



Adverse Effects of Organochlorine Pesticide Residues on Biochemical Parameters and Oxidative Stress in Libyan Agricultural Workers

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Abstract: Agricultural workers are in danger of being exposed to toxic substances such as pesticides. To estimate the individual danger of handling pesticides, the bio-monitoring of effects on agricultural workers is required. There has been no such research previously conducted among Libyan agricultural workers. This research was designed to study the biochemical parameters impacts of the pesticide contamination among Libyan agricultural workers at Aljebel Alakhtar, Libya. 45 blood samples were taken from male agriculture workers at Aljebel Alakhtar who had been exposed to pesticides in crop fields for a long time, while 25 blood samples were taken from a group of people who had not been exposed to pesticides (control). Kits were used to assess plasma ALT, AST, ALP, GST, SOD, total protein, albumin, globulin, total bilirubin, total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C, urea, and creatinine. The thiobarbituric acid reactive substances (TBARS) assay was used to evaluate lipid peroxidation in serum. Using a gas chromatography-electron capture detector, the blood samples were tested for organochlorine pesticide residues (GC-ECD). In comparison to controls, workers had significantly higher SOD, GST, ALP, AST, and ALT activities, as well as higher levels of lipid profile, total bilirubin, creatinine, and urea, as well as significantly higher TBARS concentrations. Furthermore, long-term pesticide exposure was also related to reducing total protein, albumin, and globulin, as well as reduced HDL-C levels. Pesticide exposure seems to influence various biochemical markers in general. These biomarkers appear to be indicative of pesticide-related deleterious effects in agricultural workers, indicating that they should be used for routine monitoring of impacts.

Keywords: Lipid profile; Liver function; Kidney function; Oxidative stress biomarkers.

INTRODUCTION

The negative effects of persistent organic pollutants (POPs) on the environment have been a prominent topic of debate around the world. These chemicals are poisonous, and because of their propensity to be transported long distances and released by water or wind,

they have a variety of effects on biota and people (Buccini, 2003). Due to their omnipresent nature, lipophilic properties, and persistence within the environment, they tend to accumulate in the tissues of living organisms (Olisah, Adeniji, et al., 2019) Pesticide use has risen in recent years around the world. Farmers and agricultural workers are fre-

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quently the most exposed individuals among those involved in the formulation and final distribution of pesticide combinations (Alves et al., 2016). Because of their versatility, chemicals in this group, such as organochlorine pesticides (OCPs), are employed for pest management (Olisah, Okoh, et al., 2019).

These include insecticides such as aldrin, dieldrin, endrin, chlordane, dichlorodiphenyltrichloroethane (DDT), mirex, toxaphene, heptachlor, fungicides, and hexachlorobenzene (HCB). Other chemicals in this group that are inadvertently delivered are polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The high quantity of pesticides detected in the atmosphere, water bodies, and soils is due to inappropriate treatment of these chemicals by farmers and other end users, posing a hazard to human health and the ecosystem as a whole. Pesticides prevalent in the environment (particularly organochlorine kinds) have caused a variety of difficulties in Asia, America, Europe, and Africa (Ali et al., 2013; Elibariki & Maguta, 2017; Olisah et al., 2020). These incorporate DDTs, endosulfans, HCB, and Drins (aldrin, endrin, and dieldrin).

Despite being banned, most African countries continued to use OCPs as of 2009. Researchers in Africa and other regions of the world have found extremely high amounts of these pollutants in a variety of matrices, including biota, sediment, soil, water, and food products. (Bempah & Donkor, 2011; Kolani et al., 2016). Pesticide poisoning affects around 25 million agricultural laborers in underdeveloped nations each year, according to the World Health Organization (WHO, 2009). Low-level pesticide exposure can cause a range of biochemical changes, some of which may be responsible for the documented negative biological effects in humans and animals (Banerjee et al., 1999; Hernández et al., 2006). Some metabolic changes, on the other hand, may not always result in clinically detectable symptoms.

The study of molecular markers of human pesticide exposure has received much interest in recent years. These markers are used to detect the effects of pesticides before they have a negative impact on human health. Biological markers must be present in conveniently accessible and ethically acceptable tissues in humans, such as blood or urine. Pesticide poisoning may have an impact on biological factors related to organ function in humans. Hepatic or renal cytotoxicity could be the cause of the biochemical dysfunctions. A mild nephrotoxic alteration in pesticide-exposed workers has been described (Hernández et al., 2006; Olisah et al., 2020). Pesticide workers exposed to various pesticides have reported altered liver enzyme activity, such as serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Altuntas et al., 2002; Khan et al., 2008).

