

Biochemical And Hematological Evaluation Pattern Of Pentostam- Induced Hepatotoxicity And Nephrotoxicity In Albino Mice

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Abstract

This study was designed to examine the effect of different dose of Pentostam administration in male albino mice as a model, to determine their effect on some biochemical and hematological parameters. 28 adult male of BALB/c wild mice aged 8- 12 weeks and weighing 25-39 gm were used and i.p. injected with 10mg/kg, 20mg/kg, and 40mg/kg pentostam in addition to a control group. After 28 therapeutic days, blood sample were collected from each animal. The result of Biochemical Index of the serum obtained showed that there were increase significant differences between different concentration doses on AST, ALT, ALP, Urea, Creatinine and BUN as compare to control group at ($p > 0.01$). Conversely in case of Albumin and Total protein were the result showed decrease significant difference between different concentration doses on this parameter as compare to control group of the same at ($p > 0.01$). while no significant differences between the different concentrations group of doses on Total bilirubin, where the F value was (1.00). The result of Hematological Index of blood sample showed that there were decrease significant difference at ($P \leq 0.01-0.05$) in hemoglobin, HCT, WBCs, LYM and MON concentration in the different concentrations dose of treatment as compared to control group. Conversely, for MCHC, NEU and EOS there were increase significant difference at ($P \leq 0.05-0.01$) as compared to the control group. While no significant changes ($P \leq 0.0-0.05$) in the parameters of RBCs, MCV, MCH, BAS count and Platelet were found when compared with control animals. Biochemical and hematological evaluation pattern still an important role to evaluate drug toxicity. However, the results of this study concluded that, different doses of Pentostam can cause hepatotoxicity and nephrotoxicity. Therefore, it will be important of change in anti-leishmanial regimen to reduce their side effect.

INTRODUCTION:

Leishmaniasis is a major vector-borne disease, it is distributed worldwide and threatens 350 million people, and belonging to the group of Neglected Tropical Diseases (NTD). It is devastating protozoan disease caused by obligate intracellular protozoan parasites of genus *Leishmania* (Zavitsanou *et al.*, 2008), it is containing nearly 30 species, 20 of which infect man (Ashford, 2000). It's represents a major public health problem due to lack of vaccine.

There are five main diseases caused by this parasite: Cutaneous leishmaniasis (CL), Visceral leishmaniasis (VL), Mucocutaneous leishmaniasis (MCL), Post-kala azar dermal leishmaniasis (PLDL) and Diffuse cutaneous leishmaniasis. there is no morphological difference between of all species of *Leishmania* parasite and differentiation is done biochemically (Sherris *et al.*, 1984). Leishmaniasis affects people living all over the world except Australia and Antarctica. In Libya, as the record of National Center for Disease Control-Libya, CL is endemic in most parts of the country while VL is limited to the North Eastern part of the country.

The drugs recommended in the management of all types of leishmaniasis are pentavalent antimonials such as Sodium stibogluconate (SSG) or Pentostam which is drug of choice and has been successfully used for treatment of leishmaniasis diseases worldwide for decennia (Croft and Coombs, 2003).

Previous study recorded that there is a transitory abnormalities of serum alanine aminotransferase (ALT), by using sodium stibogluconate as treatment of CL (Behrens & Doherty, 1993). Furthermore, a study by (Hepburn *et al.*, 1994) revealed an increase in both ALT and glutathione S- transferase B1 (GST) and a fall in the caffeine clearance (CCL) on CL patients treated by using the same drug. while other studied on using the same drug as treatment to VL patients reported various adverse effects such as reversible elevation of liver enzymes, acute kidney injury, hepatic necrosis (Gupta, 1953; Harrison *et al.*, 1998; Thakur *et al.*, 1988).

As the recommended regimen of SSG has evolved, the doses and duration have been increased, with the current recommendation being 20 mg/(kg) for 20 days for cutaneous disease and 28 days for visceral or mucosal disease (Herwaldt and Berman, 1992). It requires a prolonged course of treatment and has been losing its efficacy in some regions and leads to multiple acute and chronic adverse effects, which can be minimized by using the lowest effective dose, consequently previous reports of SSG toxicity at a dosage of 20 mg/kg have been limited and varied (Bryceson, 1987 & Herwaldt and Berman, 1992), hence was shown to decrease over the world but still used in Libya. The aim of the present study was to investigate the side effect of Pentostam administration in male albino mice as a model

Key words: Leishmaniasis- Pentostam- male albino mice-biochemical parameters -hepatotoxicity – nephrotoxicity

2. MATERIAL AND METHODS:

2.1. Animals and Experimental Design:

28 adult male of BALB/c wild mice aged 8- 12 weeks and weighing 25-39 gm were used.

