and yield of peas has been investigated in many studies (Hernhdeza et al., 1995; Martí, 2011; Pandolfi, 2012; Shahid, 2012; Husen et al., 2016; Wang et al., 2016; Desoky et al, 2017), however the impact of salinity on germination and seedling growth has not been well investigated and the relative importance of the effects of salt stress on seed germination of peas is not clear. Therefore, the aim of the present study was to evaluate the effect of salinity on germination and growth of pea plants.

MATERIAL AND METHODS

This study was carried out at the Department of Plant Science, University of Zawia. Pea seeds were obtained from the local market. Salinity concentrations were (0, 50, 100, and 150mM NaCl) prepared using NaCl and fresh water. The electrical conductivities of NaCl solutions were 4.4, 8.3, and 15.3 dS m^{-1}, and fresh water served as a control. For this research, a pair of experiments were conducted. A laboratory experiment to evaluate the effect of salinity stress on seed germination of peas, and a pot experiment to evaluate the influence of salinity stress on seedling growth of pea plants.

Experiment I: Pea seeds were first sterilized for 5 min with 5 % commercial bleach (Pandolfi e al., 2012), then thoroughly washed with distilled water. Germination tests were conducted in Petri dishes (containing one Whatman filter papers with 20 ml of respective test solutions) with four treatments by four replications (10 seeds per replication). The Whatman filter papers were replaced every 2 days to prevent the accumulation of salts. Seeds were allowed to germinate at room temperature and in darkness for 10 days. During this period, the Petri dishes were monitored daily, and 5 ml of the appropriate solution was added to the Petri dishes. A seed was considered to have germinated when the emerging radicle was 10 mm long (Cokkizgin, 2013).

Experiment II: A pot experiment was conducted to investigate the effect of salinity on the growth of pea plants. Pea seeds were

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grown in pots filled with loamy soil, which was collected from the soil surface (0-10 cm). The soil was air-dried and passed through a 5-mm mesh screen, and filled in small plastic pots without a leaching possibility. Four pea seeds were sown in each pot and irrigated with fresh water for 10 days. After seedling establishment, only two seedlings of each pot were kept. Pots were then divided into four groups with four replications. Each group represented one saline treatment, which includes 0, 50, 100, and 150 mM NaCl. Pots were kept under semi-controlled conditions and irrigated with an appropriate saline solution for 30 days. At the end of the experiment, one plant from each pot was collected and used for data collection.

Data Collection:

Germination traits: Germinated seeds were counted daily for 10 days, and the number of germinated seeds was recorded every 24 h for each replicate of the treatment. After 10 days the germination percentage (GP) was calculated using the formula below (Nasri et al., 2011).

$$GP\ \% = (NSG \div TNSS) \times 100$$

Where *NSG* is the number of seeds germinated. *TNSS* is the total number of seeds sown.

The germination speed (GS) was calculated according to the equation given by Rubio-Casal *et al*. (2003). The number of germinated seeds was recorded every day from sowing, and lasted for 10 days, and was used to calculate GS. The following formula was used to calculate GS:

$$GS = n1/d1 + n2/d2 + n3/d3 + \cdots$$

Where n_1 is the number of seeds germinated in day one of sowing, *d1* is the number of days taken for germination from the day of sowing.

Mean daily germination (MDG) was calculated as per Gairola *et al* (2011). The following formula was used to calculate MDG:

$$MDG = TNGS \div TNDG$$

Where *TNGS* is the total number of germinated seeds and *TNDG* is the total number of days taken for final germination.

Growth traits: Morphological traits include plant height, number of branches per plant, number of leaves per plant, leaf area, and aboveground fresh biomass weight per plant, and their dry weight. Aboveground fresh and dry biomass weight were subsequently measured from 4 uniform seedlings per each treatment at the seedling stage. The plant height was measured using a measuring ruler from the surface of the soil to the top of the last leaf blade. The leaf area was calculated by measuring each leaf. Each leaf has two leaflets (left, and right), and the traits length (L) and maximum width (W) of leaflets were measured using a measuring ruler. The product of the length times width (LW) of the leaflet was calculated. The fresh weight of aboveground biomass was recorded using a weighing balance and then dried in an oven at 50 °C till a stable weight had been attained. Subsequently, the aboveground dry weights were recorded using a weighing balance. Using the morphological traits, the salinity tolerance index (STI) and seedling vigor index (SVI) were calculated as follows.

Salinity tolerance index (STI) was calculated according to the equation given by Tregay et al. (2014). STI = Seedling dry weight of NaCl treated/seedling dry weight in control x 100.

Seedling vigor index (SVI) was calculated according to the equation given by Abdoli1 et al. (2013) SVI = Seedling length (cm) x germination percentage / 100.

Statistical analysis:

The experimental design was completely randomized (CRD) with four replications. Analysis of variance was performed using the generalized linear model (GLM) procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for seed germination and growth related traits. Separation of means was carried out using the least significant differences (LSD; $P < 0.05$). The means were compared using Duncan's multiple range test.

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RESULTS

The Probability values for germination and plant growth traits obtained with SAS PROC GLM are presented in Table 1. The obtained results clearly illustrated that salinity stress induced a significant reduction in germination and growth parameters in pea plants. Significant reduction (P<0.05) was observed in almost all the studied germination and plant growth traits when pea plants were grown under salinity stress. Germination percent, mean daily germination, germination speed, plant height, number of branches, number of leaves, leaf area, fresh and dry branches weight, salinity tolerance index and vigor index were the traits which showed significant differences as shown in Table 2.

Table (1): Probability values of the effects of Salinity (S) on germination and plant growth traits of pea plants.

Traits	Salinity (S)
Germination percent (%)	0.0458
Mean daily germination (MDG)	0.0453
Germination speed	0.0425
Plant height (cm)	0.0495
number **of branches**	0.0357
number **of leaves**	0.0416
Leaf area (cm^2)	0.0477
Shoot fresh weight (g)	0.0504
Shoot dry weight (g)	0.0370
Salinity tolerance index	0.0355
Vigor index	0.0348

Germination traits: Although, the germination percentage and speed of germination were not significantly affected at a low salinity level (50mM NaCl.), however, germination traits including germination percentage, speed of germination, and mean daily germination, decreased gradually with increasing salinity stress levels. Under a salinity level of 150mM NaCl, germination percentage, speed of germination, and mean daily germination decreased by 37 %, 45 %, and 43 % respectively (Fig 1a, b, and c).

Table (2) The effect of Salinity (S) on germination and plant growth traits of pea plants..

Traits	Salinity Level mM NaCl			
	0	50	100	150
Germination percent (%)	95^{a}	90^{a}	83^{ab}	60^{b}
Mean daily germination	1.5^{a}	1.3^{ab}	1^{ab}	0.8^{b}
Germination speed	1.9^{a}	1.6^{a}	1.4^{ab}	1^{b}
Plant height (cm)	14.9^{a}	13^{a}	8.6^{ab}	5.6^{b}
number **of branches**	4.5^{a}	3.5^{ab}	2^{b}	1.5^{b}
number **of leaves**	10.5^{a}	8.5^{ab}	5.5^{bc}	3.5^{c}
Leaf area (cm^2)	23^{a}	19^{ab}	12^{ab}	8^{b}
Shoot fresh weight (g)	4.8^{a}	4^{ab}	2.8^{ab}	2^{b}
Shoot dry weight (g)	2.6^{a}	1.9^{ab}	1.3^{ab}	0.7^{b}
Salt tolerance index	100^{a}	84^{a}	54^{ab}	27^{b}
Vigor index	14^{a}	12^{a}	7^{ab}	4^{b}

* Individual value is the mean of 4 plants under different salinity levels. Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

Growth traits: Growth data presented in Tables 1 and 2 show that plant growth traits were significantly affected (P<0.05) by salinity stress. The data pointed out that under increased salinity levels, the plant height and number of branches per plant was significantly reduced. At a high level of salinity (150mM NaCl), both traits (plant height and branch number per plant) were reduced by 62% and 67% respectively (Fig 2a and b).

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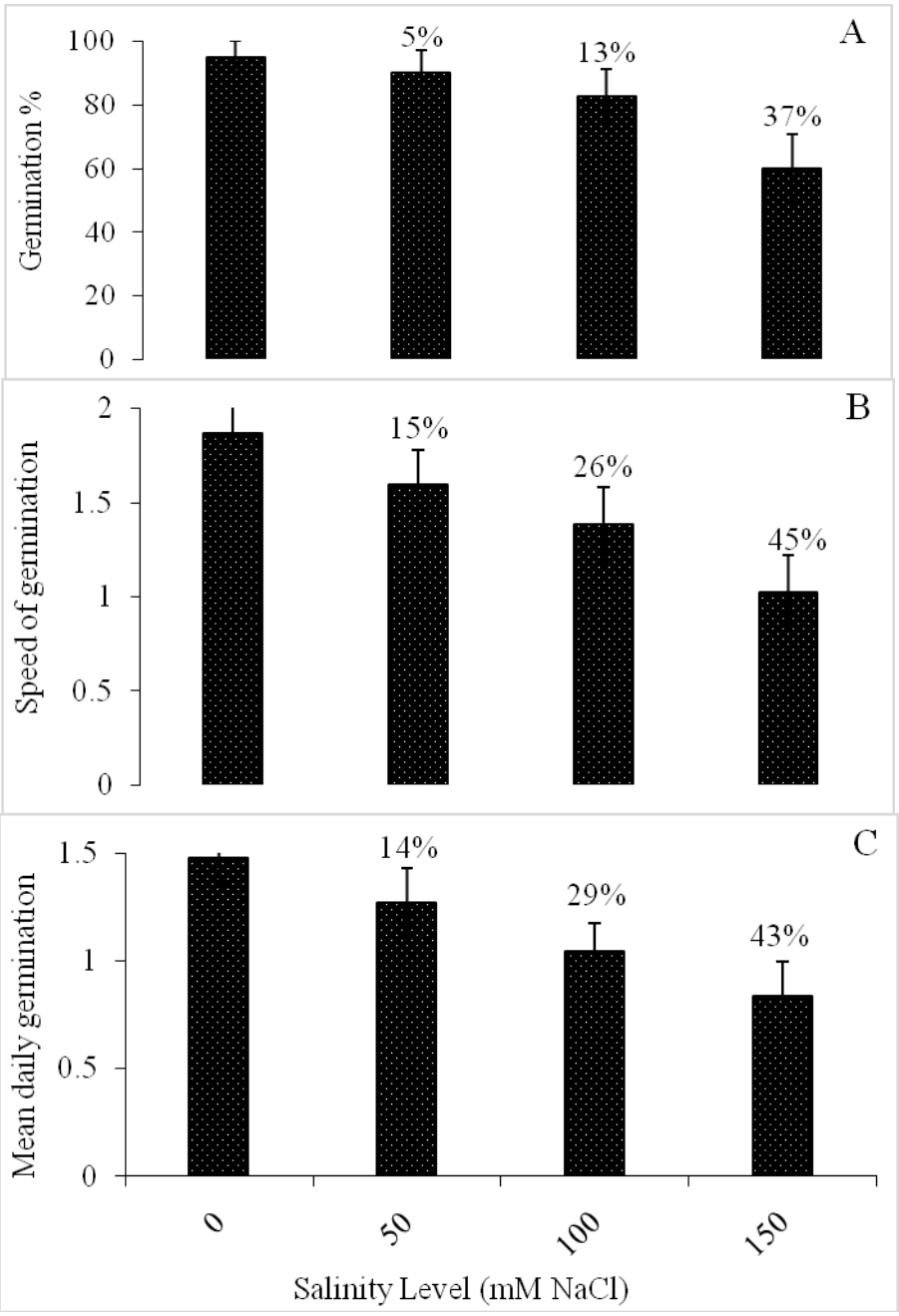

Figure (1) The effects of salinity treatments on (A) germination percentage, (B) speed of germination and (C) mean daily germination of pea plant. Each datum indicates mean value, and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

Raising the salinity level from 50 to 150mM NaCl gradually decreased the vigor index. The highest vigor index was observed in control, while salinity at 50, 100, and 150mM NaCl decreased the vigor index. A significant decrease in the vigor index was observed at 150 mM NaCl salinity, which caused a 71 % reduction over the control (Fig 2c).