Some biological parameters can be utilized to identify preclinical changes or negative health consequences caused by a compound's external exposure and absorption. These biomarkers may indicate an early stage of illness development and thus may be predictive of future disease (Benford et al., 2000). Pesticides may produce damaging and degenerative alterations in numerous organs, including the kidneys, resulting in these biochemical abnormalities (Gangemi et al., 2016; Khan et al., 2013). In Africa, there hasn't been much research done on detecting these pollutants in human fluids (Olisah et al., 2020). This study was carried out to evaluate the effects of organochlorine pesticides on biochemical parameters in agricultural workers chronically exposed to these compounds, because of the limited research in Libya related to human monitoring of organochlorine pesticides' exposure.

MATERIALS AND METHODS

Sample collections and experimental design: A total of 70 people were chosen as a sample size from various locations of El-Jabil

Al Kadar, with 45 blood samples collected from male agriculture workers who had been frequently exposed to pesticides for a long time but had no previous history of infectious diseases or other environmental exposures. Individuals for the study were enrolled in February 2015, with a mean age of 61.24 ± 5.12 and an average of 25 years of experience working in agricultural fields. Similarly, 25 healthy volunteers were recruited from the same location, but had no current or recent history of infectious illnesses, pesticide exposure, or other environmental exposures (as a control). The age distribution of the control subjects (59.14 ± 3.21 years) was nearly identical to that of the patients.

Data collection: Filling out the questionnaire, which was specified for matching the study need, was done through a meeting interview. The researcher performed all interviews in person. During the study, the interviewer clarified any of the participants' questions that were unclear to them. The majority of the questions were yes/no questions, which provided a binary option. After informing the respondents about the purpose of the study and getting informed consent, the individuals were given a questionnaire with questions about their age, smoking habits, sex, and sickness duration. The questionnaire was also used to collect information regarding medical history, such as the presence of kidney disease, cardiovascular disease, liver disease, and recurring infection signs and symptoms.

Sampling: By numbering them and the blood samples taken from them, all subjects were made anonymous. Blood samples (7 mL) were drawn from each person and collected in anticoagulant sterile vacutainer tubes. Within 2 hours of blood donation, the blood specimens were preserved in an ice-cold chamber, transported, and delivered to the laboratory for processing.

Oxidative stress biomarkers: Lipid peroxidation was estimated in serum by thiobarbituric acid reactive substances (TBARS) assay,

through a malondialdehyde (MDA) reaction with 2-thiobarbituric acid (TBA), which was optically measured. TBARS levels were expressed as nmol MDA/mg protein according to (Buege & Aust, 1978). The level of plasma MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief, to a 150 μ l plasma sample, the following were added: 1ml (17.5%) trichloroacetic acid (TCA) and 1ml of 0.66% TBA, mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added, and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, and the supernatant was taken out for spectrophotometer assay. The concentration of MDA was calculated as follows:

$$MDA (\mu\text{mol/l}) = \frac{\text{Absorbance at } 532 \text{ nm}}{L \times E_0} \times D \times 10^6$$

L: light path (1cm).

E_0 : Extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

D: Dilution factor.

Biochemical assays: Total protein, albumin, globulin, total bilirubin, total cholesterol, triglycerides, high-density Lipoprotein concentration (HDL-C), low-density Lipoprotein concentration (LDL-C), very low-density Lipoprotein concentration (VLDL-C), urea and creatinine were all measured using kits (Vitro Scient, Germany) (Tolman & Rej, 1999).

Extraction of pesticide residues from the whole blood: The procedure used by (Agarwal et al., 1976) was used for extraction. Blood (5 ml) was diluted with 25 ml distilled water and 2 mL saturated brine solution, then transferred to a separatory funnel with a capacity of 125 ml. It was extracted three times with hexane: acetone (1:1) (20 ml) by vigorously shaking the separatory funnel for 2-3 minutes, intermittently releasing the pressure. As a result, the layers were given the opportunity to separate. Using a rotary vacuum evaporator, the three mixed extracts were passed through anhydrous sodi-

um sulfate and condensed to around 1-2 ml. As a result, whole blood was used.

Sample analysis: The samples were analyzed for organochlorine using gas chromatography at Cairo University's Faculty of Science. Organochlorine pesticide standard solutions were prepared in n-hexane (Clarke, 1986).

Statistical analysis: Student's t-test was used to assess and compare data from agriculture workers and healthy controls from several experiments. The data was presented as a mean standard deviation. The significant test was used with a p-value of less than 0.05.

RESULTS

The current study was carried out to determine the effects of pesticides on the health of farmers in Aljebal Alakhtar, north-east Libya, by determining their levels of biochemical markers. For this reason, the individuals missing any history of disease were examined after conducting preparatory investigations. Table 1 shows the characteristics of the population under consideration. Mean age of the control and spraying peoples were 59.14 ± 3.21 and 61.24 ± 5.12 years, respectively, which were not significantly different. All farm workers were exposed to pesticides for an average of 21 ± 3.7 years. Personal protection equipment was not used by any of the populations surveyed (PPE).

