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

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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 respectively. 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 antimonial 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...
(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 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**

**Experimental 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), aspartateaminotransferase (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 ≤ 0.001 and P ≤ 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.

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

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).

**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 treatment 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.

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المستخلص: تعد مركبات الانتيموني العلاج الأساسي المستخدم لمرض الشمانية نوعية الجلدي، والخشي بجرعة 20 ميكروجرامًا لكل كيلوجرام من وزن الجسم لب şa 28 يومًا، وقد نشرت العديد من الدراسات أن عقار البنوتايم سبب أعراض جانبية حادة، ومزمنة للمرضى حتى في الجرعات الصغيرة. هدف هذه الدراسة اختبار إمكانية حدوث تسمم كبدى في الفئران أمنًا نموًا بعد حقنها عقار البنوتايم. واعتمدت التجربة على استخدام فئران الألبينو البالغة، وتقييمها الى أربعة مجموعات. المجموعة الأولى: هي المجموعة الضابطة، ولم تحقن بالدواء، في حين حققت الفئران في المجموعات الأخرى بالبنوتايم بجرعة 20 ميكروجرامًا لكل كيلوجرام من وزن الجسم في التجويف البروتوتي لمدة 14 يومًا، ثم ذبحت المجموعات الثلاثة، وهي: الثانية، والثالثة، والرابعة، حسب الترتيب التناثر، بعد أسبوع، وبعد ثلاثة أسابيع، وبعد ستة أسابيع من آخر جرعة، وجمعت عينات الدم لكل مجموعة على حدة، بالإضافة إلى الأنسجة الكبدية لإجراء التحليل البكيميواسي، والنسجية. وقد أظهرت الدراسة ارتفاعًا في أنزيم دالفة أكلاينين، وأنزيم ناقلة الأبيسينات، وإندوز 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 البنوتايم سبب تسممًا كبدًا في الفئران.

الكلمات المفتاحية: الشمانية، البنوتايم، التسمم الكبدى، فئران الألبينو.