Mice were housed in controlled environment; the room temperature was maintained at 25±2°C and 10 hrs dark/ 14 hrs light cycle two weeks prior to the initiation of the experiment. They were held in plastic cages and maintained on commercial basal food and water ad libitum to all groups. Mice were received human care were allowed to acclimatize for two weeks prior to the start of the study. All the experiments were carried out in compliance with the guide for the care and use of laboratory animals. After 2 weeks of adaptation, all animals were randomly divided into four groups of six mice each and treated intraperitoneally (i.p) as below for 28 consecutive days:

- a- **Group 1 (Control):** served as control group and received intraperitoneally 1 ml/kg of normal saline once daily without any ingredient of Pentostam.
- b- **Group 2 [first treated group (1T)]:** animals in this group received intraperitoneally 0.25 mg Pentostam in 0.25 ml sterile distilled water which equivalent 10 mg/kg.
- c- **Group 3 [Second treated group (2T)]:** animals in this group received intraperitoneally 0.5 mg Pentostam in 0.25 ml sterile distilled water which equivalent 20 mg/kg.
- d- **Group 4 [Third treated group (3T)]:** animals in this group received intraperitoneally 1.0 mg Pentostam in 0.25 ml sterile distilled water which equivalent 40 mg/kg.

Fresh dilutions water made each day and the remaining undiluted drug was stored in the refrigerator at 4°C.

2.2. Chemicals:

The drugs used in the experiment include Anti-leishmaniasis –Pentostam®- (Sodium Stibogluconate Injection B.P.) each ml contains 100 mg are available in 30 ml bottle, which obtained from Health ministry of Libya. The drugs were diluted to get required concentrations (doses) (10mg/kg, 20mg/kg and 40mg/kg) using sterile double distilled water. Considering the average weight of an adult mouse to be 25 grams. The different concentration doses have been given intraperitoneally (i.p), each mouse received the determined dosage once a day for 28 consecutive days.

2.3. Evaluated Parameters:

Serum biochemical analysis: at the end of 28 days, blood sample were collected from each animal (the control and experimental animals) by sacrificed by cervical dislocation followed by decapitation, the blood samples obtained were collected into plain sample tubes and centrifuged at 3000 rpm for 15 minutes to separate the serum. Serum was preserved in eppendorf tubes and sent to Al- Saleem Medical Laboratory-Benghazi- Libya, to estimate of:

2.3.1. **Liver Function Test:** serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total Bilirubin (TBIL), Albumin and Total Protein.

2.3.2. **Renal Function Test:** Serum Urea, Creatinine and BUN

2.3.3. **Hematological profile**

2.4. Statistical analysis:

Data were statically evaluated by using one-way ANOVA. Wherever the ANOVA values were found to be significant, Duncan's new multiple range test (DMRT) was applied (SPSS computer software). The values were considered significant at the level of ($P \leq 0.01-0.05$).

3. RESULT

After 28 i.p Pentostam therapeutic days and finishing the serum estimated parameters of collected serum, the obtained results were as the follows:

3.1. Biochemical Index:
3.1.1. Liver Function test:

Table 1: Effects of intraperitoneal administration of different doses of Pentostam on serum liver functions.

Parameters (unit)	Control	1 th T Pentostam 10mg/kg	2 nd T Pentostam 20mg/kg	3 th T Pentostam 40mg/kg	F-test	P-value
AST (U/L)	144.25±2.71d	386.93±39.36c	594.63±48.34b	778.15±56.90a	125.295	.000
ALT (U/L)	49.19±20.70b	61.43±18.12b	93.63±13.94a	113.17±8.58a	10.065	.004
ALP (U/L)	129.46±7.67c	224.27±23.76b	270.62±36.39b	366.83±48.82a	27.007	.000
Total bilirubin(TBIL) (mg/dl)	0.10±0.0a	0.10±0.00a	0.10±0.00a	0.13±0.06a	1.000	.441
Albumin (mg/dl)	3.67±0.64a	3.97±0.20a	2.52±0.12b	1.69±0.15c	27.387	.000
Total protein (mg/dl)	5.57±0.15a	4.89±1.09ab	3.26±0.37c	4.23±0.38bc	7.817	.009

Values are expressed as mean±SEM.