Table (1). Characteristics of the study population (Mean \pm SD)

Characteristic	Control group [n=25]	Exposed farm workers [n=45]
Gender	Male	Male
Age (year)	59.14 ± 3.21	61.24 ± 5.12
Wt (Kg)	76.36 ± 6.75	74.76 ± 7.34
Years of exposure	-	21 ± 3.7
Personal protective equipment	-	-

There has been no research on blood levels of organochlorine pesticides in the Libyan population. Organochlorines and their isomer residue levels in the whole blood of agricul-

turalists and non-agriculturalists were quantified. An analytical method of GC-electron-capture detection using a capillary column was implemented to determine dichlorodiphenyltrichloroethane (DDT) and its metabolites (p,p-DDD and p,p-DDE), as well as other organochlorine pesticides in whole blood samples from 45 farmers and 25 non-occupationally exposed workers. As shown in Table 2, we detected four (o,p-DDE, o,p-DDD, o,p-DDT, and p,p-DDT) of the 16 organochlorine compounds tested for in the whole blood samples (Table 2).

Table (2). The distribution concentrations (ppm) of organochlorine pesticide residues detected in whole blood samples collected from Libyan farm workers (Mean \pm SD)

Pesticides detected in farmers group	Concentrations of pesticide residues (ppm)
Dichlorodiphenyltrichloroethane DDT/metabolites	
p,p'-DDT	0.169 ± 0.001
o,p'-DDT	0.129 ± 0.003
Dichlorodiphenyldichloroethylene DDE total	
o,p'-DDE	0.328 ± 0.007
Dichlorodiphenyldichloroethane DDD total	
p,p'-DDD	0.510 ± 0.01

Quantitative examination of serum total bilirubin, total protein, albumin, globulin, ALP, ALT, and AST, which were used as biochemical markers of liver damage, was used to evaluate hepatotoxicity (Table 3).

Table (3). Effects of chronic exposure to a mixture of pesticides on liver function parameters measured in plasma of controls and exposed group (Mean \pm SD)

Parameters	Control (n=25)	Exposed farm workers (n = 45)
ALP (U/L)	59.38 ± 6.32	$98.21 \pm 19.84^*$
ALT (U/L)	29.76 ± 2.11	$41.02 \pm 6.72^*$
AST (U/L)	31.12 ± 3.54	$53.27 \pm 6.91^*$
Total protein (g/dl)	7.19 ± 0.28	$5.43 \pm 0.71^*$
Albumin (g/dl)	5.41 ± 0.23	$3.90 \pm 0.18^*$
Globulin (g/dl)	1.78 ± 0.08	$1.53 \pm 0.17^*$
Total bilirubin (mg/dl)	0.99 ± 0.03	$1.39 \pm 0.07^*$

* $p < 0.05$ for exposed group compared to control group.

The values of biochemical parameters determined for the exposed workers and the control group are shown in Table 4.

Table (4). Effects of chronic exposure to a mixture of pesticides on kidney function parameters measured in plasma of control and exposed group (Mean ± SD)

Parameters	Control (n= 25)	Exposed farm workers (n = 45)
Urea (mg/dl)	26.32 ± 1.03	33.58 ± 3.61*
Creatinine (mg/dl)	0.64 ± 0.02	0.97 ± 0.06*

**p*<0.05 for exposed group compared to control group.

The effects of chronic exposure to a mixture of pesticides on biochemical plasma lipid parameters are also shown in Table 5.

Table (5). Effects of chronic exposure to a mixture of pesticides on the biochemical plasma lipid parameters measured in plasma of controls and exposed group (Mean ± SD)

Parameters	Control (n= 25)	Exposed farm workers (n = 45)
Total Cholesterol (mg/dl)	185.16 ± 11.09	219.02 ± 21.17*
Triglycerides (mg/dl)	160.72 ± 11.13	199.0 ± 25.98*
HDL-C (mg/dl)	39.61 ± 1.16	21.10 ± 3.93*
LDL-C (mg/dl)	101.25 ± 10.23	148.85 ± 19.87*
VLDL-C (mg/dl)	32.14 ± 2.22	39.85 ± 7.19*

**p*<0.05 for exposed group compared to control group.

Table 6 shows the effects of chronic pesticide exposure on enzymatic antioxidants and plasma thiobarbituric acid reactive substances (TBARS) concentrations.

