Al-Mukhtar Journal of Sciences **37 (1): 22-28, 2022**
ISSN: online 2617-2186 print 2617-2178
Journal Homepage https://omu.edu.ly/journals/index.php/mjsc/index

Evaluation of Hepatotoxicity Effect of Sodium Stibogluconate (Pentostam) in Mice Model

Kaula. A. Saad[1], Intisar. O. Abdalla[2] , Hanan. A. Alkailani [3], Ahmed. M. Elbakush[1] and Zena. A. Zreiba [1]

[1]Administration of Zoonotic Diseases Control-National Center of Diseases Control - Tripoli-Libya
[2]Department of Basic Veterinary Medical sciences (Pathology branch)- Faculty of Veterinary Medicine-Omar Al-Mukhtar University-Albayda-Libya
[3] Department of Basic Veterinary Medical sciences (Pharmacology branch)- Faculty of Veterinary Medicine-Omar Al-Mukhtar University-Albayda-Libya

Received: 13 October 2021/ Accepted: 01 March 2022
Doi: https://doi.org/10.54172/mjsc.v37i1.738

Abstract: The recommended treatment for visceral and cutaneous leishmaniasis is pentavalent antimony at a dosage of 20 mic/kg/day for 28 days. Some studies suggested that antimonial pentostam has multiple acute and chronic adverse effects, which can be minimized by using the lowest effective dose. The present study is aimed to evaluate the hepatotoxicity effect of sodium stibogluconate (pentostam) in mice as a model. Adult male albino mice were divided into three groups, seven mice each, injected with 20 mic/kg pentostam in addition to a control group. Later 14 days, groups II, III, and IV were tested after one, three, and six weeks respecttively. The mice's serum and liver tissues were collected, and biochemical and histopathological measurement were carried out. Biochemical analysis of the serum obtained showed a significant increase in the levels of AST, ALT, and ALP in groups II and III when compared with the control group. In parallel, the histopathological assessments of the liver tissue proved hepatocytic necrosis. From this study, it can be concluded that the antimonial pentostam has a hepatotoxicity effect on treated mice.

Keywords: Leishmaniasis, Pentostam, Hepatotoxicity, Albino Mice.

INTRODUCTION

Leishmaniasis is an important tropical disease targeted by the world health organization (WHO) (Meeting & Organization, 2010) that affects both humans and animals (Dantas-Torres, 2006). There are several different forms of leishmaniasis in people. It comes mainly in two forms: cutaneous (CL) and visceral leishmaniasis, (VL) (Pearson & de Queiroz Sousa, 1996). The natural hosts of leishmaniasis are forest rodents, while the disease is transmitted by sandflies that frequently feed in the early evening (Killick-Kendrick, 1990). Leishmaniasis, like most protozoan diseases, is largely a problem of developing countries. Therefore, there is a need to improve cheap and effective anti-leishmanial drugs.

Pentavalent antimonal agents (Sbv), such as sodium stibogluconate (pentostam) and N-methylglucamine antimonite (glucantime) are first-line drugs for treating leishmanial infection (Gupta, 1953). Although the pentavalent antimonial sodium stibogluconate

***Corresponding author:** Kaula. A. Saad *mohamedkaula@gmail.com* , Administration of Zoonotic Diseases Control-National Center of Diseases Control - Tripoli-Libya

(Pentostam) was first recognized as clinically effective in 1947 (Berman, 1988). Several studies have confirmed that pentavalent antimonials, in adequate doses, are effective against several types of leishmaniasis.

Pentavalent antimonials are usually used despite their toxicity, intricate administration, a quite high cost of treatment, and the appearance of resistant parasite strains. There is a study that recorded transitory abnormalities of serum alanine aminotransferase (ALT) which are dose related, during treatment of CL with sodium stibogluconate (Behrens & Doherty, 1993). While another study recorded on CL patients an increase in both ALT and glutathione S-transferase B1 (GST) and a fall in the caffeine clearance (CCL) (Hepburn et al., 1994). On the other hand, various studies in patients with VL have been reported that sodium stibogluconate (Pentostam) caused various adverse effects such as anorexia, abdominal pain, diarrhea, nausea, vomiting, pancreatitis, reversible elevation of liver enzymes, myalgia, arthralgia, proteinuria, phlebitis, optic atrophy, acute kidney injury, hepatic necrosis, and bone marrow hypoplasia (Gupta, 1953; Harrison et al., 1998; Thakur et al., 1988).