Mean± standard error based on ANOVA analysis. Means in the same row followed by the same letters refer to not significantly different, while Means in the same row followed by different letters refer to significantly different ($P \leq 0.01-0.05$) according to Duncan Multiple Range Test (DMRT).

-The results showed that there were increase significant differences between different concentration doses on AST, ALT and ALP (where the F value were 125.295- 10.065 and 27.007 respectively) as compare to control group at ($p > 0.01$) and the highest increase was in concentration group 40mg/kg, followed by group 20mg/kg and then 10mg/kg. Conversely in case of Albumin were the result showed decrease significant difference between different concentration doses on this parameter (where the F value was 27.387) as compare to control group of the same at ($p > 0.01$), the highest decrease was in concentration group 10mg/kg, followed by group 20mg/kg and then 40mg/kg, while decrease Total protein were significant different at ($p > 0.01$) in 20mg/kg-40mg/kg and 10mg/kg respectively.

The results showed that there were not significant differences between the different concentrations group of doses on Total bilirubin, where the F value was 1.00

3.1.2. Renal function test:

Table 2: Effects of intraperitoneal administration of different doses of Pentostam on serum renal functions.

Parameters	Control	1 th T Pentostam 10mg/kg	2 nd T Pentostam 20mg/kg	3 th T Pentostam 40mg/kg	F-test	P-value	L.S.D
Urea mg/dl	36.66±2.84c	59.86±1.73b	127.82±8.49a	132.13±8.92a	171.027	.000	5.21
Creatinine mg/dl	0.36±0.07d	0.69±0.18c	1.14±0.10b	2.24±0.16a	112.297	.000	0.25
BUN	11.03±0.1b	12.98±1.33b	23.97±1.17a	24.70±2.08a	82.366	.000	2.58

Values are expressed as mean±SEM.

Mean± standard error based on ANOVA analysis. Means in the same row followed by the same letters refer to not significantly different, while Means in the same row followed by different letters refer to significantly different ($P \leq 0.05-0.01$) according to Duncan Multiple Range Test (DMRT).

The results showed that there were increase significant differences between different concentration doses of Urea, Creatinine and BUN (where the F value was 171.0- 112.297 and 82.366 respectively) as compare to control group at ($p > 0.01$) and the highest increase was in concentration group 40mg/kg, followed by group 20mg/kg and then 10mg/kg (table 2).

3.1.3. Hematological Index:

Parameters	Control	1 th T Pentostam 10mg/kg	2 nd T Pentostam 20mg/kg	3 th T Pentostam 40mg/kg	F-test	P-value
Hemoglobin(g/dl)	13.73±0.29a	10.70±0.17c	12.47±1.10b	11.63±0.57b	12.062	.002
RBCs ($10^6/\mu\text{L}$)	9.26±0.85a	8.78±0.59ab	8.34±0.90ab	7.42±0.52b	3.430	.073
HCT(g/dl)	46.10±0.87a	35.07±0.64b	39.37±3.36b	36.03±2.76b	14.925	.001
MCV	49.17±2.90a	47.87±1.97a	49.90±2.74a	48.53±2.40a	.355	.787
MCH	15.40±1.28a	15.33±0.91a	16.03±1.10a	16.37±0.25a	.809	.524
MCHC (g/dl)	31.23±0.75b	35.07±1.25a	33.60±1.25a	34.53±1.93a	4.649	.037
WBCs ($10^3/\mu\text{L}$)	7.83±1.93a	6.30±1.73a	4.70±1.39b	3.47±0.38b	4.922	.032
NEU (%)	9.98±0.46b	38.50±7.90a	36.79±6.84a	42.84±2.95a	22.601	.000
LYM(%)	73.57±3.45a	48.80±6.55b	50.03±5.82b	42.77±2.18c	23.617	.000
MON(%)	16.93±0.40a	9.56±2.22b	9.74±1.54b	10.64±1.22b	16.414	.001
EOS (%)	0.50±0.02c	2.13±0.17b	2.22±0.27ab	2.54±0.23a	65.911	.000
BAS (%)	0.95±0.03a	1.01±0.03a	1.22±0.24a	1.21±0.38a	1.060	.418
Platelet($10^3/\mu\text{L}$)	876.00±94.69b	920.00±3.61a	895.67±45.98ab	921.67±40.50a	.446	.727