Table (6). Effects of chronic exposure to a mixture of pesticides on antioxidant enzymes and TBARS measured in plasma of control and exposed group (Mean ± SD)

Parameters	Control (n= 25)	Exposed farm workers (n = 45)
TBARS (nmol/ml)	2.14 ± 0.03	3.97 ± 0.29*
GST (µmol/hr)	0.52 ± 0.005	0.68 ± 0.009*
SOD (U/ml)	0.69 ± 0.004	1.21 ± 0.01*

**p*<0.05 for exposed group compared to control group.

DISCUSSION

During their work, pesticide sprayers in this study are exposed to various pesticides (insecticides, fungicides, and herbicides). All agricultural workers in the exposed group were regularly exposed to complicated pesticide mixes (2-3 times per week). Our research farmers used a wide range of pesticides, generally in the "moderately dangerous" to "somewhat hazardous" categories. World Health Organization (WHO) (very toxic) pesticides should not be used in developing countries, according to the Food and Agriculture Organization (FAO). It also recommended that pesticides classified as class II (moderately dangerous) be avoided. Individuals are routinely exposed to a variety of pesticides or pesticide mixtures, either concurrently or sequentially, making it difficult to distinguish between their effects. The link between pesticide-related cytotoxicity and overt clinical organ dysfunction remains unknown. In this case, biomarkers could be used to detect pesticide impacts before they cause adverse clinical health effects (Hassanin, 2009). Organochlorines have long been a source of concern for ecologists due to their resistance to biodegradation. Organochlorine deposition in biolipids is caused by their low water solubility due to their high lipophilicity. These characteristics explain why organochlorine accumulates to amounts that are regarded as significant organochlorine residual loads in human adipose tissue (Kreiss et al., 1981). Despite the fact that organochlorines are extremely long-lasting, some biodegradation can occur, yielding the metabolites indicated in Figure 1.

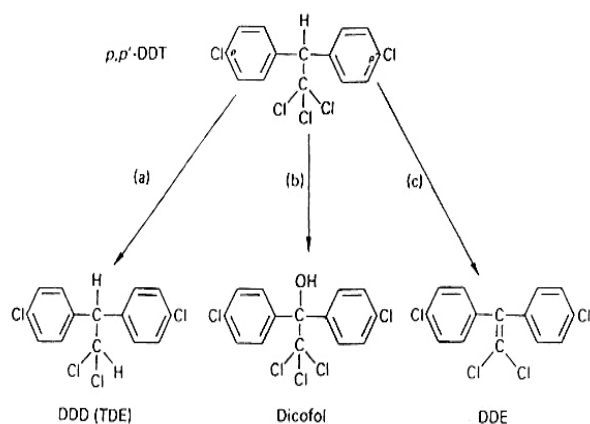


Figure (1). Metabolites formed from DDT, (a) reductive dechlorination, (b) oxidation, (c) dehydrochlorination (Kresis et al., 1981).

Because different analytical methods were used, the concentration of dichlorodiphenyldichloroethylene (DDE) in whole blood samples from Libyan farm workers was 0.328 ppm. Other authors used packed columns, while we used capillary column separation and GC/MS (El Zorgani et al., 1994; Manal, 1997). Despite the limitations mentioned above, it can be concluded that the blood concentrations of Libyan farm workers were extremely high when compared to values determined for workers in other countries (Bouwman et al., 1991; Dogheim et al., 1996; Mazzarri B & Lauschner, 1988). All Egyptian and international published data, such as those of (Barkatina et al., 1998; Cok et al., 1999; Pardio et al., 1998; Saleh et al., 1996; Salem & Ahmed, 2002; Waliszewski et al., 2001), showed that *p, p'*-DDE was the most prevalent compound among DDT. This could be related to DDT's high solubility and tendency for accumulating and being stored within fatty tissues (Waliszewski et al., 2001). DDT is normally converted into its more stable metabolite DDE in natural conditions in a living organism (Waliszewski et al., 2001). The study found that DDT and DDE measured levels were lower than those previously reported by (Dogheim et al., 1996; Saleh et al., 1996; Salem & Ahmed, 2002) in several Egyptian governorates. In countries where DDT is still used, the DDT level is usually greater. As a result, DDT has been

prohibited in most Western countries since the 1970s (Travis & Arms, 1988). Despite the fact that DDT has been banned in Libya for over a decade, its traces can still be found in the environment. This is owing to the high level of persistence. The persistence and long-range transport characteristics of DDT and its metabolite DDE, as well as their capacity to bioaccumulate and biomagnify in the food chain, make this concentration useful in detecting the source of contamination (Zhang et al., 2018).