The aim of the present study is to evaluate the Hepatotoxicity of Sodium Stibogluconate (Pentostam) in mice as a model.

MATERIALS AND METHODS

E**xperimental design:** Adult albino mice weighing 25-35g at the age of 8-10 weeks. The animals were housed in standard laboratory conditions of temperature, 12 h light and dark places, with food and water ad libitum. The animals were separated into four groups of seven animals each. Group I (control group), was treated with distilled water as a vehicle for 14 days. All the animals of groups II, III & IV were administered with pentostam at a dose of 20 mg/kg b.w. once a day intra-peritoneal (IP) injection for 14 days. Seven days after receiving the final dose, the mice of group II were tested, while group III were tested after three weeks, and group IV were tested after six weeks.

Biochemical analysis: Blood samples were placed into ice-chilled disposable siliconized glass tubes. Centrifugation of the blood samples was carried out at 4000 rpm at 4°C for 15 min to get serum, which was kept at -20 °C for assessing serum enzyme levels. The concentration of the serum enzyme, like alanine aminotransferase (ALT), was measured by Hitachi-902 fully automated chemistry analyzer by Roche diagnostics (Bergmeyer et al., 1986a), aspartateam-inotransferase (AST) by Hitachi-902 fully automated chemistry analyzer by Roche diagnostics (Bergmeyer et al., 1986b), and alkaline phosphatase (ALP) levels were measured by Hitachi-902 fully automated chemistry analyzer by Roche diagnostics (Tietz et al., 1983).

Histopathological Studies: After blood collection, the livers of each animals group were dissected and preserved in the formalin solution. The specimens were fixed in paraffin to prepare for sectioning (4-5 μm) then subjected to hematoxylin and eosin stain (H & E) for photomicroscopic observations (Galigher & Kozloff, 1971).

Statistical analysis: The data are expressed as Mean ± SEM. The study results were analyzed using one-way ANOVA followed by a Tukey test (SPSS version19). The level of significance was $P \leq 0.001$ and $P \leq 0.0001$.

RESULTS

Effect of IP injection of Sodium Stibogluconate (Pentostam) on Liver enzyme ALT, AST, and ALP levels (U/L) in albino mice: The results of liver function tests in this study revealed a significant increase in the levels of AST, ALT, and ALP

enzymes in group II that was put down after seven days of IP injection of pentostam, in comparison with the control group (Table 1). While the serum AST level decreased to normal levels in administrated group III that were put down after 21 days of treatment, on the contrast of ALT and ALP enzymes concentration in the same group compared to the normal group. In addition, there was no significant change in these parameters in the animals of group IV compared to the control group.

Table (1): Effect of IP injection of Sodium Stibogluconate (Pentostam) on liver enzymes.

Enzyme	Control group	Group II	Group III	Group IV
ALT (U/L)	205.14 ± 1.9	308.00 ±4.2 *	277.42 ± 4.6*	215.42 ± 2.3
AST (U/L)	83.85 ± 1.4	142.42 ± 2.3**	88.71 ± 0.5	84.57 ± 0.9
ALP (U/L)	112.00 ±2.1	202.71 ± 0.86*	171.00 ± 0.84*	115.85 ± 1.2

All values are presented as Mean ± SEM, n =7,
*P ≤ 0.001, **P ≤ 0.0001.

Histopathological study: Histopathological studies of mice liver tissue from Group I animals show normal hepatic cells with a central vein (Fig.1).

In the pentostam treated group (Group II), severe hepatotoxicity was observed by congestion of the central vein and portal blood vessels with marked hepatocellular vacuolar degeneration and severe necrosis. A mild degree of restoration of a large number of hepatic cells with mild vacuolar degeneration and scattered necrotic cells was observed in Group III, and normal liver architecture with normal hepatocytes arranged in normal sheets or cord around the central vein in Group IV (H&E, x400) (Fig 1).