Mean \pm standard error based on ANOVA analysis. Means in the same row followed by the same letters refer to not significantly different, while Means in the same row followed by different letters refer to significantly different ($P \leq 0.05-0.01$) according to Duncan Multiple Range Test (DMRT).

Effect of different concentration dose of Pentostam on hematological parameters were depicted in Table (3). The results showed that there were decrease significant difference at ($P \leq 0.01-0.05$) in hemoglobin concentration in the different concentrations dose of treatment as compared to control group, and the highest decrease was in treatment group 10mg/kg, followed by treatment group 40mg/kg and then 20mg/kg.

In the same table there is no significant changes ($P \leq 0.0-0.05$) in the parameters of RBCs, MCV and MCH were found when compared with control animals with a gradual decrease in the rate of RBCs in different doses from the lowest concentration to the highest concentration. While decrease concentration level of MCV and MCH in doses was 10mg/kg-40mg/kg and 20mg/kg respectively, conversely, for HCT and MCHC, there were significant difference at ($P \leq 0.05-0.01$) as compared to the control group.

The results in Table (3) also showed a significant decrease ($P \leq 0.01-0.05$) in the total number of white blood cells (WBCs), as well as in the percentages of LYM and MON in the different concentrations of treatment compared to the control group, while significant increase ($P \leq 0.01-0.05$) in in the percentage of NEU and EOS in the different concentrations of treatment compared to the control group.

On the other hand, the results showed that there were no significant differences between the value of different concentrations doses of Basophiles count and Platelet as compared to control group, where the F value was 0.418 and 0.727 respectively.

4. DISCUSSION

New approaches in the treatment of *Leishmania* spp. infections are well studied in BALB/c wild mice. However, treatment efficacy studies may overestimate the beneficial effect of the interventions. The approach applied here consists in an effort to identify and describe toxicity profile of a Pentostam by measurement of some parameters that may help to explain the effect of Pentostam in non-*Leishmania*-infected in murine models. Here the parameters measured in this study allowed to extend the indirect evaluation of liver and kidney function. Blood chemistry Analyzes that measure liver enzymes, as AST, ALT, ALP, TBIL, Albumin and Total protein to evaluate liver function, as well as measuring urea, creatinine and urea nitrogen (BUN) concentrations, are the most commonly used to assess the kidneys in general. Measuring these parameters and comparing the results of groups treated with the drug with the control group gives an indication of the health status of vitality, and thus any change in the results of the drug-treated groups compared to the control group indicate the presence of a condition that either appears only or actually exists, but at a lower stage, (Murray, *et al.*, 2010) The liver and kidney are important and sensitive organs to drug actions. Specifically, the liver is an organ for the metabolism sensitive to toxic substances, while the kidney was the main organ for excretion (Wallig *et al.*, 2017 and Ezeja, *et al.*, 2014)

Liver enzymes as AST and ALT are released into the serum after relative cells and tissues were damaged, in this study, there marked elevation in the level of AST and ALT ($P > 0.01$) with increase of concentration doses of Pentostam. In most toxicological studies, increased ALT is generally considered a practical and specific enzyme indicator of hepatocyte injury. This increase is not specified and AST often parallels serum ALT activity.

Similarly, an elevation in AST concentration is related to the number of hepatocytes affected and does not reflect the severity of the lesion or its reversibility on a pathological basis. This elevation of serum liver enzymes is similar to that reported by previous studies (Siddiqui, 2004; Rahman and Siddiqui, 2005; Wang, *et al.*, 2019; Saad, *et al.*, 2022)

Consequently, in this study there is gradually increase of ALP level with different concentrations of Pentostam doses from low concentration to high of drug during the 28-day study ($p < 0.01$). These results indicate that Pentostam may cause hepatotoxicity. The elevation of ALP is similar to that reported by previous studies which the serum of ALP has toxic effects on the liver in experimental mice and it is related to hepatic cell damage. Increased ALP activity is indicative of decreased bile, and may also be the result of intrahepatic bile duct obstruction or extrahepatic obstruction caused by swelling of hepatocytes. Drug-induced hepatocellular necrosis can also increase the activity of ALP, our finding agree with (Walley, 1966 ; Moss and Butterworth, 1974; Abdel Dayem, 2002 ; Abdel-Mageed, *et al.*, 2003 ; Foster, 2005 and Saad, *et al.*, 2022), on the other hand the level of TBIL in this study is not affected by Pentostam (Vítek, 2012).