This disparity is thought to be attributable to various pesticide spraying practices in the fields. Pesticides are frequently administered manually in Libya, particularly in the Aljebal Alakhtar area, without any protective devices. DDT is a nonsystemic insecticide with a lot of influence. 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene (*p, p'*-DDE), a significant DDT metabolite, was found at mean levels of 0.328 ppm in whole blood samples taken from Libyan farm workers. DDE has a longer half-life than DDT. *p, p'*-DDD In whole blood samples, another DDT metabolite was found at mean levels of 0.510 ppm. DDT was found in blood samples, probably due to its long-lasting nature. Because DDT is known to undergo metabolic conversion and dehydrochlorination, the detection of DDT metabolites such as DDD and DDE in our study could be the result of these metabolic processes. Biochemical alterations are essential biological indicators since they are the body's sensitive response to hazardous exposures. These modifications enable you to keep track of the level of exposure and prevent irreversible consequences. AST and ALT are liver enzymes that are utilized as biological indicators of liver disease (Machado et al., 2021). Cirrhosis, biliary obstruction, infectious and toxic hepatitis, and ischemia all raise AST levels, as can cirrhosis, biliary obstruction, and infectious and toxic hepatitis (Wang et al., 2006). Furthermore, the increase in ALT occurs solely as a result of hepatic alterations (Murphy et al., 2018).

Table 3 shows that the mean values of the liver enzymes ALT, AST, and ALP, as well as serum total bilirubin, were significantly higher in pesticide-exposed workers than in the control group ($P < 0.05$). Pesticide-exposed workers, on the other hand, had considerably lower serum total protein, albumin, and globulin concentrations than the control group, with 95.0 percent significance (Table 3). Air Force veterans who were involved in aerial herbicide spraying in Vietnam had a higher risk of liver dysfunction, which was attributed to increased AST and ALT levels (Michalek et al., 2001). Glyphosate and paraquat have been shown to suppress the activity of two enzymes in vitro: ALT and AST (Yousef et al., 2003).

(Azmi et al., 2006) found that pesticide exposure caused a considerable increase in enzyme levels (ALT, AST, and ALP) in several fruit and vegetable farm-station workers. The activity of serum transaminases may be enhanced in many diseases due to increased release from non-liver tissue sources (Kobayashi et al., 2020). A high level of impaired liver function in agricultural workers could indicate pesticide toxicity and pesticide residues in the blood. Occupational workers exposed to organophosphorus insecticides alone or in combination with organochlorines have been shown to have altered liver enzyme activity (Muñoz-Quezada et al., 2016). According to (Awad et al., 2014), agriculture workers had a significant increase in serum liver enzymes (AST, ALT, and ALP) as compared to controls (Awad et al., 2014). Other researchers have documented high levels of AST, ALT, and ALP in pesticide-exposed people (Azmi et al., 2006; Khan et al., 2013). The end product of heme catabolism, total bilirubin, possesses antioxidant and anti-inflammatory effects (Maines, 1988). (Vitek et al., 2019) identified bilirubin as a compound with high antioxidant and anti-inflammatory effects. As a result, it's not surprising that bilirubin levels have risen in this study because it has protective benefits against oxidative stress and associated reper-

cussions. A study by (Fahimul-Haq et al., 2013) found that total bilirubin levels in both groups were not only within the normal range, but also comparatively near the upper normal limit among pesticide industrial workers. It could be related to long-term pesticide exposure, which disrupted normal red blood cell metabolism, producing hepatic dysfunction and raising bilirubin levels in the blood, resulting in hyperbilirubinemia, which could be caused by the creation of more bilirubin than the typical liver can eliminate (Awad et al., 2014). When pesticide-exposed workers were compared to healthy controls, blood total protein albumin and globulin levels were found to be lower. The decrease in serum protein was thought to be mostly attributable to a decrease in albumin rather than the globulin fraction (Yousef et al., 2003). Furthermore, it was revealed that protein deficiency in the blood was primarily caused by severe nephrosis loss (Yousef et al., 2003). Furthermore, a decline in blood protein could be attributable to protein loss, which could be caused by decreased protein synthesis, increased proteolytic activity, or breakdown (Shakoori et al., 1990). The decrease in serum protein was mostly owing to lower serum albumin and serum globulin levels (Table 3).

The farmers' lower serum albumin levels could be due to their dietary situation and/or liver synthetic function. The farmers appeared to be in good health and were not starving. The farmers' reduced albumin levels could be the result of a slight alteration in liver expression levels (Aroonvilairat et al., 2015). Other authors, on the other hand, found lower serum total protein and albumin levels in pesticide sprayer farmers (Singh & Singh, 2014). Nevertheless, the current findings show that pesticide exposure causes changes in protein metabolism. Pesticide toxicity can change serum protein concentrations by impairing protein synthesis in hepatocytes and disrupting kidney function (Mostafalou & Abdollahi, 2013). Also, as evidenced by the increased activities of serum AST, ALT,

and ALP, the observed decrease in serum protein could be attributable in part to the damaging effect on pesticide-exposed workers' liver cells (Table 3). These are the hepatocellular injury markers that are employed in primary screening (Giannini et al., 2005). The serum urea and serum creatinine values are used to determine whether or not there is a concern with renal function (Kanwar et al., 2015). When there are issues with renal filtration, blood levels of creatinine and urea rise, while total protein levels are reduced (Murphy et al., 2018). Furthermore, creatinine, urea, and total protein are used as biomarkers of renal changes, assessing the ability of the kidneys to filter plasma in the glomeruli by measuring the clearance of the same (Murphy et al., 2018). As a result, these markers can be used to determine whether renal function has been affected by agricultural pesticide exposure (Calvert, 2016).