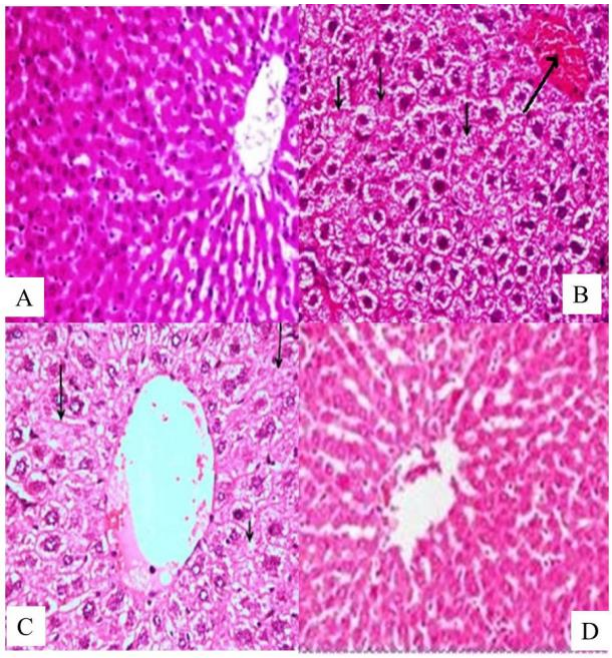

Figure (1): (H&E, ×400): A: Photomicrograph of liver tissue of control group showing normal hepatic cells with central vein.
B: Photomicrograph of Group II showing severe necrosis (short arrow) with congestion of the central vein (long arrow) and disappearance of nuclei.
C: Photomicrograph of Group III showing normal hepatocytes with mild vacuolar degeneration (short arrow) and scattered necrotic cells (long arrow).
D: Photomicrograph of Group IV showing normal liver architecture with normal hepatocytes.

DISCUSSION

The liver is considered to be highly sensitive to toxic agents. The study of different enzyme activities, such as ALT, AST, ALP, total bilirubin, and total protein have been found to be of great value in clinical and experimental liver damage assessment (Vaishwanar et al., 1976). It was observed that the animals treated with pentostam resulted in significant hepatic damage, as shown by the elevated levels of liver enzymes. These changes in the enzymes' levels will reflect in hepatic structural integrity. The elevation in the AST is usually accompanied by a rise in ALT levels, which plays a vital role in the conversion of amino acids to keto acids (Sallie & Tredger, 1999). On the other hand, the serum ALP level is related to hepatic cell damage. An increase in

serum levels of ALP is due to increased synthesis in the presence of increasing biliary pressure (Moss & Butterworth, 1974). Our results are in agreement with the study which reported increases in AST and ALT levels in the treatment of CL in US military personnel with sodium stibogluconate (Wortmann et al., 2002). Six weeks after treatment, ALP, ALT, and AST levels had almost returned to normal. Our findings indicate that sodium stibogluconate is associated with acute hepatocellular damage.

This damage ceased after therapy stopped and was rapidly reversible. These results are consistent with the results of the research which compare the efficacy and adverse effects of sodium stibogluconate and the aminoglycoside aminosidine in CL patients and reported increases in ALT and AST during treatment with sodium stibogluconate, associated with a fall in caffeine clearance (Hepburn et al., 1993). After six weeks of treatment, ALT and AST had almost returned to pretreatment levels in every patient (Hepburn et al., 1994)

The histological examination of the liver tissue of albino mice was shown severe necrosis with hepatocellular vacuolar degeneration after seven days of pentostam injection, and a mild degree of necrosis with normal cells after three weeks of treatment. A recent study has published hydropic degeneration, focal and hepatocytic necrosis in the 20 mg/kg pentostam treatment group, and an irregular area of hepatocytes with condensed pyknotic nuclei (hepatocyte necrosis) in the 40 mg/kg pentostam group (Elammari & Sariti, 2021). Our findings are in agreement with the reality that the liver is highly oversensitive to being affected by toxic chemicals (Afshar et al., 2008). Moreover, other researchers reported that accumulation of pentostam is directly toxic to hepatocytes, this hepatocyte toxicity is increased by increasing the pentostam dose and duration time (Al-Jahdali & Bisher, 2007).