These changes in the liver enzymes may be attributed to the ability of Pentostam to induce severe physiological and histological alterations in the liver cause liver syndrome's intensity correlated with the increase in dose and duration time, and it's affected by toxic chemicals that resulting in an increase in serum enzymes levels (Hassan, 2005, Al-Jahdali & Bisher, 2007, Afshar *et al.*, 2008, Elammari & Sariti, 2021 and Saad, *et al.* , 2022).

Kidneys are responsible for the elimination of metabolic waste and the control of the amount and composition of the body fluids. Nephrotoxicity is toxic to the kidneys. The present results showed that different doses of Pentostam induced change of Urea, Creatinine and BUN levels with increase the doses of treatment. (Hole, 1992 and Iqbal, 2004). In the previous reports, the results of histological examination of kidney tissues in male wild-type BALB/c mice ranged from showing mild cloudy swelling (reversible hydronephrosis) in the 10 mg/kg pentostam group, to showing stromal clumps of inflammatory cells (nephritis), in the Pentostam 20 mg/kg group, and finally showed the presence of renal tubules and necrosis in the Pentostam 40 mg/kg group Elammari and Sariti, 2021. Similar results obtained by Forhan *et al.*, where their study showed that hematopoietic tissue necrosis, tubule cell emptying, and capillary dilation Glomerular, endothelial cell degeneration are some of the pathological changes observed in hematopoietic cells. Kidneys from various toxins (Iqbal, 2004). Consequently, in the results of this study, high blood urea levels were observed in animals treated with different doses of pentostam, has an influence on renal function and indicates kidney damage (Wallig, *et al.*, 2017), as well as the level of creatinine (Wang, 2019).

Creatinine is one of the products of the secretion of muscle activity that circulates in the blood. Its elimination is exclusively renal, so there is a correlation between creatinine levels and renal function. That is, most of the creatinine excreted by the kidneys is freely filtered into the renal glomeruli, and a small part is filtered by the tubular component, which is a good indicator of glomerular renal function (Afshar, *et al.*, 2008), Moreover, the level of urea and creatinine were increased in the high-dose group, which may imply the Pentostam has an influence on renal function (Wallig *et al.*, 2017).

Similar to urea and creatinine, the BUN was significantly increased in animal that received Pentostam, indicating impending kidney damage. BUN is the amount of nitrogen circulating in the form of urea through the circulation. In healthy animals, urea is cleared from the plasma by the renal glomerulus. It returns to the blood through the renal tubules, but most of it is excreted through the urine. However, if the kidneys are not functioning properly, then no enough urea can be removed from the plasma, resulting in elevated BUN levels (Marrugal-Lorenzo, *et al.*; Maiden, *et al.*, and Pic, *et al.*, 2019)

Regarding the effect of Pentostam on the hematological parameters in our study, there was significant decrease in hemoglobin concentration and HCT with the different doses of Pentostam, and this finding has been also reported in other studies (Ahmed, *et al.*, 2022 and Rambaldi, *et al.*, 2018). The Pentostam injection was associated with significant suppression of the total WBCs and lymphocyte count in our study as it was seen in other reports (An I,Harman, *et al.*, 2019 and Wise, *et al.*, 2012), while the neutrophil count has increased after different concentrations of Pentostam injection. There was no significant change in platelets count, as it was reported that thrombocytopenia is rare adverse effect of leishmania treatment by pentavalent antimonial (Oliveira, *et al.*, 2011).

5. CONCLUSION

Biochemical and heamatological evaluation pattern still an important role to evaluate drug toxicity. However, the results of this study concluded that, different doses of Pentostam can cause hepatotoxicity and nephrotoxicity. Therefore, it will be important of change in anti-leishmanial regimen to reduce their side effect.

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