The values of biochemical parameters determined for the exposed workers and the control group are shown in Table 4. In pesticide-exposed farm workers, mean levels of urea and creatinine were considerably ($P < 0.05$) higher than in controls (Table 4). An increase in serum urea may be attributed to a decrease in its synthesis as a result of impaired hepatic function and/or a disruption in protein metabolism (Elfowiris & Banigesh, 2022; Idonije et al., 2011). Creatinine is a waste product filtered from the blood and discharged in the urine. Higher creatinine levels in exposed agricultures workers may be related to changes in kidney function (Hassanin et al., 2018). Furthermore, the observed elevations in urea and creatinine could be explained by glomerular hyper-filtration caused by increased creatinine clearance from the circulation (Palatini, 2012). Glomerular Filtration Rate is measured by serum creatinine and urea (GFR). Though, when compared to urea level, serum creatinine is a more sensitive indicator of renal function. This is because creatinine meets the majority of the criteria for an ideal filtration marker (Palatini, 2012). Previous research has found a consid-

erable increase in serum urea and creatinine concentrations in pesticide-exposed workers, which has been linked to renal injury and kidney dysfunction (Hassanin et al., 2018; Mahmoud Abdul_Aal & Mahmoud, 2019; Yassin, 2015). Elevated blood urea and creatinine levels in response to pesticide exposure may be explained by: 1) increased urea and creatinine production as a result of reduced hepatic function, as seen in the current investigation. 2) A disruption in protein metabolism, as evidenced by the current findings, and 3) a decrease in renal filtration rate, as evidenced by the current findings. Pesticide toxicity could explain the observed elevations in urea and creatinine levels as a measure of renal function in the exposed group compared to the controls (Haghighizadeh et al., 2015). Lipids serve as messengers and regulators of inflammation, as well as being precursors for hormones and employed for energy storage (Watson, 2006). One of the most vulnerable targets for free radicals is lipids (Rajani & Ashok, 2009). The liver is a metabolically diverse organ that regulates the chemical environment inside the body (Behl et al., 2011). It is particularly important in the synthesis and regulation of circulating lipids, lipoproteins, triglycerides, cholesterol, cholesterol esters, and in the degradation of cholesterol and steroids. The liver is the primary location for detoxification and promotes clearance by excreting water-soluble compounds, as well as being the key organ of the antioxidant defense system (Arulmozhi et al., 2010). Organochlorine, like other pesticides, remains in the environment and bioaccumulates in human tissues (Botella et al., 2004).

Table 5 shows the serum lipid profile of pesticide-exposed farmers and controls, including cholesterol, triglycerides, HDL-C, LDL-C, and VLDL-C. The average levels of serum cholesterol, triglycerides, LDL-C, and VLDL-C were found to be higher in pesticide-exposed farmers (219.02 ± 21.17 , 199.0 ± 25.98 , 148.85 ± 19.87 , and 39.85 ± 7.19 mg/dl, respectively) compared to controls (185.16 ± 11.09 , 160.72 ± 11.13 , $101.25 \pm$

10.23, and 32.14 ± 2.22 mg/dl, respectively). The difference was statistically significant ($P < 0.05$). Pesticide-exposed farmers, on the other hand, had considerably lower blood HDL-C levels than healthy controls (21.10 ± 3.93 vs. 39.61 ± 1.16 mg/dl). Pesticide-exposed farmers had a significant increase in cholesterol and triglyceride levels, followed by a significant decrease in phospholipid levels, compared to controls, indicating severe pesticide-induced hyperlipidemia. (Sharma et al., 2010) previously reported that organochlorine insecticides cause hepatotoxicity. Previously, rats and mice fed an organochlorine pesticide-contaminated diet had higher serum levels of triglycerides, cholesterol, and phospholipids (Boll et al., 1995; Ravinder et al., 1990).