CONCLUSION

Biochemical investigation and histopathological analysis of liver tissue still have an important role to evaluate drug toxicity. The treatment of mice with therapeutic doses of pentostam induced hepatotoxicity characterized by clear histological changes in the liver. These histological changes needed about six weeks to return to normal. Consequently, pentavalent antimony pentostam may be included in the large list of offending agents that can cause drug-induced hepatotoxicity that requires more research.

REFERENCE

Afshar, S., Farshid, A., Heidari, R., & Ilkhanipour, M. (2008). Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion. Toxicology and industrial health, 24(9), 581-586.

Al-Jahdali, M. O., & Bisher, A. S. B. (2007). Testicular histopathological alterations in rats treated with sumithion® NP 25/2.5 EC, insecticide. J. Biol. Sci, 7(3), 520-525.

Behrens, R., & Doherty, J. (1993). Severe hepatitis A despite passive immunisation. The Lancet, 341(8850), 972.

Bergmeyer, H., Horder, M., Rej, R., & Committee, I. F. o. C. C. S. (1986a). Analytical Section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for alanine aminotransferase (l-alanine: 2-oxoglutarate aminotransferase, EC 2.6. 1.2). Journal of clinical chemistry and clinical biochemistry. Zeitschrift fÃ¼r klinische Chemie und klinische Biochemie, 24(7), 481-495.

Bergmeyer, H., Horder, M., Rej, R., & Committee, I. F. o. C. C. S. (1986b). Analytical Section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (l-alanine: 2-oxoglutarate aminotransferase, EC 2.6. 1.2). Journal of clinical chemistry and clinical biochemistry. Zeitschrift fÃ¼r klinische Chemie und klinische Biochemie, 24(7), 481-495.

Berman, J. D. (1988). Chemotherapy for leishmaniasis: biochemical mechanisms, clinical efficacy, and future strategies. Reviews of infectious diseases, 10(3), 560-586.

Dantas-Torres, F. (2006). Leishmune® vaccine: the newest tool for prevention and control of canine visceral leishmaniosis and its potential as a transmission-blocking vaccine. Veterinary parasitology, 141(1-2), 1-8.

Elammari, N. E., & Sariti, S. R. (2021). Anti-Leishmanial drug Pentostam induced histological changes to liver and kidney in male BALB/c wild mice. Health Sciences, 1(2), 07-14.

Galigher, A. E., & Kozloff, E. N. (1971). Essentials of practical microtechnique, Philadelphia, Lea & Fabiger Publisher, 2nd ed.

Gupta, P. S. (1953). Chemotherapy of leishmanial diseases, a resume of recent researches. The Indian Medical Gazette, 88(1), 20.

Harrison, W., Bradberry, S., & Vale, J. (1998). Sodium stibogluconate. UKPID Monograph, UK: National Poisons Information Services,(Birmingham Centre).

Hepburn, N., Siddique, I., Howie, A., Beckett, G., & Hayes, P. (1993). Hepatotoxicity of sodium stibogluconate in leishmaniasis. The Lancet, 342(8865), 238-239.

Hepburn, N., Siddique, I., Howie, A., Beckett, G., & Hayes, P. (1994). Hepatotoxicity of sodium stibogluconate therapy for American cutaneous leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 88(4), 453-455.

Killick-Kendrick, R. (1990). The life-cycle of Leishmania in the sandfly with special reference to the form infective to the vertebrate host. Annales de Parasitologie humaine et comparée, 65, 37-42.

Meeting, W. E. C. o. t. C. o. t. L., & Organization, W. H. (2010). Control of the Leishmaniases: Report of a Meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22-26 March 2010. World Health Organization.

Moss, D. W., & Butterworth, P. J. (1974). Enzymology and medicine. Pitman Medical.

Pearson, R. D., & de Queiroz Sousa, A. (1996). Clinical spectrum of leishmaniasis. Clinical infectious diseases, 1-11.

Sallie, R., & Tredger, J. (1999). Willaiam. Drugs and the liver. Biopharm Drug Dispos, 12, 251-259.

Thakur, C., Kumar, M., Kumar, P., Mishra, B., & Pandey, A. (1988). Rationalisation of regimens of treatment of kala-azar with sodium stibogluconate in India: a randomised study. Br Med J (Clin Res Ed), 296(6636), 1557-1561.