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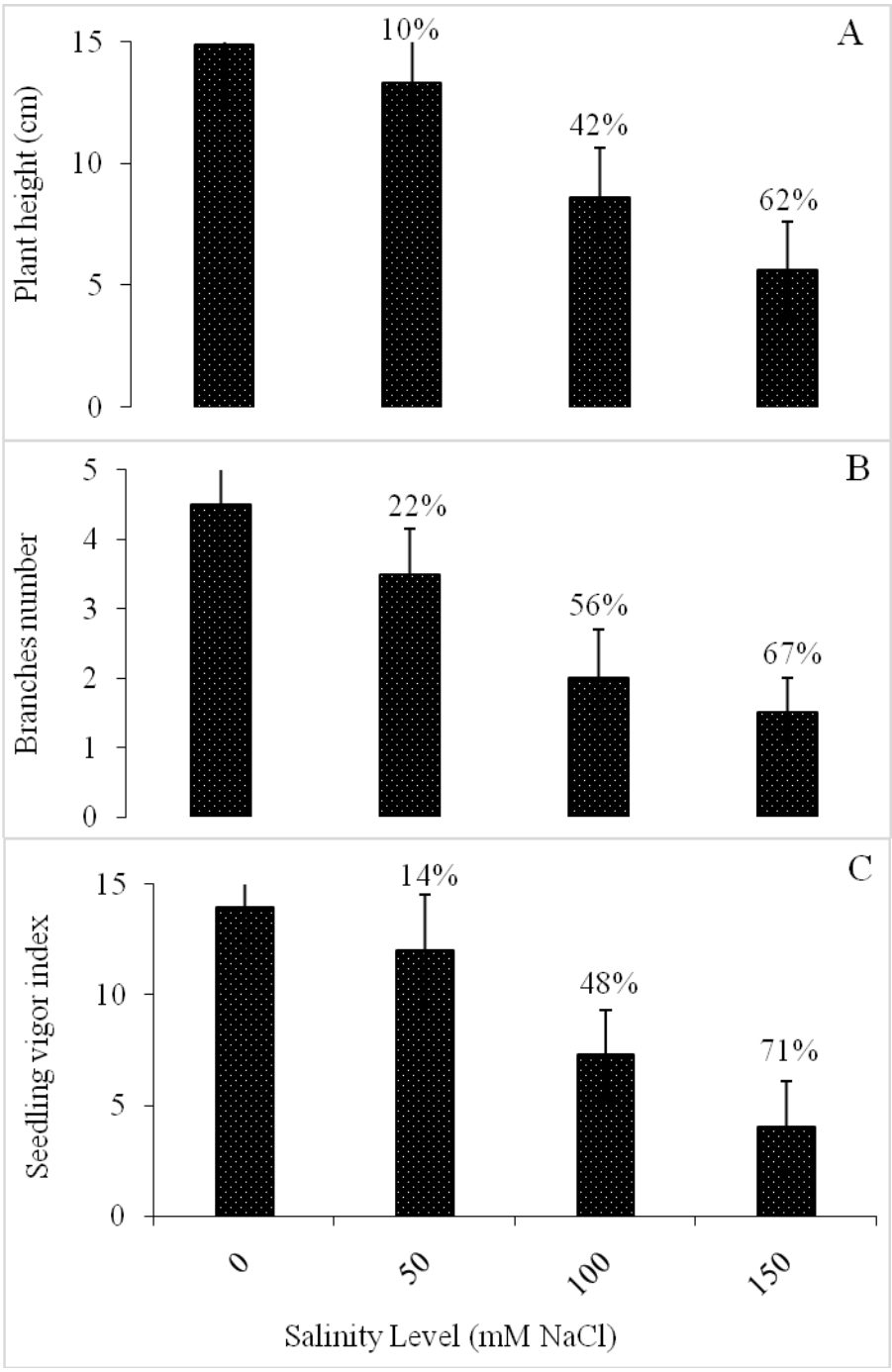

Figure (2): The effects of salinity treatments on (A) plant height (cm), (B) branch number and (C) seedling vigor index of pea plant. Each datum indicates mean value, and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

The same reduction tendency was seen in leaf number per plant and leaf area per plant. As shown in Figure 3a and b, both traits, leaf number per plant and leaf area per plant, were strongly influenced by the high salinity level. The percent reduction over the control of leaf number per plant and leaf area per plant were 65 % and 67 % respectively (Fig 3a and b).

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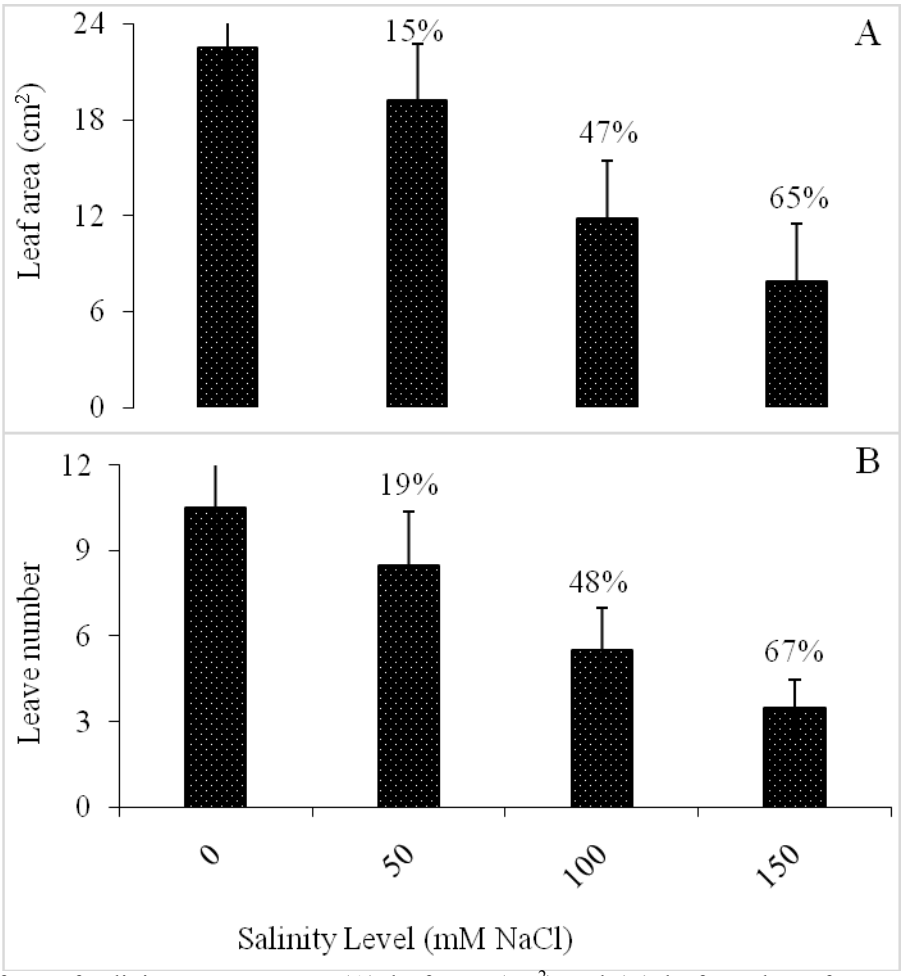

Figure 3 The effects of salinity treatments on (A) leaf area (cm^2) and (B) leaf number of pea plant. Each datum indicates mean value, and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

In addition, the result reported that salinity stress significantly decreased aboveground biomass in pea plants in terms of shoot fresh and dry weight per plant. Nevertheless, the shoot dry weight was more strongly affected than shoot fresh weight. At a high salinity level (150mM NaCl), the aboveground fresh weight decreased by 58 % compared with control (0mM NaCl), and likewise, aboveground dry weight decreased by 74 % compared with control (Fig 4a, and b). Results regarding the salt tolerance index (STI) of pea plants showed that pea plants were able to deal with a low salinity level (50mM NaCl), however as the salinity level increased, pea plants became more sensitive to salinity stress. As shown in the result. At 150mM NaCl, the salt tolerance index decreased by 73 % as compared with control (Fig 4c.).

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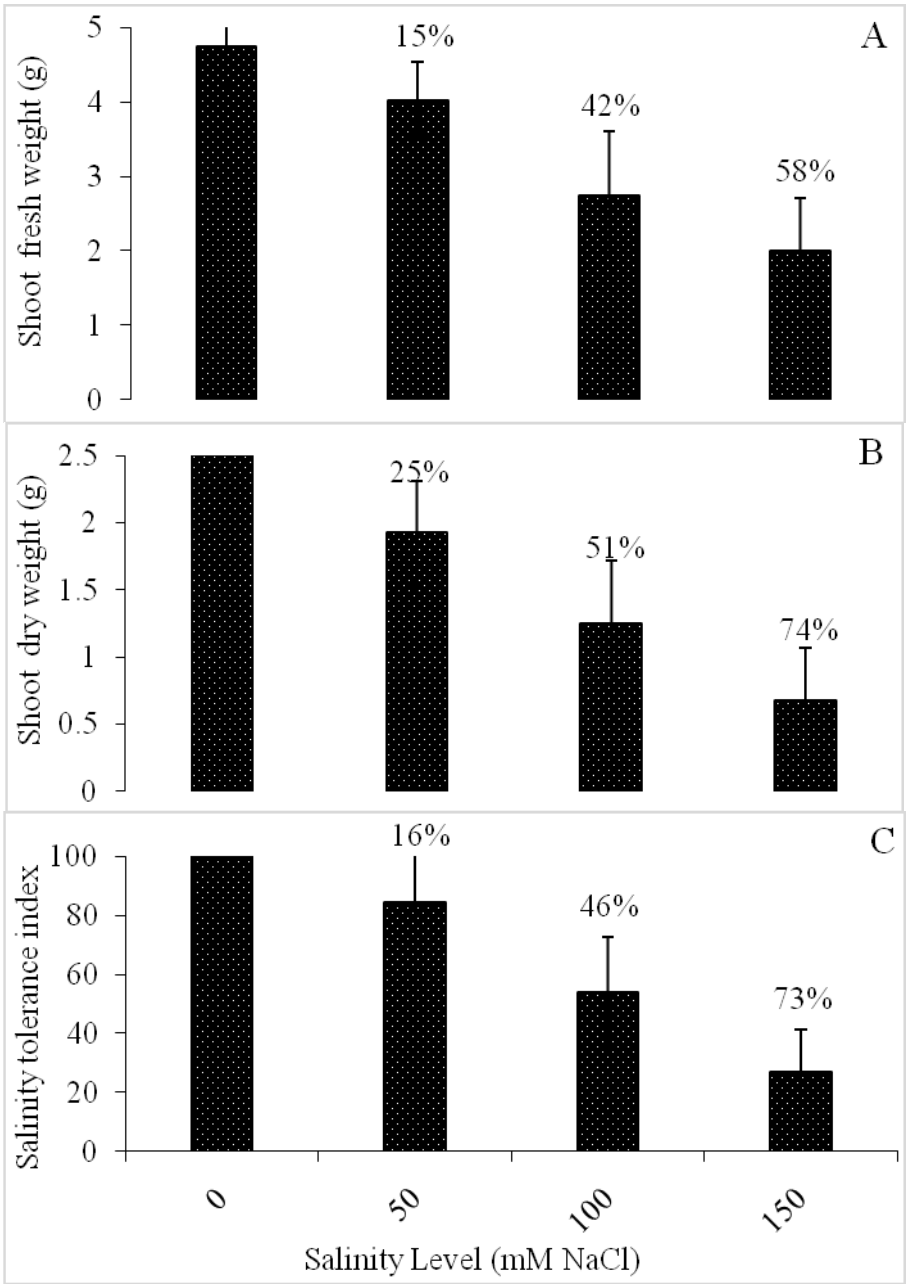