The findings of this study (Table 5) are consistent with the reports mentioned above. Excessive synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS), among other reactive species, causes oxidative stress. Such chemicals are found in a variety of physiological conditions and constitute an important part of human metabolism (Marrocco et al., 2017). Through oxidative and antioxidant indicators, researchers can monitor the prevalence of oxidative stress. The amounts of Thiobarbituric acid reactive substances (TBARS) and protein carbonyls are measured to determine oxidative indicators. Considering that, lipid peroxidation (LPO), which is the leading cause of cell death, as well as DNA damage, enzyme inactivation, and other factors, Oxidation of hormones and DNA are both markers of oxidative cell damage (Ruas et al., 2008). LPO, in particular, has been proposed as one of the pesticide-induced toxicity pathways (Santi et al., 2011). Pesticide-induced oxidative stress has been the subject of toxicological research for more than a decade as a potential toxicity mechanism. The direct measurement of lipid peroxidation by-product malondialdehyde (MDA; the end result of lipid peroxidation) can demonstrate the toxic effects of pesticides on humans, especially by eliminating

radical generation (Muniz et al., 2008). When compared to control subjects, there was a significant rise in TBARS or MDA in the exposed population (Table 6). The increased generation of MDA or TBARS in the sprayer population could be attributable to enhanced membrane peroxidation. The basic process by which any and related radicals, including superoxide and hydroxy radicals, produce membrane damage in lipid peroxidation is documented in the literature (LPO). LPO can be triggered by free radical intermediates as well as superoxide hydroxy radicals. LPO causes bio-membrane and subcellular organelle degradation. The oxidation of microsomal membranes high in polyunsaturated fatty acids is a concern (Freeman & Crapo, 1982). However, pesticides were found to enhance lipid peroxidation in the liver and kidneys of experimental animals (Pawar & Kachole, 1978). After eight weeks of therapy, oral administration of DDT (100 and 200 ppm) and lindane (40 and 80 ppm) dose-dependently raised TBARS levels in serum (Koner et al., 1998). DDT is shown to have severe acute effects on male Wistar rats, and TBARS levels were found to be considerably greater (Li et al., 2017).

The toxicity of pesticides has been linked to reactive oxygen species (ROS). Organophosphates cause oxidative stress alterations that are unique to them (Abdollahi et al., 2004). Organophosphates induce characteristic changes in oxidative stress (Abdollahi et al., 2004). When compared to controls, sprayers exposed to organophosphate, carbamate, and organochlorine pesticides had significantly higher MDA (TBARS) levels, suggesting that oxidative stress may play a role in pesticide toxicity (Prakasam et al., 2001). (Varga & Matkovics, 1997). Our findings are in agreement with other research that suggested pesticides cause oxidative stress in humans, showing that MDA, the last product of lipid peroxidation, was found to be significantly higher in sprayers compared to controls, and that serum MDA or TBARS levels were 1.8 times higher in farm workers compared to

controls. These findings suggest that free radicals may play a role in organochlorine-induced immunotoxicity (Li et al., 2017). Organisms have evolved mechanisms to minimize the impacts of radicals produced in the cellular membrane. The antioxidant system, such as superoxide dismutase, is involved in this mechanism (SOD). Antioxidant systems work by converting highly reactive oxygen species into less reactive intermediates that are no longer harmful to the cell (Birben et al., 2012). However, for healthy biological integrity to be maintained, there must be a balance between oxidation and antioxidant levels in the system. SOD is one of the most active enzymes in the body, with sufficient activity to dismutate superoxide anions formed during oxidative stress in cells (Birben et al., 2012). SOD and Glutathione-S-transferase activity were used to assess the antioxidant profile (GST).

The exposed group's SOD activity was substantially higher than the control group's (Table 6). SOD is an important antioxidant enzyme that converts superoxide anion to peroxide. The enhanced activity of this enzyme indicates a higher production of superoxide anion as a result of pesticide exposure. When the amount of reactive oxygen species (ROS) produced in a cell or tissue exceeds the cell's antioxidant capacity, oxidative stress occurs (Sevanian & Peterson, 1984). Chronic pesticide exposure has already been linked to increased SOD activity, according to (Shadnia et al., 2005). Other studies have found that pesticide exposure to various categories, such as organophosphates, carbamates, or pyrethroids, causes oxidative stress in pesticide sprayers (Da Silva et al., 2012; Prakasam et al., 2001). Glutathione S-transferase (GST) is a detoxification gene family that plays a key role in the detoxification of exogenous chemicals (Gui et al., 2009). We observed increased oxidative stress in this study as a result of several pesticides used by farmers. As a result of the oxidative stress, GST enzyme gene expression may have risen, resulting in increased GST activity in our study (Table 6).