Tietz, N., Rinker, A., & Shaw, L. (1983). International Federation of Clinical Chemistry. IFCC methods for the measurement of catalytic concentration of enzymes. Part 5. IFCC method for alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1. 3.1). IFCC Document Stage 2, Draft 1, 1983-03 with a view to an IFCC Recommendation. Clinica chimica acta; international journal of clinical chemistry, 135(3), 339F-367F.

Vaishwanar, I., Kowale, C., & Jiddewar, G. (1976). Effect Of 2 Ayurvedic Drugs Shilajeet And Eclinol On Changes In Liver And Serum-Lipids Produced By Carbon-Tetrachloride (Vol. 14, pp. 58-61): Council Scientific Industrial Research Publ & Info Directorate, New Delhi ….

Wortmann, G., Miller, R. S., Oster, C., Jackson, J., & Aronson, N. (2002). A randomized, double-blind study of the efficacy of a 10-or 20-day course of sodium stibogluconate for treatment of cutaneous leishmaniasis in United States military personnel. Clinical infectious diseases, 35(3), 261-267.

تقييم التسمم الكبدي بعقار ستبجلوكونات الصوديم (البنتوستام) في الفئران

خوله احمد سعد[1] ، انتصار عثمان عبدالله [2]، حنان علي الكيلاني[3]، احمد البكوش [1]، زينه عبد الفتاح زرايبه[1]

[1] إدارة الأمراض المشتركة، المركز الوطني لمكافحة الأمراض، طرابلس– ليبيا

[2] قسم العلوم الطبية البيطرية الأساسية، شعبة الأمراض، كلية الطب البيطري، جامعة عمر المختار، البيضاء– ليبيا

[3] قسم العلوم الطبية البيطرية الأساسية، شعبة الأدوية، كلية الطب البيطري، جامعة عمر المختار، البيضاء – ليبيا

تاريخ الاستلام: 13 أكتوبر 2021 / تاريخ القبول: 01 مارس 2022

Doi:https://doi.org/10.54172/mjsc.v37i1.738

المستخلص: تعد مركبات الانتيمونيال العلاج الأساسي المستخدم لمرض اللشمانيا بنوعيها الجلدي، والحشوي بجرعة 20 ميكروجراماً لكل كيلوجرام من وزن الجسم لمدة 28 يوما، وقد نشرت العديد من الدراسات أن عقار البنتوستام يسبب أعراضا جانبية حادة، ومزمنة للمرضى حتى في الجرعات الصغيرة. هدف هذه الدراسة اختبار إمكانية حدوث تسمم كبدي في الفئران أنموذجاً بعد حقنها بعقار البنتوستام. واعتمدت التجربة على استخدام فئران الالبينو البالغة، وتقسيمها إلى أربعة مجموعات. المجموعة الأولى: هي المجموعة الضابطة، ولم تحقن بالدواء، في حين حقنت الفئران في المجموعات الأخرى بالبنتوستام بجرعة 20 ميكروجراماً لكل كيلوجرام من وزن الجسم في التجويف البروتوني لمدة 14 يوما، ثم ذبحت المجموعات الثلاثة، وهي: الثانية، والثالثة، والرابعة، حسب الترتيب بعد أسبوع، وبعد ثلاثة أسابيع، وبعد ستة أسابيع من آخر جرعة، وجمعت عينات الدم لكل مجموعة على حدة، بالإضافة إلى الأنسجة الكبدية لإجراء التحاليل البيوكيميائية، والنسيجية. وقد أظهرت الدراسة ارتفاعاً في أنزيم ناقلة ألألانين، وأنزيم ناقلة الاسبرتات، وانزيم الفوسفاتاز القلوي في المجموعة الثانية، والثالثة، بالمقارنة مع المجموعة الضابطة، بالإضافة إلى حدوث تنخر، ونزيف في الأنسجة الكبدية في هذه المجموعات مما يدل على أن عقار البنتوستام سبب تسمماً كبدياً في الفئران.

الكلمات المفتاحية: اللشمانيا، البنتوستام، التسمم الكبدي، فئران الالبينو.

*خوله احمد سعد mohamedkaula@gmail.com، إدارة الأمراض المشتركة، المركز الوطني لمكافحة الأمراض، طرابلس– ليبيا