Figure: (4). The effects of salinity treatments on (A) shoot fresh weight (g), (B shoot dry weight (g) and (C) salinity tolerance index of pea plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control

DISCUSSION

In the present study, the adverse effect of salinity decreased germination and growth of pea plants as shown in Table 2. Germination and seedling stages are critical life stages for plant survival and appropriate seedling establishment, particularly under stress conditions. The findings of this study indicated that seeds germination and establishment of pea seedlings were inhibited gradually by increasing salinity stress. At a high salinity level of 150mM NaCl, seed germination was completely inhibited. In this respect, many studies reported that increasing salinity level decreased germination percentage and germination speed in field pea (Wolde and Adamu, 2018)), chick-pea (Ashraf and

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Waheed, 1992) wheat (Majid et al., 2013) and other legumes (Esechie, 1995; Morais et al., 2012; Piwowarczyk et al, 2016). Salinity delayed and prevented seed germination through various factors, for instance, a decrease in water uptake, changes in the mobilization of stored food, and disturbing the structural organization of proteins (Ibrahim, 2016). In addition, the present study showed that plant height, branch number, leaf number and area, fresh and dry biomass was severely reduced as salinity level increased and the death of plants was noticed at the high salinity concentration (150mM NaCl) after 4 weeks of plant establishment. These results were consistent with previously published research (Grozeva et al., 2019). From the result of this study, it is evident that the toxicity in the salinity treatments is expressed more clearly in dry weight. This finding supports early findings which indicated that growth inhibition by NaCl treatments was greater for dry biomass production (Cordovilla et al, 1999; Hussain et al, 2002; López-Aguilar et al., 2003). The reduction of growth traits may be attributed to the osmotic effect of salinity stress which causes a decrease of growth promoters (Desoky et al., 2017). Also, the growth inhibition could be due to water deficit, ion toxicity, and nutrient imbalance due to the blockage of other nutrients such as N, P, K, Ca, and NO_3 (Hasegawa et al., 2000). Other studies have shown similar result in pea plants (Grozeva et al., 2019), and other legumes plants such as pea plant (Hernandez et al., 1999), chick-pea (Ashraf and Waheed, 1992), and sesbania (Mahmood et al., 2008) when grown under salinity condition. The result showed a decreasing leaf number per plant, physiologically, salinity stress has a negative impact on many processes, however, the most significant effect is reducing cell division and expansion, which caused a reduction in leaf number. Moreover, the result herein pointed out that a high salinity level caused a reduction in leaf area, which may have resulted due to a reduction in cell division and cell extension. These results agree with another result that reported that salt stress causes a reduction in leaf surface expansion ratio, leading to the cessation of expansion as salt concentrations keep increasing (Wang and Nii, 2000).

CONCLUSIONS

Food productivity is decreasing due to the effect of various abiotic stresses. Cold, heat, salinity, and drought are among the major stresses, which adversely affect plants' growth and productivity. For that reason, reducing these losses is a main area of concern for all crop producers to manage increasing crop production. This study was aimed to investigate the impact of salinity stress on pea plants. The study showed that high salt stress inhibited and delayed seed germination and growth of pea plants. The study concluded that pea plants (*Pisum sativum* L.) are resistant to 50 and can withstand salinity at 100 mM NaCl, but this cultivar is strongly sensitive to 150mM NaCl, and damages of salt-stress were significantly observed. However, plant growth was more sensitive to salt stress than germination. Future research must concentrate on molecular, physiological, and metabolic changes induced by salinity stress. Also, comprehensive information is required to understand the physiological responses of this plant under field conditions.

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REFERENCES

Abdel-Haleem A. &El-Shaieny H. (2015). Seed germination percentage and early seedling establishment of five [*Vigna unguiculata* (L.) Walp.] genotypes under salt stress. *European J. Exp. Biol.,* 5(2): 22-32

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Ashraf, M., & Waheed, A. (1992). Screening chick-pea (Cicer arietinum L.) for salt tolerance. *Der Tropenlandwirt-Journal of Agriculture in the Tropics and Subtropics*, *93*(1), 45-55.

Bhattacharjee, S. (2008). Triadimefon pretreatment protects newly assembled membrane system and causes up-regulation of stress proteins in salinity stressed Amaranthus lividus L. during early germination. *J. Environ. Biol*, *29*(5), 805-810.

Cheng, X., Deng, G., Su, Y., Liu, J. J., Yang, Y., Du, G. H., ... & Liu, F. H. (2016). Protein mechanisms in response to NaCl-stress of salt-tolerant and salt-sensitive industrial hemp based on iTRAQ technology. *Industrial Crops and Products*, 83, 444-452.

Chinnusamy, V., Jagendorf, A., & Zhu, J. K. (2005). Understanding and improving salt tolerance in plants. *Crop science*, *45*(2), 437-448.

Cokkizgin, A. (2013). Effects of Hydro and Osmo-Priming on seed Vigor of pea (Pisum sativum L). *seed*, *10*, 100.

Cordovilla, M. D. P., Ligero, F., & Lluch, C. (1999). Effect of salinity on growth, nodulation and nitrogen assimilation in nodules of faba bean (Vicia faba L.). *Applied Soil Ecology*, *11*(1), 1-7.

Desoky, E. M., Merwad, A. M., & Elrys, A. S. (2017). Response of pea plants to natural bio-stimulants under soil salinity stress. *Am. J. Plant Physiol*, *12*, 28-37.

Esechie, H. A. (1995). Partitioning of chloride ion in the germinating seed of two forage legumes under varied salinity and temperature regimes. *Communications in soil science and plant analysis*, *26*(19-20), 3357-3370.

Gairola, K. C., Nautiyal, A. R., & Dwivedi, A. K. (2011). Effect of temperatures and germination media on seed germination of Jatropha curcas Linn. *Advances in bioresearch*, *2*(2), 66-71.

Grozeva, S., Kalapchieva, S., & Tringovska, I. (2019). Evaluation of garden pea cultivars to salt stress tolerance. *Mechanization in agriculture & Conserving of the resources*, *65*(4), 150-152.

Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annual review of plant biology*, 51(1), 463-499.

Hayward, H. E., & Wadleigh, C. H. (1949). Plant growth on saline and alkali soils. In *Advances in Agronomy* (Vol. 1, pp. 1-38). Academic Press.

Hernhdeza, J. A., Olmosa, E., Corpasb, F. J., Sevilla, F., & de1 Riob, L. A. (1995). Salt-induced oxidative stress in chloroplasts of pea plants. *Plant Science*, *105*(151167), 04047-8.

Hernandez, J. A., Campillo, A., Jimenez, A., Alarcon, J. J., & Sevilla, F. (1999). Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytologist,* 141(2), 241-251.

Hubbard, M., Germida, J., & Vujanovic, V. (2012). Fungal endophytes improve wheat seed germination under heat and drought stress. *Botany*, *90*(2), 137-149.

Husen, A., Iqbal, M., & Aref, I. M. (2016). IAA-induced alteration in growth and photosynthesis of pea (L.) plants grown under salt stress Pisum sativum. *Journal of Environmental Biology*, *37*, 421-429.

Hussain, N., Mujeeb, F., Tahir, M., Khan, G. D., Hassan, N. M., & Bari, A. (2002). Effectiveness of Rhizobium under salinity stress. *Asian J Plant Sci*, *1*(1), 12-14.

ISSN: online 2617-2186 print 2617-2178

Ibrahim, E. A. (2016). Seed priming to alleviate salinity stress in germinating seeds. *Journal of Plant Physiology*, 192, 38-46.

Johnston, A. M., Tanaka, D. L., Miller, P. R., Brandt, S. A., Nielsen, D. C., Lafond, G. P., & Riveland, N. R. (2002). Oilseed crops for semiarid cropping systems in the northern Great Plains. *Agronomy Journal*, *94*(2), 231-240.

Kaya, M. D., Ç İ Ftci, C. Y., & Kaya, M. (2002). Bakteri Aşılaması ve Azot Dozlarının Bezelye (*Pisum sativum L.*)'de Verim ve Verim Öğelerine Etkileri.A.O. Ziraat. Fakültesi Tarım Bilimleri Dergisi 8(4): 300-305.

Khan, H. A., Siddique, K. H., Munir, R., & Colmer, T. D. (2015). Salt sensitivity in chickpea: growth, photosynthesis, seed yield components and tissue ion regulation in contrasting genotypes. *Journal of plant physiology*, *182*, 1-12.

Konuşkan, Ö., Gözübenli, H., Atiş, İ., & Atak, M. (2017). Effects of salinity stress on emergence and seedling growth parameters of some maize genotypes (Zea mays L.). *Turkish Journal of Agriculture-Food Science and Technology*, *5*(12), 1668-1672.

López-Aguilar, R., Orduño-Cruz, A., Lucero-Arce, A., Murillo-Amador, B., & Troyo-Diéguez, E. (2003). Response to salinity of three grain legumes for potential cultivation in arid areas. *Soil science and plant nutrition*, *49*(3), 329-336.

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. *Archives of biochemistry and biophysics*, 444(2), 139-158.

Mahmood, A., Athar, M., Qadri, R., & Mahmood, N. (2008). Effect of NaCl salinity on growth, nodulation and total nitrogen content in Sesbania sesban. *Agriculturae Conspectus Scientificus*, *73*(3), 137-141.

Majid, A., Mohsen, S., Mandana, A., Saeid, J. H., Ezatollah, E., & Fariborz, S. (2013). The effects of different levels of salinity and indole-3-acetic acid (IAA) on early growth and germination of wheat seedling. *Journal of Stress Physiology & Biochemistry*, *9*(4).

Martí, M. C., Florez-Sarasa, I., Camejo, D., Ribas-Carbó, M., Lázaro, J. J., Sevilla, F., & Jiménez, A. (2011). Response of mitochondrial thioredoxin PsTrx o 1, antioxidant enzymes, and respiration to salinity in pea (Pisum sativum L.) leaves. *Journal of Experimental Botany*, *62*(11), 3863-3874.

McKay, K., Schatz, B. G., & Endres, G. (2003). *Field pea production*. NDSU Extension Service.

Morais, M. C., Panuccio, M. R., Muscolo, A., & Freitas, H. (2012). Does salt stress increase the ability of the exotic legume Acacia longifolia to compete with native legumes in sand dune ecosystems?. *Environmental and Experimental Botany*, *82*, 74-79.

Mujeeb-Kazi, A., Munns, R., Rasheed, A., Ogbonnaya, F. C., Ali, N., Hollington, P., ... & Saddiq, M. S. (2019). Breeding strategies for structuring salinity tolerance in wheat. In *Advances in Agronomy* (Vol. 155, pp. 121-187). Academic Press.

Munns, R. (1993). Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell & Environment*, *16*(1), 15-24.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, cell & environment*, *25*(2), 239-250.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59, 651-681.

ISSN: online 2617-2186 print 2617-2178

Nasri, N., Kaddour, R., Rabhi, M., Plassard, C., & Lachaal, M. (2011). Effect of salinity on germination, phytase activity and phytate content in lettuce seedling. *Acta physiologiae plantarum*, *33*(3), 935-942..

Noreen, Z., & Ashraf, M. (2009). Assessment of variation in antioxidative defense system in salt-treated pea (Pisum sativum) cultivars and its putative use as salinity tolerance markers. *Journal of plant physiology*, *166*(16), 1764-1774.

Nuttonson, M. Y. (1961). *The Physical Environment and Agriculture of Libya and Egypt with Special Reference to Their Regions Containing Areas Climatically and Latitudinally Analogous to Israel: A Study Based on Official Records, Material, and Reports of Various Agencies of the United Nations and Several National Administrations*. American Institute of Crop Ecology.