The addition of glutathione to endogenous xenobiotics is catalyzed by GST (Satheesh et al., 2010). GSH and GST have been used as markers of toxic effects of exposure to diverse xenobiotics due to their high sensitivity to environmental contaminants (Manno et al., 1985). In the current study, GST levels were found to be significantly higher ($P < 0.05$) in pesticide-exposed individuals (Table 6). GST activity was significantly increased ($p < 0.05$) in serum workers exposed to pesticides. In rats exposed to the insecticide, (Otitoju & Onwurah, 2007) found an increase in plasma GST activity. Increased GST activity has been associated with pesticide resistance in all of the major classes. Increased GST activity in tissues could signal the development of a defense mechanism to counteract pesticide effects, as well as the prospect of more effective pesticide toxicity prevention (Ranson & Hemingway, 2005).

CONCLUSION

Some of the organochlorine pesticides assessed in this study have lower and higher levels than previously reported. The prevalence and levels of several pesticides, particularly p, p'-DDT, o, p'-DDT, o, p'-DDE, and p, p'-DDD, remain high, requiring more effective and continuous defenses. Food of animal origin, fish, vegetables, fruits, and cereals from polluted areas will be monitored frequently and continuously, allowing consumers to take precautions while consuming these pesticides. The findings of this study revealed that some organochlorine pesticide residues are still present in the environment. 4 out of 16 pesticides were detected in blood samples from Aljebal Alakhtar, indicating that each person is exposed to and carries a body burden of multiple pesticides, which could be due to a combination of direct and indirect pesticide exposure. Organochlorine pesticides make up the majority of total pesticide concentrations in blood samples from Aljebal Alakhtar. The presence of organochlorine pesticides in the blood indicates that they stay in the body for a long period. Long-term

exposure to various pesticides causes cytotoxicity, resulting in biochemical changes in particular, according to this study. Several biomarkers could be used to monitor the early impacts of pesticides on agricultural workers' health.

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ETHICS

The Libyan National Committee for Biosafety and Bioethics gave their clearance to this retrospective study.

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الآثار الضارة لبقايا المبيدات العضوية الكلورية على العوامل البيوكيميائية لدى العمال الزراعيين الليبيين

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المستخلص: يتعرض عمال الزراعة لخطر التعرض للمواد السامة مثل مبيدات الآفات. ولتقدير الخطر الفردي لمناولة مبيدات الآفات، يلزم الرصد البيولوجي لتأثيراتها على العمال الزراعيين. لم يتم إجراء أي بحث بين عمال الزراعة الليبيين. لذلك صممت هذه الدراسة لدراسة تأثير المعايير البيوكيميائية للتلوث بالمبيدات بين عمال الزراعة الليبيين في الجبل الأخضر، ليبيا. وفي منطقة الجبل الأخضر، تم أخذ 45 عينة دم من عمال زراعيين ذكور تعرضوا للمبيدات في حقول المحاصيل لفترة طويلة، فيما تم أخذ 25 عينة دم من مجموعة لم يتعرضوا للمبيدات (مجموعة المقارنة). تم استخدام المحاليل القياسية لتقدير البلازما ALT، AST، SOD، GST، ALP، البروتين الكلي، والألبومين، والجلوبيولين، والبليروبين الكلي، والكوليسترول الكلي، والدهون الثلاثية HDL-C، LDL-C، VLDL-C، واليوريبا، والكرياتينين أيضاً كالكوليسترول الكلي، والدهون الثلاثية، HDL-C، LDL-C، VLDL-C، اليوريبا، والكرياتينين. تم استخدام المواد التفاعلية المنتجة من حمض ثيوباربيتوريك (TBARS) لتقدير بيروكسيد الدهون في مصل الدم. باستخدام كروماتوغرافيا الغاز (GC-ECD) تم تحليل عينات الدم من بقايا المبيدات العضوية الكلورية. بالمقارنة مع مجموعة التحكم، كان لدى العمال أنشطة SOD، GST، ALP، AST، ALT أعلى بشكل ملحوظ، بالإضافة إلى مستويات أعلى من إجمالي الدهون، والبليروبين، والكرياتينين، واليوريبا، بالإضافة إلى تركيزات أعلى بشكل ملحوظ من TBARS. علاوة على ذلك، كان التعرض لمبيدات الآفات على المدى الطويل مرتبطاً أيضاً بتقليل البروتين الكلي، والألبومين، والجلوبيولين، فضلاً عن تقليل مستوى HDL-C. يبدو أن التعرض لمبيدات الآفات يؤثر على مختلف العوامل البيوكيميائية بشكل عام. وهذه المؤشرات الحيوية تشير إلى الآثار الضارة المرتبطة بمبيدات الآفات على العمال الزراعيين، مما يشير إلى أنه ينبغي استخدامها للرصد الروتيني لتأثيراتها.

الكلمات المفتاحية: مستوى الدهون؛ وظائف الكبد؛ وظائف الكلى؛ مؤشرات الاجهاد التأكسدي.

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