Pandolfi, C., Mancuso, S., & Shabala, S. (2012). Physiology of acclimation to salinity stress in pea (Pisum sativum). *Environmental and Experimental Botany*, *84*, 44-51.

Piwowarczyk, B., Tokarz, K., & Kamińska, I. (2016). Responses of grass pea seedlings to salinity stress in in vitro culture conditions. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *124*(2), 227-240.

Rubio-Casal, A. E., Castillo, J. M., Luque, C. J., & Figueroa, M. E. (2003). Influence of salinity on germination and seeds viability of two primary colonizers of Mediterranean salt pans. *Journal of Arid Environments*, *53*(2), 145-154.

Sadak, M. S. (2016). Mitigation of salinity adverse effects on wheat by grain priming with melatonin. *International Journal of ChemTech Research*, *9*(2), 85-97.

Sarker, A., Hossain, M. I., & Kashem, M. A. (2014). Salinity (NaCl) tolerance of four vegetable crops during germination and early seedling growth. *Int. J. Latest Res. Sci. Technol*, *3*(1), 91-95.

Schatz, B., & Endres, G. (1999). Field pea production.

Wang, Y., & Nii, N. (2000). Changes in chlorophyll, ribulose bisphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. *The Journal of Horticultural Science and Biotechnology*, *75*(6), 623-627.

Wang, Q., Dodd, I. C., Belimov, A. A., & Jiang, F. (2016). Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase growth and photosynthesis of pea plants under salt stress by limiting Na+ accumulation. *Functional Plant Biology*, *43*(2), 161-172.

Wolde, G., & Adamu, C. (2018). Impact of salinity on seed germination and biomass yields of field pea (Pisum sativum L.). *Asian J. Sci. Tech*, *9*, 7565-7569.

Yan, S., Tang, Z., Su, W., & Sun, W. (2005). Proteomic analysis of salt stress-responsive proteins in rice root. *Proteomics*, 5(1), 235-244.

Yan, M. (2015). Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. *South African Journal of Botany*, 99, 88-92.

Zahran, H. H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol. Mol. Biol. Rev.*, *63*(4), 968-989.

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تأثير إجهاد الملوحة على إنبات ونمو نباتات البازلاء *Pisum sativum* L

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المستخلص: الهدف من الدراسة هو تقييم تاثيرات إجهاد الملوحة علي انبات ونمو نبات البازلاء. التجارب المعملية اجريت على نبات البازلاء .*Pisum sativum* L حيث زرعت بذور البازلاء في أطباق بيتري وفي أصص وعولجت بأربع مستويات مختلفة من الملوحة (0 ، 50 ، 100 ، و 150 ملي مولار من كلوريد الصوديوم). نفذت التجارب وفق التصميم العشوائي الكامل بأربعة مكررات. أظهرت النتائج أن بذور البازلاء كانت قادرة على الإنبات عند مستويات ملوحة منخفضة (50 ملي مولار من كلوريد الصوديوم) دون انخفاض ملحوظ في الإنبات، وبعض صفات النمو، وفي الوقت نفسه أشارت النتائج إلى وجود انخفاض حاد في هذه الصفات عند مستويات ملوحة أعلى (100 و 150 ملي مولار من كلوريد الصوديوم). كما أوضحت النتائج أن ارتفاع مستوي الملوحة قد نتج عنه تثبيط لعملية إنبات البذور وتطور البادرات وذلك بسبب انخفاض الجهد المائي مما أدى إلى انخفاض امتصاص الماء بواسطة البذور. وتم منع إنبات البذور بسبب مستوى عالٍ من إجهاد الملوحة (150 ملي مولار من كلوريد الصوديوم). وتشير النتائج إلى وجود اختلاف في كل من نسبة الإنبات، ومتوسط الإنبات اليومي، وسرعة الإنبات، ومؤشر قوة البادرة تحت مستويات الملوحة المتوسطة والعالية. كما تشير النتائج إلى انخفاض جميع الصفات المدروسة بزيادة تركيز محلول كلوريد الصوديوم. حيث سجلت النتائج أن الحد الأقصى لكل من نسبة الإنبات، ومتوسط الإنبات اليومي، وسرعة الإنبات، ومؤشر قوة البادرة كانت تحت ظروف (0 ملي مولار من كلوريد الصوديوم) والحد الادنى الصفات نفسها كان عند أعلى مستوى للملوحة (150 ملي مولار من كلوريد الصوديوم). وقد لوحظ التأثير نفسه مع صفات النمو الأخري والتي تشمل: طول النبات ،وعدد التفرعات، وعدد الأوراق، مساحة الورقة، والوزن الطازج والوزن الجاف للمجموع الخضري. توفر نتائج هذه الدراسة معلومات مرجعية مهمة للبحث عن تأثير الملوحة على إنبات ونمو البازلاء.

الكلمات المفتاحية: البازلاء(*Pisum sativum* L.)، الإجهاد الملحي، الإنبات، النمو، مؤشر قوة البادرة.

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Experience of the Orthopedic Surgeon in the Management of War Victims

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Abstract: Surgery for victims of war is different from surgery for civilian injuries. War wounds are always extremely contaminated, and missiles may cause massive destruction of soft tissues, bones, and other structures. The principles of surgery for war wounds have been known for decades but need to be relearned by each new generation of surgeons working in a war situation. People were not at all prepared for a physical fight. Similarly, the medical institutes were not specially prepared for such a sea of casualties all of a sudden. The study's aim was to analyze the result of our experience in the surgical management of the gunshot patients admitted to *Al-Bayda* teaching hospital during the first month of the Libyan revolution of February 2011. The total number of patients admitted to Al-Bayda teaching hospital in the period from 16\2\2011 to 16\3\2011 was 288 patients. We have done debridement and repair of the wounds and fixation of fractures in different settings and follow-up was carried out for 7 months. Our orthopedic morbidity rate was 9 %, and infection was recorded in 30 patients 21%. We, as a team of orthopedic, vascular, and general surgeons, gained a very good exposure and experience to manage war or civilian unrest victims.

Keywords: Orthopedic; Gunshot; Civilian; Fractures.

INTRODUCTION

The evacuation and care of wounded people have evolved throughout the history of warfare. In the recent conflicts in Iraq and Afghanistan, an echeloned approach to trauma care has been instituted (Bagg *et al.,* 2006).

The first aid provided at the point of wounding or at the safest place near the battlefield and rapid evacuation are of vital importance, because mortality and morbidity increase with delay between wounding and treatment. The more effective the first aid and the quicker the evacuation to a hospital, the better will be the final results. If first aid is inadequate or unavailable and the evacuation chain is long, then the outcome will be more disaster (Dufour *et al.,* 1998). Even though the people of Libya were keen for liberation and freedom, the unrest was unprepared and unprecedented. People were not at all prepared for a physical fight. Similarly, the medical institutes were not specially prepared for such a sea of casualties all of a sudden. The maximum number of patients was hospitalized on 17/02/2011 approximately 60% of war injuries involve the limbs and musculoskeletal system, the orthopedic surgeons suppose a pivotal role in the frontline treatment of these injuries, Providing battlefield orthopedic care poses special challenges, because lack of experience and working under difficult conditions, many wounds are unlike those encountered in civilian practice (Covey, 2006).Our teaching hospital was not prepared and did not have enough beds, medicines, materials, necessary equipment, and instruments to meet a large number of patients at a time with various types of gunshot injuries. Our reason for conducting this study is to highlight the unexpected load on or-

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thopedic surgeons' performance during the management of civilian war victims.

MATERIALS AND METHODS

Our materials depended on the patients admitted to the Al-Bayda teaching hospital managed by what we have at that time without any preparation and lack of instrument, beds, and experience in the management of gunshot patients all of a sudden. The total number of patients admitted to Al-Bayda teaching hospital in the period from 16\2\2011 to 16\3\2011 was 288 patients, Exclusively 140 patients had musculoskeletal related gunshot injuries. Associated injuries include general surgery, fall-down, and road traffic accidents (Table.1).

Table(1). Number of patients admitted during the period of study (16\2\2011 – 16\3\2011)

Type of trauma	Number of patients
Gunshot [Musculoskeletal]	140
Gunshot [Abdomen]	16
Gunshot [abdomen and chest]	21
Gunshot [Head and neck]	19
Gunshot [scrotum]	2
Road traffic accident	2
Fall down	6
Combined [General surgery + Musculoskeletal]	82
Total	288

The patients' ages varied from 15 to 60 years. One 15-year-old patient sustained a gunshot to the left little finger, and there was only one female patient aged 30 yrs who had a gunshot injury to the left shoulder (Table.2).

Table:(2). Age of the patient with type of trauma

Age of the patient	No. of patients	Type of trauma
5 – 10	3	Road traffic accident + fall down
11 – 15	3	Fall down + gunshot
16 – 20	35	Gunshot + Fall down
21 -25	67	Gunshot
26 -30	82	Gunshot
31 -35	40	Gunshot
36 – 40	29	Gunshot
41 – 45	20	Gunshot
46 – 50	4	Gunshot
51 - 55	2	Gunshot
56 - 60	3	Gunshot
Total	288	

The majority of patients were admitted in the first week of the revolution, and the maximum number was on battle of the Shahhat battalion (the head battalion in Jabal-Al-Akhdar) on 17th of February 2011 were 56 patients hospitalized (Fig. **1**).

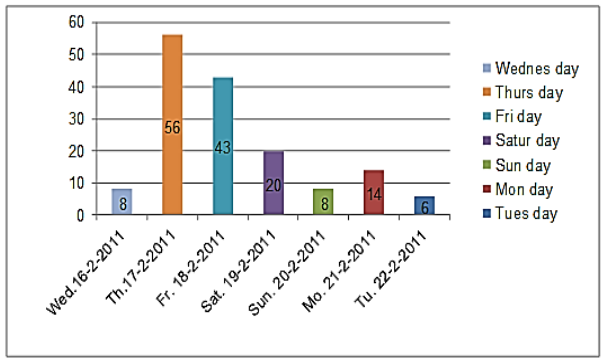

Figure: (1**).** Number of patient admitted in the first week of revolution.

We, as a team of orthopedic, vascular, and general surgeons, gained the exposure necessary to manage the different types of gunshot injuries explained in (Table.3).

Table: (3). Operations done by the general, vascular, and orthopedic surgeons.

Type of injury	Surgical operation	Number of patient
Abdominal gunshot (16) patients	Nephroctomy	(4) patients
	Nephroctomy + spleenectomy	(1) patients
	Lapratomy with Bowel injury	(11) patients
Thoracic gunshot (21) patients	Chest tub thoracotomy	(11) patients
	Chest superficial	(5) patients
		(5) patients
Vascular injury (12) patients	Vascular repair With fracture fixation	(7) patients
	Vascular repair without fracture	(5) patients
	Above knee amputation	(2) patients

The following treatment was offered to the patients by the orthopedic team; External fixation in 37 cases (27%), Ilizarov external fixation 5 cases (4%), K-wire in 5 cases (4%), screw in 2 cases (1%), dynamic compression plate (DCP) in 1 case (0.5%), proximal femoral nail (PFN)

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in 2 cases (1%), debridement and gypsum splint application in 35 cases (24%), and superficial wound management done in 53 cases (38%) (Table. 4). Following treatment and stabilization of immediate life-threatening conditions, all patients were given antibiotics, but tetanus prophylaxis was not available.

Table: (4). Type of orthopedic fixations

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Type of fixation	No. of patient
External Fixation	37
Ilizarov Ext.Fix	5
K.Wire	5
Screw	2
DCP	1
PFN	2
Debridement + Gypsum	35
Superficial injury (wounds)	53
Total	140

RESULTS

After a period of follow-up for 7 months, we get the following result; 12 patients had nerve injury, sciatic nerve in three cases, common peroneal nerve in two cases, median nerve in one case, radial nerve in five cases, and ulnar nerve in one case (Table.5).

Table: (5). Number of patients with nerve injury

Nerve injury	Number of patients
Sciatic nerve	3
Common peroneal nerve	2
Median nerve	1
Radial nerve	5
Ulnar nerve	1
Total	12

Our complications, encountered in our series of exclusively 140 patients, had musculoskeletal related gunshot injuries, vascular injury post-gunshots were 12 patients. Seven patients had fractures and 5 patients were without fractures, 2 patients had upper limb vascular injury and 10 patients had lower limb vascular injury; out of the 12 patients, 2 patients had above-knee amputations (Table.6).

Table:(6). Complications in the 140 musculoskeletal related gunshot patients

complications	No, of patients	Percentage
Infection after 2 weeks	30 patients	21%
Nonunion (7 months full up)	5patients	3%
Failed nerve repair (7 months full up)	5patients	3%
Stiff knee (7 months full up)	2 patients	1%
Amputation	2 patients	1%
Our orthopedic morbidity rate	12 patients	9%

One patient had a bad mutilating injury of the hand, which usually leads to amputation. We performed debridement and repair, including fixation of fractures by K.wires and plastic surgery in different settings which has resulted in a partially functioning cosmetically acceptable hand.

DISCUSSION

The range of primary blast injuries includes fractures, amputations, crush injury, burns, cuts, lacerations, acute occlusion of an artery, air embolism– induced injury, compartment syndrome, and others. Secondary, tertiary, and quaternary injuries are also commonly seen in extremity blast injuries. Severe contamination and tissue damage are the major problems (Bumbaširevic et al*.,* 2006). During our work, we have done timely debridement, fracture fixation, post-operative follow-up for seven months, found reasonably results, and we agree with MSF USA (2016). Still, adequate staff, doctors, beds, materials, medicines, instruments, equipment, and orthopedic appliances would have helped us to achieve a much better result, and with the onset of armed conflict in 2011, many foreign workers left the country, so the health system is presently in a critical state.

The AAOS/OTA Extremity War Injuries (EWI II): Development of Clinical Treatment Principles symposium, held in January 2007.
EWI II sessions focused specifically on four separate areas (Defense Casualty Report 2007):

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(1) Prehospital management of extremity wounds
(2) Initial debridement
(3) Early stabilization
(4) Postoperative wound management during their evacuation process.
In comparison to the (EWI II) in our study, we missed the first stage because of the lack of experience and working under difficult conditions and we focused on initial debridement, early stabilization, and postoperative wound management.

Gunshot injuries to the musculoskeletal system in our study constituted 65%. (EWI II) published that 26% of these individuals had sustained fractures (Ficke and Pollak. 2007). Whereas (Covey, 2006) published that approximately 60% of war injuries involve the musculoskeletal system

Amputee care remains complex and challenging. Recent medical, surgical, rehabilitative, and prosthetic advancements have raised both patient and caregiver expectations for outcomes. The rate of major amputation, as a percentage of all battle injuries, was 1% in our study as two patients had above –knee amputations, which was 12% during the American Civil War (Potter and Scoville 2006).

We agree with Owens et al. (2006) Current treatment protocols favor the inclusion of timely and stable axial limb fixation, radical debridement of all compromised soft tissues and osseous structures, and early wound closure, in an attempt to minimize overall morbidity and to achieve the highest level of function possible.

CONCLUSION

Treatment of war or civilian unrest wounds, many of which are devastating in the scope of soft-tissue and bony injury, requires a team approach using hypotensive resuscitation, damage-control orthopedics, newer techniques of hemostasis and vacuum-assisted wound closure, and advanced reconstruction. Current challenges include prevention of infection, heterotopic ossification, good cosmetic and functional results, adequate rehabilitation, physical therapy, and occupational therapy services, as well as psychiatric, or other services, when necessary

We strongly recommend having a specialized trauma center with adequate facilities, material, and trained qualified personal.

We, as a team of orthopedic, vascular and general surgeons, gained very good exposure and experience to manage the war or civilian unrest victims in 2011.

REFERENCES

Bagg, M. R., Covey, D. C., & Powell, E. T. I. (2006). Levels of Medical Care in the Global War on Terrorism. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons, 14*(10), S7-S9. https://journals.lww.com/jaaos/Fulltext/2006/00001/Levels_of_Medical_Care_in_the_Global_War_on.3.aspx

Bumbaširevic, M., Lesic, A., Mitkovic, M., & Bumbaširevic, V. (2006). Treatment of Blast Injuries of the Extremity. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons, 14*(10), S77-S81. https://journals.lww.com/jaaos/Fulltext/2006/00001/Treatment_of_Blast_Injuries_of_the_Extremity.18.aspx

Covey, D. C. (2006). Combat Orthopaedics: A View from the Trenches. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons, 14*(10), S10-S17. https://journals.lww.com/jaaos/Fulltext/2006/00001/Combat_Orthopa dics_ A_ View_ From_the _Trenches.4.aspx

Dufour, D. , Kromann, S. Jensen, Owensmith,M. , Salmela, J., Stening, G.F.and zetterstr zetterstr, B. (1998). Surgery for victims of war. International Com-

ISSN: online 2617-2186 print 2617-2178

mittee of the Red Cross Geneva, October (third edition) .p225

Defense Casualty Report 20 August (2007) .Available at :http://www.defenselink.mil/news/casualty.

Ficke, J. R., & Pollak, A. N. (2007). Extremity War Injuries: Development of Clinical Treatment Principles. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons, 15*(10), 590-595. https://journals.lww.com/jaaos/Fulltext/2007/10000/Extremity_War_Injuries__Development_of_Clinical.3.aspx

Medicines Sans Frontiers. MSF USA, April 26, 2016

Owens, B. D., Wenke, J. C., Svoboda, S. J., & White, D. W. (2006). Extremity Trauma Research in the United States Army.*JAAOS - Journal of the American Academy of Orthopaedic Surgeons, 14*(10), S37-S40. https://journals.lww.com/jaaos/Fulltext/2006/00001/Extremity_Trauma_Research_in_the_United_States.8.aspx

Potter, B. K., & Scoville, C. R. (2006). Amputation is not isolated: an overview of the US Army Amputee Patient Care Program and associated amputee injuries. *J Am Acad Orthop Surg, 14*(10 Spec No.), S188-190. https://doi.org/10.5435/00124635-200600001-00041

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خبرة جراحة العظام في معالجة ضحايا الحروب

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المستخلص: جراحة ضحايا الحرب تختلف عن الجراحات التي تم التدرب عليها من إصابات المدنيين، فالجروح التي تحدث من إصابات الحروب دائما ملوثة للغاية مع تهتك في الأنسجة وكسور في العظام بسبب الصواريخ. إن مبادئ الجراحة الخاصة بمعالجة جروح الحرب كانت معروفة لعقود من الزمن ولكنها تحتاج إلى إعادة التعرف عليها من قبل الجيل الجديد من الجراحين العاملين في حالة حرب. لم يكن الناس مستعدين للقتال الجسدي. وبالمثل، لم تكن المراكز الطبية مستعدة بشكل خاص لبحر من الضحايا وبشكل فجائي. الهدف من الدراسة كان تحليل النتائج من خلال خبرتنا في المعالجة الجراحية للإصابات الناتجة عن الأعيرة النارية والتي تم ادخالها لمستشفى البيضاء التعليمي خلال الشهر الأول من الثورة الليبية لسنة 2011 م. حيث أن العدد الإجمالي للمرضى خلال الفترة من 2011\2\16 إلى 2011\3\16 وصل إلى 288 مريضاً. لقد قمنا بتنظيف الجروح وتثبيت الكسور على مراحل متعددة لمدة وصلت إلى 7 شهور من المتابعة، حيث وصل معدل اعتلال العظام إلى 9% بينما معدل الالتهاب والتي تم تسجيلها في 30 مريضاً 21%. وقد اكتسب فريق جراحة العظام والأوعية الدموية والجراحة العامة خبرة واسعة في التعامل مع ضحايا الحرب واصابات المدنيين.

الكلمات المفتاحية: طبيب العظام، الطلقات النارية، المدنيين، الكسور.

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دراسة تأثير بعض المساحيق النباتية على حياتية خنفساء الدقيق المتشابهة (Coleoptera: Tenebrionidae) *Tribolium confusum* du Val

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المستخلص : استهدفت هذه الدراسة اختبار الفعالية الحيوية لثلاثة مساحيق نباتية (ثمار الفلفل الأسود *Piper nigrum* L.، بذور جوزة الطيب *Myristica fragrans* Houtt وأوراق الرند *Laurus nobilis* L.) عند خلطها مع دقيق القمح بتراكيز 0.2، 0.5، 0.75، 1 و 1.5 % (وزن/وزن) ضد خنفساء الدقيق المتشابهة *Tribolium confusum*. أظهرت النتائج إن مسحوق ثمار الفلفل الأسود سبب انخفاضاً في إنتاجية اليرقات بلغت 65.36%، بينما سبب مسحوق بذور جوزة الطيب انخفاضاً قدره 50.93% مقارنة بمسحوق أوراق الرند سبب انخفاضاً قدره 38.20%. و سجل انخفاضاً في إنتاجية أفراد الجيل الأول (F1) بلغ 90.75% و 75.34% عند المعاملة بمسحوقي ثمار الفلفل الأسود و بذور جوزة الطيب على التوالي، بينما مسحوق أوراق الرند كان أقل تأثيراً حيث سجل انخفاضاً بلغ 30.13%.

الكلمات المفتاحية : حياتية خنفساء الدقيق المتشابهة، *Tribolium confusum* ، المساحيق النباتية.

المقدمة

تعتبر الحبوب ومنتجاتها من أكبر مصادر الغذاء للإنسان والحيوان، وفي بعض البلدان تمثل حوالي 80% من الغذاء (Neethirajan وآخرون2009 ., 2007; Carver,) تتعرض الحبوب المخزونة ومنتجاتها إلى كثير من الآفات التي تسبب اضرار اقتصادية كبيرة (Hagstrum and Subramanyam, 2006; Kumar, 2017)، وحسب ما أشارت إليه منظمة الأغذية والزراعة فإن الحشرات تسبب تلف ما يقارب 10% من حبوب المحاصيل المخزونة في العالم (Wolpert, 1967). وقد يصل الفقد في الحبوب إلى حوالي 50% في الدول النامية (, Brader وآخرون2002 .,). تعد خنفساء الدقيق المتشابهة *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) أحد الآفات الحشرية المهمة في مناطق عديدة من العالم، إذ تهاجم العديد من المواد الغذائية مثل أنواع الدقيق، الحبوب، البسكويت، والفواكه المجففة وغيرها(Rees, 1995; Christian Olsson وآخرون، 2006؛ Kavallieratos وآخرون.، 2015). وتعمل الحشرة على تلويث الحبوب المخزونة ومنتجاتها وإتلافها وجعلها غير صالحة للاستهلاك عن طريق أجسام أطوار الحشرة المختلفة وكذلك جلود انسلاخها وبرازها ويمتاز الدقيق المصاب بهذه الحشرة برائحة مميزة وطعم متعفن نتيجة لإفرازات مركبات الكينون (Yezerski وآخرون، 2004؛ Rees,1995). تزداد الكثافة العددية لحشرة خنفساء الدقيق المتشابهة *T. confusum* سريعاً، حيث تضع الأنثى عدداً كبيراً من البيض، ما بين 2-10 بيضة يومياً وحوالي 970 بيضة خلال فترة حياتها. الخنافس طويلة الحياة ويمكن أن تبقى على قيد الحياة لمدة سنتين ولها 4-5 أجيال في السنة (Rees,1995).

ونظراً للخسائر الكبيرة التي تسببها الآفات بما فيها حشرات المواد المخزونة والحبوب ونتيجة للاعتماد الكلي على المبيدات الحشرية في المكافحة وما ينتج عنها من خلل في التوازن الطبيعي وتدهور مكونات البيئة (Tavares وآخرون .،

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2010)، اتجهت الأبحاث إلى استخدام أساليب أخرى في المكافحة (Yusof and Ho, 1992; Subramanyam and Hagstrum, 1995; Zoubiri and Baaliouamer, 2014)، كاستخدام الزيوت النباتية (Brari and Kumar, 2019) والمستخلصات النباتية (Boff وآخرون.، 2006؛ Udo, 2011; Fouad وآخرون .، 2012؛ Iram وآخرون.، 2013) و كذلك المساحيق النباتية (Rajendran and Sriranjini, 2008; Singh, 2017) كبديل عن المبيدات الكيماوية المصنعة في مكافحة العديد من الآفات، وأحد الاتجاهات الحديثة الإدارة المتكاملة للآفات (Hill and Schoonhoven, 1981; Sule and Ahmed, 2009).

تمت دراسة تأثير مسحوق بذور الشبت *Nethum graveolens* على الحشرات الكاملة لحشرة سوسة الأرز *Sitophilus oryzae*، وثاقبة الحبوب الصغرى *Rhizopertha dominica* وخنفساء الدقيق الحمراء *Tribolium castaneum* عند تراكيز 0.5، 1، 2، 4% وأدى إلى خفض عدد أفراد الجيل الأول بنسبة 59–100% و42–93% في حشرة سوسة الأرز S. oryzae وثاقبة الحبوب الصغرى *R. dominica* بينما انخفض عدد أفراد الجيل الأول لحشرة خنفساء الدقيق الحمراء *T. castaneum* عند تركيز 4% إلى 69% (اللقوة وآخرون، 1992).

قام العراقي وآخرون (2008) باختبار فعالية ثلاث مساحيق نباتية وهي السذاب *Ruta graveoleus*، النعناع *Mentha piperita* والعطر *Odovatissium pelargonium* عند خلطها مع حبوب القمح عند التراكيز 0.5، 1.0 و2.5 جم/كجم في حياتية خنفساء الخابرا الشعرية *Trogoderma granarium* (Everts) ولمدة جيلين متتاليين، أظهرت النتائج أن المساحيق المستخدمة أثرت في حياتية الحشرة وخاصة في أفراد الجيل الثاني حيث انخفض معدل عدد اليرقات الخارجة، كما سببت المساحيق نسبة موت لليرقات والعذارى خلال نموها وتطورها مما أدى إلى خفض في عدد الحشرات الخارجة في كلا الجيلين، وأدت المعاملة بالمساحيق إلى إطالة فترة التطور لأفراد الجيلين.

قام إبراهيم والناصر (2009) بتقدير كفاءة بعض المستخلصات النباتية مثل الكمون *Cuminum cyminum* L.، الشمرة *Foeinculum vulgare*، الشبت *Anethum graveolens* L.، الزعتر *Thymus capitatus* L.، الثوم *Allium sativum* L. ، الزنزلخت *Melia azedarch* L.، الكينيا *Eucalyptus ssp.* والفلفل *Capsicum annuuum* L. كمواد مانعة لوضع البيض لخنفساء اللوبيا *Callosobruchus maculates* (Fab.).

أوضحت دراسة قاما بها Derbalah و Ahmed (2011) بأنه عند معاملة حبوب القمح بزيت ومسحوق نبات النعناع سجل تأثير واضح على سوسة الأرز *S. oryzae* حيث أدت معاملتا الزيت ومسحوق النعناع إلى انخفاض في معدل النسل الناتج بسبب موت البالغات وبالتالي انخفاض في معدل البالغات الخارجة.

أختبر الأعرجي وآخرون (2012) تأثير مستخلص المركبات القلوانية الخام لأزهار نبات القرنفل Dianthus caryophylus في بعض جوانب الأداء الحياتي لخنفساء الدقيق المتشابهة *T. confusum* وأوضحت نتائج الدراسة أن مستخلص المركبات القلوانية الخام للأزهار قد أثر تأثيراً معنوياً في قتل الأطوار المختلفة لخنفساء الدقيق المتشابهة *T. confusum* إذا بلغت نسبة القتل للعمرين اليرقي الثاني والسادس والعذراء والبالغات 83.8، 63.9، 68.8، 49.0% على التوالي عند التركيز 2% بالمقارنة مع 21.1، 16.8، 14.2، 9.9% على التوالي في معاملات الشاهد. كذلك أوضحت الدراسة أن هناك علاقة طردية بين تركيز المستخلص ونسبة الموت، وكان العمر اليرقي السادس أكثر حساسية.

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هدفت الدراسة الحالية إلى استخدام مساحيق بعض النباتات وهي: ثمار الفلفل الأسود *Piper nigrum* L.، بذور جوزة الطيب *Myristica fragrans* Houtt. و أوراق الرند (ورق الغار) *Laurus nobilis* L. للكشف عن تأثيرها على بعض الجوانب الحياتية لخنفساء الدقيق المتشابهة *T. confusum*.

المواد وطرق البحث

أجري البحث في مختبر الحشرات قسم وقاية النبات/ كلية الزراعة/ جامعة طرابلس.

تربية الحشرات: جمعت بالغات *T. confusum* من الدقيق المصاب بالحشرة من عينات الدقيق بالأسواق وتم التعريف بواسطة قرن الاستشعار باستخدام المجهر الضوئي، وتمت تربية الحشرة في حضان عند درجة حرارة 28± 2°م، ورطوبة نسبية 60–70%. ووضع 200 جرام من الدقيق المعقم في برطمانات زجاجية، ووضعت الحشرات الكاملة في الدقيق، وغطيت بشاش وتم تثبيت الشاش برباط مطاطي لتجنب هروب البالغات وبقائها لمدة أسبوع لوضع البيض وتم غربلة الدقيق وإزالة الحشرات بواسطة فرشاة ناعمة، و يتم تجديد المزرعة باستمرار بعد كل جيل و مراقبة خروج الحشرات الكاملة لاستخدامها في التجارب اللاحقة.

تحضير المساحيق النباتية: طحنت ثمار الفلفل الأسود *P. nigrum*، بذور جوزة الطيب *M. fragrans* و أوراق الرند *L. nobilis* بواسطة مطحنة كهربائية وحفظت المساحيق داخل برطمانات زجاجية كتب عليها البيانات التي تشير لنوع النبات وحفظت إلى حين الاستعمال.

اختبار تأثير بعض المساحيق النباتية على حياتية خنفساء الدقيق المتشابهة *T. confusum*:

خطوات التجربة:

1. تجهيز البيئة لتربية الحشرات للحصول على العذارى.
2. فصل العذارى باستخدام المجهر للتمييز بين الذكور و الإناث لبداية التجربة (Park, 1934).

وضع 10 جرام من الدقيق المخلوط بمساحيق ثمار الفلفل الأسود، بذور جوزة الطيب و أوراق الرند بتراكيز 2، 5، 7.5، 10، 15% (وزن/وزن). حيث خلط وزن 0.2، 0.5، 0.75، 1، 1.5 جم من المساحيق مع وزن 9.8، 9.5، 9.25، 9، 8.5 جم من الدقيق المعقم بمعدل 3 مكررات لكل معاملة عدد (2 ذكور و 2 أناث من عذارى خنافس الدقيق المتشابهة *T. confusum*). وضعت في أنابيب اختبار بالإضافة إلى معاملة شاهد تحتوي 10 جرام دقيق معقم وعدد (2 ذكور و 2 أناث من عذارى خنافس الدقيق المتشابهة *T. confusum*) بدون اضافة مساحيق النباتات المختبرة، وغطيت الأنابيب بقطعة شاش مثبت برباط من أعلى الأنبوب. و وضعت كل الأنابيب في حضان تحت درجة حرارة 28 ± 2°م و رطوبة نسبية تتراوح بين 60– 70%. و بعد أسبوع تمت إزالة الآباء من الأنابيب ويتم مراقبتها يوميا إلى حين خروج الحشرات الكاملة. تم متابعة حياتية الحشرة لمدة 49 يوماً من بداية التجربة.

حسب عدد اليرقات الخارجة، النسبة المئوية لمقدار الانخفاض في عدد اليرقات، عدد البالغات الخارجة والنسبة المئوية لنقصان أفراد الجيل الأول للبالغات تبعاً لـ El-Lakwah و آخرون (1996) و حسب المعادلات التالية:

النسبة المئوية لمقدار الانخفاض في اليرقات= عدد اليرقات في الشاهد– عدد اليرقات في المعاملة/عدد البيض في الشاهد × 100

النسبة المئوية لنقصان أفراد الجيل الأول= عدد البالغات في الشاهد–عدد البالغات في المعاملة/ عدد الحشرات في الشاهد × 100

صممت التجربة وفق التصميم الكامل العشوائية (C.R.D) و استخدم لمقارنة النتائج الفرق المعنوي الأصغر (L.S.D) و على مستوى معنوي 0.05%.

النتائج

تأثير المساحيق النباتية لثمار الفلفل الأسود *P. nigrum*، بذور جوزة الطيب *M. Fragrans* و أوراق الرند *L.*

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nobilis* علـى حياتيــة خنفســاء الـدقيق المتشـابهة *T. confusum : أشارت نتائج التحليل الإحصائي (شكل 1) إن معاملـة الوسـط الغـذائي بمسـحوق ثمـار الفلفـل الأسـود أظهـر كفاءة جيدة في خفض أعداد اليرقات والبالغات. وسجلت فروقا معنويـة عاليـة بـين التراكيـز عنـد مسـتوى ($p<0.05$) بـين متوسطات أعداد اليرقات والبالغات في المعاملات باختلاف التراكيـز فكلمـا زاد التركيـز قلـت أعـداد اليرقـات و الحشـرات الخارجة. وكان أعلى معدل لعدد اليرقات الخارجة عند تركيز 0.2% (وزن/وزن) 16 يرقة، وكان أقل عدد 0.67 يرقة عند تركيز 1.5% (وزن/وزن) مقارنة بـ .43.33 يرقة في معاملة الشـاهد و نلاحـظ مـن شـكل (1) لا توجـد فـروق معنويـة بـين التركيزين 1 و 1.5 % (وزن/وزن) عند مستوى ($p<0.05$).

سـجل اختـلاف فـي عـدد الحشـرات الخارجـة بـاختلاف التركيـز بدرجة معنوية عالية، حيث بلغ عدد الحشرات الخارجة 3.33، 2.67، 0.69، 0 و0 حشـرة لكـل مـن التراكيـز 0.2، 0.5، 0.75، 1 و 1.5 % (وزن/وزن) علـى التـوالي مقارنـة بمعاملـة الشـاهد حيـث بلـغ متوسـط عـدد البالغـات الخارجـة 34.37 حشرة.

أظهرت نتائج التحليل الإحصائي (شكل 2) أن متوسط عدد اليرقـات الناتجـة فـي الوسـط الغـذائي المعامـل بمسـحوق بـذور جـوزة الطيـب عنـد التركيـز 0.2%، 0.5%، 0.75%، 1%، 1.5% (وزن/وزن) كان 45، 12.33، 14.33، 4.33، 6.33 يرقة على التوالي مقارنة بمعاملة الشاهد 35.67 يرقة.

أما عدد البالغات الخارجة بلغ 23.67، 9.67، 0.33، 0.67 و 0.67 حشـرة لـنفس التراكيـز علـى التـوالي مقارنـة بمعاملـة الشـاهد بلـغ متوسـط عـدد البالغـات الخارجـة 29.67 حشـرة. يلاحـظ مـن النتـائج أن تركيـز 0.2% (وزن/وزن) لـم يكـن لـه تأثير معنوي على عدد اليرقات الناتجة، في حين بقية التراكيز سـجلت فروقـاً معنويـة عاليـة مقارنـة بمعاملـة الشـاهد. كـذلك نلاحـظ مـن الشـكل (2) بأنـه لا توجـد فـروق معنويـة بـين التركيـزين 0.5 و 0.75 % (وزن/وزن) فـي إنتاجيـة اليرقـات، فـي حـين كـان هنـاك فـرق معنـوي عـالي بـين التركيـزين فـي متوسط أعداد البالغات الخارجة.

أظهـرت النتـائج (شـكل3) تـأثير إضـافة تراكيـز مختلفـة مـن مسـحوق أوراق الرنـد إلـى الوسـط الغـذائي، حيـث كـان متوسـط عـدد اليرقـات الناتجـة لحشـرة خنفسـاء الـدقيق المتشـابهة *T. confusum* عنـــد تركيـــز 0.2%، 0.5%، 0.75%، 1%، 1.5% (وزن/وزن) 27.33 ، 22.67 ، 16.67 ، 16.33 يرقـة، 8.00 يرقـة، بالمقارنـة مـع معاملـة الشـاهد بلـغ متوسـط اليرقـات 28.33 يرقـة. مـن خـلال نتـائج التحليـل الإحصـائي يتضـح عـدم وجـود فـرق معنـوي بـين التركيـزين 0.75 و 1% (وزن/وزن) فـي متوسـط عـدد اليرقـات الناتجـة. فـي حـين كـان متوسـط عـدد البالغـات الخارجـة 22.33، 16.00، 16.33، 6.00 و 1.33 حشـرة للتراكيـز 0.2، 0.5، 0.75، 1 و 1.5 % (وزن/وزن) علـى التـوالي. بالمقارنـة مـع معاملـة الشـاهد إذ بلـغ متوسـط عـدد البالغـات الخارجـة 24.33 حشـرة. يـذكر بأنـه يوجـد فـرق معنـوي بـين التركيـزين 0.75 و 1% (وزن/وزن) في متوسط أعداد البالغات الخارجة، كذلك من خلال النتائج يتضح بأنه لا توجد فروق معنوية بين التركيزين 0.5 و 0.75 % (وزن/وزن) مـن مسـحوق أوراق الرنـد فـي متوسـط عـدد البالغات الخارجة.

تأثير المساحيق النباتية لثمار الفلفل الأسود *P. nigrum*، بذور جوزة الطيب *M. fragrans* و أوراق الرند *L. nobilis* على النسب المئوية لمقدار الانخفاض في عدد اليرقات و نقصان أفراد الجيل الأول (F1): يتضح من نتائج التحليل الإحصـائي فـي الشـكلين (4 و 5) أن معاملـة الوسـط الغـذائي بالمساحيق النباتيـة لثمـار الفلفل الأسـود ، بـذور جـوزة الطيـب و أوراق الرند أظهـرت كفـاءة عاليـة فـي انخفـاض متوسـط عدد اليرقات و البالغات الخارجة (F1)، و كانت أعلى نسبة خفض لأعداد اليرقات و البالغات سجلت لمسحوق ثمار الفلفل الأسـود بمعـدل 65.36 و 90.75 % ، يليـه مسـحوق جـوزة الطيـب بمعـدل 50.93 و 75.34 % ثـم مسـحوق أوراق الرنـد بمعدل 38.2 و 30.13% لليرقات و البالغات على التوالي، و

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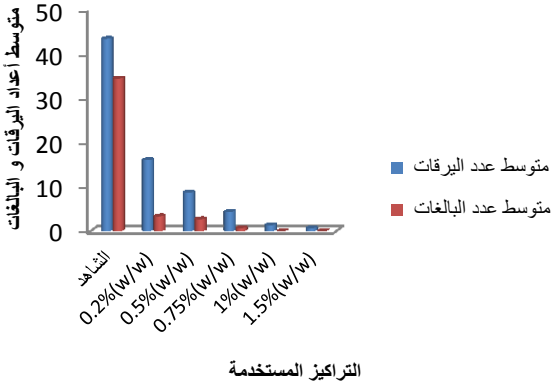
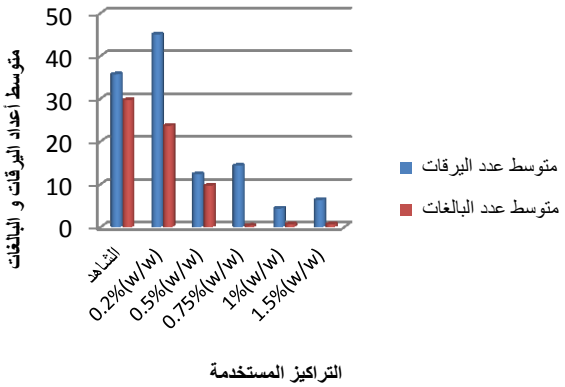
يفسر ذلك باختلاف تركيب المواد الفعالة في كل مسحوق نباتي.

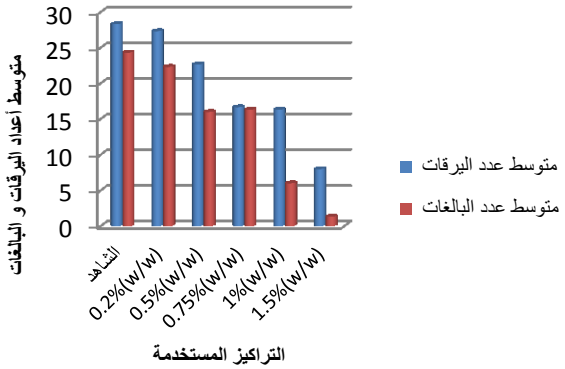
يتبين من النتائج في الشكلين (4 و 5) ان كفاءة المساحيق النباتية المستخدمة في خفض نسب أعداد اليرقات و البالغات الناتجة تزداد كلما ازدادت التراكيز المستخدمة من المساحيق النباتية، حيث أظهرت نتائج التحليل الإحصائي وجود فروقاً معنوية عند مستوى ($p<0.05$) بين متوسطات أعداد اليرقات والبالغات لجميع مساحيق النباتات و ذلك باختلاف التراكيز. كما يلاحظ من الشكل (5) ان عند التراكيز 0.75، 1 و 1.5 % (وزن/وزن) لمساحيق نباتات ثمار الفلفل الأسود و بذور جوزة الطيب لا توجد فروق معنوية في انخفاض أفراد الجيل الأول (F1)، و كذلك عند التركيز 1.5 % (وزن/وزن) لا توجد فروق معنوية بين جميع المساحيق النباتية المستخدمة في انخفاض أفراد الجيل الأول (F1) و كلها أظهرت كفاءة جيدة في خفض أعداد البالغات الخارجة.



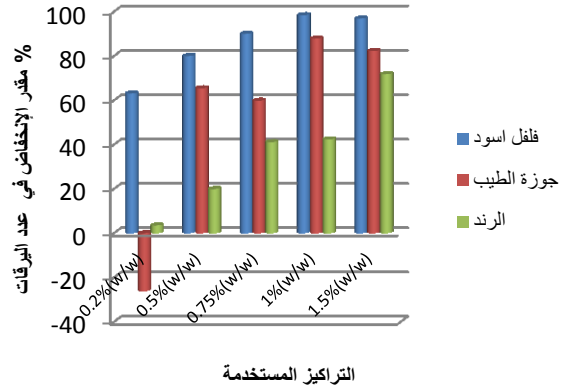
شكل (1). تأثير التراكيز المختلفة لمسحوق ثمار الفلفل الأسود *Piper nigrum* L. على إنتاجية اليرقات و البالغات لحشرة خنفساء الدقيق المتشابهة *Tribolium confusum*



شكل (2). تأثير التراكيز المختلفة لمسحوق بذور جوزة الطيب *Myristica fragrans* Houtt. على إنتاجية اليرقات و البالغات لحشرة خنفساء الدقيق المتشابهة *Tribolium confusum*



شكل (3). تأثير التراكيز المختلفة لمسحوق أوراق الرند *Laurus nobilis* L. على إنتاجية اليرقات و البالغات لحشرة خنفساء الدقيق المتشابهة *Tribolium confusum*



شكل (4). تأثير التراكيز المختلفة للمساحيق النباتية المستخدمة على مقدار الانخفاض في عدد اليرقات لحشرة خنفساء الدقيق المتشابهة *Tribolium confusum*

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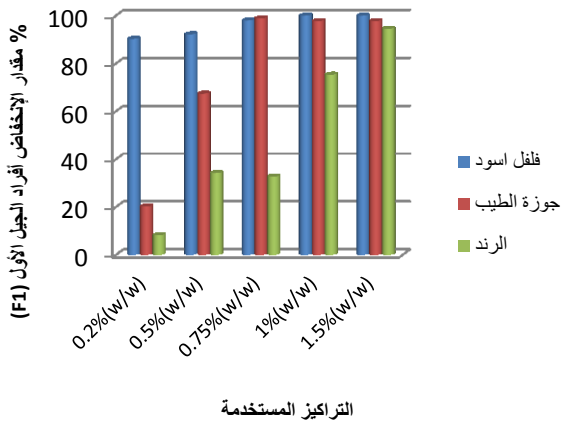

شكل (5). تأثير التراكيز المختلفة للمساحيق النباتية المستخدمة على مقدار الانخفاض لأفراد الجيل الأول (F1) لحشرة خنفساء الدقيق المتشابهة *Tribolium confusum*

المناقشة

بينت النتائج (الأشكال1،2 و3) وجود علاقة عكسية، فكلما زاد تركيز المسحوق قل عدد اليرقات والبالغات الناتجة، يعتقد إن سبب هذا النقص في أعداد اليرقات والبالغات الناتجة يعود للتأثير القاتل أو الطارد لهذه المساحيق، لذلك أثرت بشكل كبير على البيض واليرقات حديثة الفقس والبالغات (أبو النور وخليفة، 2019)، أو ربما يعود سبب هلاك اليرقات نتيجة وصول مركبات سامة لجهازها الهضمي في حال تغذيتها على الغذاء المعامل بالمساحيق، حيث تعرقل المساحيق إفراز الإنزيمات الهاضمة وبالتالي موت اليرقات أو أن هذه المركبات تؤثر في الجهاز العصبي لليرقات وتحدث شللاً ومن ثم الموت (Bowers, 1984). أشار Elhag (2000)، اذ ثبت إن المستخلصات النباتية لأوراق الحرمل *Rhazya stricta*، بذور النيم *Azadirachta indica*، الفلفل الأسود *P. nigrum*، قشور البرتقال *Citrus peels* ونبات الرمرام *Heliotropium bacciferum* كان لها تأثيرُ مانع لوضع البيض، وعلى نسبة الإنتاجية وحدوث انخفاض في النسل الناتج لخنفساء اللوبيا *Callosobruchus maculatus* بمقدار 11.5، 11.9، 12.4، 13.4 و 14.8% على التوالي، وقد يعود تأثير هذه المساحيق إلى احتواء النباتات على مركبات ثانوية و فينولية و تربينية وأشباه الجلايكوسيدية و مركبات فعالة طاردة للحشرات تعمل كمانعات تغذية أو طاردة تؤدي لقتل الحشرات البالغة أو ربما تسبب خللاً في التوازن الهرموني يؤدي إلى تثبيط عملية وضع البيض وفقسه وتؤثر في عملية النمو وانسلاخ الأطوار اليرقية للحشرة (Halawa وآخرون.، 1998؛ Ouchikhوآخرون .، 2011).

و يلاحظ من النتائج في الشكلين (4 و 5) وجود علاقة طردية بين تراكيز المساحيق النباتية المستخدمة ونسبة الانخفاض في عدد اليرقات والبالغات الخارجة، كلما زاد التركيز زاد مقدار الانخفاض في عدد اليرقات الناتجة و أفراد الجيل الأول (F1)، هذه النتائج تتوافق مع ما توصل إليه Up Adhyay و Jaiswal (2007) في دراستهما لفعالية الزيت العطري للفلفل الأسود *P. nigrum* كمبيد إحيائي ضد *T. castaneum* حيث أشارا إلى أن الزيت العطري للفلفل الأسود عمل على تخفيض نسبة تطور طور العذراء إلى الطور البالغ مع زيادة تركيز الزيت. في حين أشار (عبدالحميد و عبد المجيد، 1988) أن يكون سبب هلاك اليرقات يعزى لنفاذ المركبات السامة إلى داخل الجسم عن طريق جدار الجسم أثناء انسلاخها وبعده.

الاستنتاج

نستنتج من هذه الدراسة أن استخدام المساحيق النباتية لثمار الفلفل الأسود *P. nigrum*، بذور جوزة الطيب *M. Fragrans* و أوراق الرند *L. nobilis* له تأثير على حياتية حشرة خنفساء الدقيق المتشابهة *T. confusum*مما أدى إلى خفض ملحوظ في متوسط عدد اليرقات والبالغات الخارجة. وأن مسحوق ثمار الفلفل الأسود يسبب انخفاضا في إنتاجية اليرقات بلغ 65.36%، بينما سبب مسحوق بذور جوزة الطيب إنخفاضاً قدره 50.93% مقارنة بمسحوق الرند الذي سبب انخفاضا قدره 38.20%. وسجل انخفاضاً في إنتاجية أفراد الجيل الأول (F1) بلغ 90.75 و 75.34 % عند المعاملة

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بمسحوقي ثمار الفلف الأسود و بذور جوزة الطيب على التوالي و كان مسحوق أوراق الرند أقل تأثيراً حيث بلغ 30.13%.

الشكر والتقدير

خالص الشكر و التقدير إلى الدكتور خالد العيساوي بقسم المحاصيل على مساعدته في التحليل الإحصائي. كما أتقدم بالشكر الجزيل إلى كل من الدكتور خليفة دعباج و الدكتورة نجاة الغرياني بقسم وقاية النبات على ملاحظاتهما القيمة حول الورقة.

المراجع

إبراهيم، محمد و زكريا الناصر. (2009). دراسة كفاءة بعض المستخلصات والزيوت النباتية والمساحيق الخاملة في الوقاية من خنفساء اللوبياء. مجلة جامعة دمشق للعلوم الزراعية، 25 (1): 107-120.

أبو النور، نجاة علي و فادية إبراهيم محمد خليفة. (2019). دراسة تأثير بعض التوابل في مكافحة بالغات حشرة خنفساء الدقيق المتشابهة (المحيرة) *Tribolium confusum* du Val (Coleoptera: Tenebrionidae). المجلة الليبية لوقاية النبات، 9: 64-75.

الأعرجي، حمزة أحمد عزيز؛ عبدالعزيز إبراهيم ياسين الموسوي و أزال عبدالعزيز إبراهيم الموسوي. (2012). دراسة تأثير مستخلص المركبات القلوانية الخام لأزهار نبات القرنفل *Dianthus caryophyllus* L. في الأدوار المختلفة لخنفساء الطحين المتشابهة *Tribolium confusum* Duv.. مجلة الكوفة للعلوم الزراعية، 4 (2): 427-436.

العراقي، رياض أحمد؛ أزهار عبد الجبار حامد وإبراهيم خليل إبراهيم الحديدي (2008). تأثير بعض المساحيق النباتية في حياتية خنفساء الحبوب الشعرية (الخابرا). مجلة تكريت للعلوم الصرفة، 13 (1): 60-64.

اللقوة، فارس أمين محمد؛ أحمد عبد الفقار درويش و أمينة محمد خالد. (1992). فعالية مسحوق بذور الشبت *Anethum graveolens* L. على بعض حشرات المواد المخزونة. مجلة معهد بحوث وقاية النبات. مركز البحوث الزراعية. وزارة الزراعة. مصر. 30 (4): 2031- 2037.

عبد الحميد، زيدان هندي و محمد إبراهيم عبدالمجيد (1988). الاتجاهات الحديثة في المبيدات و مكافحة الحشرات، الجزء الأول، الدار العربية للنشر و التوزيع، القاهرة، 572 صفحة.

Bowers, W.S. (1984). Insect plant interactions: Endocrine defences. Pitman book, London.

Brader, B., Lee, R. C., Plarre, R., Burkholder, W., Kitto, G. B., Kao, C., Polston, L., Dorneanu, E., Szabo, I., Mead, B., Rouse, B., Sullins, D. and Denning, R. (2002). A comparison of screening methods for insect contamination in wheat. *Journal of Stored Products Research*, 38 (1): 75-86.

Brari, J. and Kumar, V. (2019). Antifeedant activity of four plant essential oils against major stored product insect pests. *International Journal of Pure and Applied Zoology*, 7(3): 41-45.

Boff. M. I. C., Sartoari D. V. and Bogo A. (2006). Effect of extracts of

Piper nigrum L. on the bean weevil, *Acanthoscelides obtectus* (Say). *Revista*

ISSN: online 2617-2186 print 2617-2178

Halawa, Z.A., Mohamed, R. and El-kashlan, I. H. (1998). Laboratory evaluation of some plants and insecticides against the *beetle Callosobruchus maculates* infesting stored product. *Egyptian Journal of Agricultural Research*, 76 (1): 85-93.

Hill, J. M. and Schoonhoven, A. V. (1981). The use of vegetable oil in controlling insect infestations in stored grains and pulses. *Recent Advances in Food Science and Technology*, 1: 473-481.

Iram, N., Muhammad, A. and Naheed A (2013). Evaluation of Botanical and Synthetic

Insecticide for the Control of *Tribolium castaneum*(Herbst)(Coleoptera:Tenebrionidae). *BioAssay*, 8: 3.

Kavallieratos, N.G.; Athanassiou, C.G.; Korunic, Z. and Mikeli, N.H. (2015). Evaluation of three novel diatomaceous earths against three stored-grain beetle species on wheat and maize. *Crop Protection*, 75: 132-138.

Kumar, R. (2017). Insect Pests on Stored Grain. Biology, Behavior, and Management Strategies; Apple *Academic Press: Waretown*, NJ, USA.

Neethirajan, S., Karunakaran, C., Jayas, D. S. and White, N. D. G. (2007). Detection techniques for stored-product insects in grain. *Food Control*, 18 (2): 157-162.

Ouchikh, O., Chahed, T., Ksouri, R., Taarit, M.B., Faleh, H., Abdelly, C., Kchouk, M.E. and Marzouk, B. (2011). The effects of extraction method on the measured tocopherol level and antioxidant activity of L. nobilis vegetative organs. *Journal of Food Composition and Analysis*, 24 (1): 103–110.

Brasileria de Armazenamento, 31(1): 17-22.

Derbalah, A.S.and Ahmed, S.I. (2011). Oil and powder of spear mint as an alternative to *Sitophilus oryzae* chemical control of wheat grains. *Journal of Plant Protection Research*, 51(2):146-150.

Carver, F. B. (2009). Wheat Science and Trade. Wiley-Blackwell, USA.

Christian Olsson, P. –O., Ryne, C., Wallén, R., Anderbrant, O. and Löfstedt, C. (2006). Male-produced sex pheromone in *Tribolium confusum*: Behaviour and investigation of pheromone production locations. *Journal of Stored Products Research*, 42(2): 173-182.

El-hag, E. A. (2000). Deterrent effects of some botanical products oviposition of the cowpea Bruchid, Callosobruchus maculates (Coleoptera: Bruchidae). *International Journal of Pest Management*, 46 (2): 109-113.

El-Lakwah,,F.A., Darwish,A.A.and Halawa, Z.A.(1996).Toxic effect of extracts and powders of some plants against the cowpea beetle *Callosobrucus maculatus*. *Annals of Agricultural Science Moshtohor*, 34 (4): 1849-1855.

Fouad HA, Faroni L.R.D.A., Ribeiro C, Tavares WD and Petacci F (2012). Extraction and repellent activity of *Lepidoploa aurea* and *Memora nodosa* against stored grain and byproduct pests. *Vie et Milieu*, 62 (1):11-15.

Hagstrum, D. W. and Subramanyam, Bh. (2006). Fundamentals of Stored-Product Entomology. *American Association of Cereal Chemists* Inc, St. Paul, MN, USA.

ISSN: online 2617-2186 print 2617-2178

Udo, I.O. (2011). Potentials of *Zanthaxylum xanthoxyloides* (Lam.) for the control of stored product insect pests. *Journal of Stored Products on Post Harvest Research*, 2 (3): 40-44.

Up Adhyay, R. K and Jaiswal, G. (2007). Evaluation of biological activities of *piper nigrum* oil against *Tribolium castaneum*. *Bulletin of Insectology*, 60 (1): 57-61.

Wolpert, V. (1967). Needless losses. *Far Eastern Economic Review*, 55: 411-412.

Yezerski, A., Gilmor, T. P. and Stevens, L. (2004). Genetic analysis of benzoquinone production in *Tribolium confusum*. *Journal of Chemical Ecology*, 30 (5): 1035-1044.

Yusof, O. and Ho, S.H. (1992). A survey of insecticidal resistance in *Sitophilus zeamais* Motsch. in Malaysia and Singapore. *Journal of Plant Protection in the Tropics*, 9: 219-225.

Park, T. (1934). Observations on the general biology of the flour beetle, *Tribolium confusum*. *Quarterly Review of Biology,* 9 (1): 36-54.

Rajendran, S. and Sriranjini V. (2008). Plant products as fumigants for stored-product insect control. *Journal of Stored Products Research*, 44 (2):126-135.

Rees, D. (1995). Coleoptera. In: Subramanyam, Bh. and Hagstrum, D. (Eds), Integrated Management of Insects in Stored Products. Marcel-Dekker Inc, New York, pp.1-39.

Singh, S. (2017). Natural plant products- as protectant during grain storage: A review. *Journal of Entomology and Zoology Studies*, 5 (53): 1873-1885.

Subramanyam, Bh. and Hagstrum, D. (1995). Sampling. In: Subramanyam, Bh. and Hagstrum, D. (Eds), Integrated Management of Insects in Stored Products. Marcel- Dekker Inc, New York, pp. 135-193.

Sule, H. and Ahmed, B.I. (2009). Effect of Plant product, application rates and grain type on the control of red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) on stored millet (*Pennisetum glaucum* (L.) R. Br.) *Academic Journal of Entomology*, 2 (1): 22-30.

Tavares, W.S., Costa, M.A., Cruz, I., Silveira, R.D., Serrão, J.E.and Zanuncio, J.C. (2010). Selective effects of natural and synthetic insecticides on mortality of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its predator *Eriopis connexa* (Coleoptera: Coccinellidae*). Journal of Environmental Science and Health B*, 45 (6): 557-561.

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Studying the Effectiveness of Some Plant Powders on the Biological Activities of the Confused Flour Beetle *Tribolium confusum* du Val (Coleoptera: Tenebrionidae)

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Abstract: This study aims to test the biological effect of three plant powders of (black pepper fruit *Piper nigrum* L., nutmeg seeds *Myristica fragrans* Houtt and bay leaf *Laurus nobilis* L.) when mixed with wheat flour at concentrations of; 0.2, 0.5, 0.75, 1 and 1.5 % (w/w) against the confused flour beetle *Tribolium confusum*. Results showed that the black pepper fruit caused a decrease in the production of larvae that reached 65.36%, while the nutmeg seeds powder caused a decrease of 50.93% compared with the bay leaf powder, which caused a decrease of 38.20%. The results also showed a decrease in the first generation progeny (F1) that reached 90.75% and 75.34% due to the treatment with the black pepper fruit and the nutmeg seeds powders respectively. However, the bay leaf powder was less effective, with only a decrease of 30.13%.

Keywords: Biology of Confused Flour Beetle, *Tribolium confusum*, Plant Powders.

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