Anti-Leishmanial drug Pentostam induced histological changes to liver and kidney in male BALB/c wild mice

[*]Salem Ramadan Sariti **[**]Nouara Elazirg Elammari**

Abstract: leishmaniasis still a complex disease of (sub) tropical regions of the world caused by *Leishmania* spp. Antimonial pentostam is an antileishmaniasis drug which used medically and it is the primary drug employed against leishmaniasis in Libya. It has multiple acute and chronic adverse effects which can be minimized by using the lowest effective dose. This work aimed to investigate the histological changes in liver and kidney affected by different doses of the pentostam. Adult male of BALB/c wild mice were divided into four groups, 6 mice each, and i.p. injected with 10mg/kg, 20mg/kg, and 40mg/kg pentostam in addition to a control group. After 28 therapeutic days and finishing the histological procedure to examine the collected tissue specimens, the obtained results of the liver tissue ranged between demostrating cytoplasmic vacuoles, to hydropic degeneration, focal and hepatocytic necrosis, and lastlly irregular area of hepatocytes with condensed pyknotic nuclei (hepatocyte necrosis). The histological examination of kidney tissue ranged between demostrating mild cloudy swelling (reversible hydropic degeneration), to showed stromal aggregates of inflammatory cells (nephritis), and lastlly showed renal tubule casts and necrosis. In conclusion, clear histological changes in liver and kidneys, had been seen in this study, which were dose dependent changes.

Key words: leishmaniasis, antileishmaniasis drug, pentostam, histological examination of liver tissue, histological examination of kidney tissue.

Introduction:

leishmaniasis still a complex disease of (sub) tropical regions of the world caused by *Leishmania* spp. which spread by sand fly (Ponte-Sucre, 2017). As a fact, chemotherapy is the only choice in control and management of leishmaniasis for the time being. Moreover, antimonials remain the primary drugs against different forms of leishmaniasis in several regions (Ponte-Sucre, 2017). However, Antimonial sodium stibogluconate (pentostam®) is an antileishmaniasis drug which used medically in a dose of 20mg sbv /kg per day for 28 days daily (Abyot et al., 2005), it is the primary drug employed against leishmaniasis in Libya. This toxic antimonial compound has a narrow therapeutic window (Mitropoulos, 2010; WHO, 2017). After finishing the treatment, the patient should be evaluated to determine the drug outcomes. This evaluation should include the drug toxicity specially on the liver and kidneys. Moreover, like any drug, pentostam has multiple acute and chronic adverse effects which can be minimized by using the lowest effective dose. Therefore, awareness of the danger of using

[*] Biology Department, Libyan Academy, Misurara , Libya.
To whom reprint requests should be addressed. e-mail: salemsrs@yahoo.com

[**]Department of Microbiology and Parasitology of Medicine faculty-Benghazi University.

a high dose should be taken seriously. Considering that, we think it is important to investigate the tissue damage in liver and kidney which is related to pentostam side effects.

The aim: This work had been done to investigate the histological changes in liver and kidney affected by different doses of the anti-leshmanial drug (Pentostam).

Materials and methods:

Drug for in-vivo administration: Sodium **s**tibogluconate injection B.P. (pentostam® Manufactured by AlBERT DAVID LIMITED), equivalent to pentavalent antimonite each ml contains 100mg are available in 30ml bottle, which obtained from the Libyan Health ministry. Pentostam was prepared in three concentrations 10mg/kg, 20mg/kg, and 40mg/kg and have been given intraperitonally (i.p), each mouse received the determined dosage once a day in 1ml normal saline for 28 consecutive days.

The experimental animals: Adult male of BALB/c wild mice have been used weighting 25-39g at the age of 8-12 weeks. Animals were fed a standard laboratory diet and tap water during the experiment. After 2 weeks of adaptation, all animals were randomly divided into four groups of six mice each: **Group 1:** Served as control group and received 1 ml i.p. of normal saline once daily. **Group 2:** Received 10 mg/kg/day, i.p. **Group 3:** Received 20 mg/kg/day, i.p. **Group 4:** Received 40 mg/kg, i.p.

Histological procedure: The selected tissue where R.t lobe of liver and longitudinal section from both kidneys. Moreover, the collected liver and kidney specimens of each animal were fixed in 10% formalin to prevent degradation. Then specimens were dehydrated and embedded in paraffin before microtome sectioning. However, the sections undergo tissue processing and stained by PAS stain be examined by histopathological test. After all, the stained slides were observed at 100x magnification using a Nikon's brightfield compound Nikon microscope (Model YS100). The changes in lobular architecture, fatty changes, nuclear alterations and congestion of the sinusoids were evaluated in liver specimens. Similarly, changes in cytoarchitecture of the glomeruli, proximal and distal convoluted tubules and interstitium were evaluated in kidney specimens. The assessment have been done in Al-Saleem medical laboratory-Benghazi-Libya.

Results:

After 28 i.p. pentostam therapeutic days and finishing the histological procedure of the collected tissue specimens, the obtained results were as the follows:

The liver tissue specimens:

Experimental group 1: Histological examination of liver in the Control group displayed normal microstructure and showed the arrangement of hepatocytes in the form of anastomosing plates of one to two cell thickness. These plates are separated by blood sinusoids as it is illustrated by the photomicrgraph in figure 1.

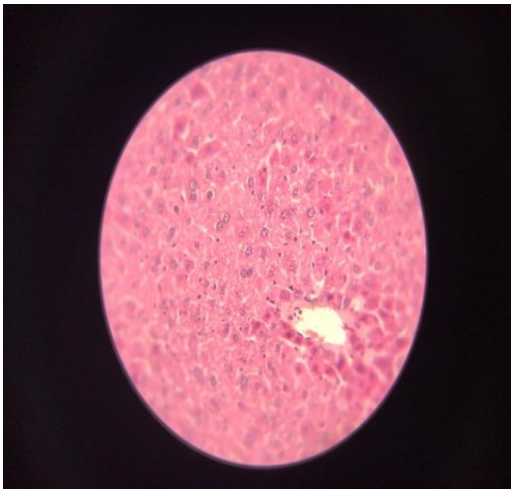

Fig. 1: Liver tissue specimen of the control group
shows normal hepatocytes and sinusoids.

Experimental group 2: Histological examination of liver in group 2, received 10mg/kg which demostrated cytoplasmic vacuoles (hydropic degeneration) as it is illustrated by the photomicrgraph in figure 2.

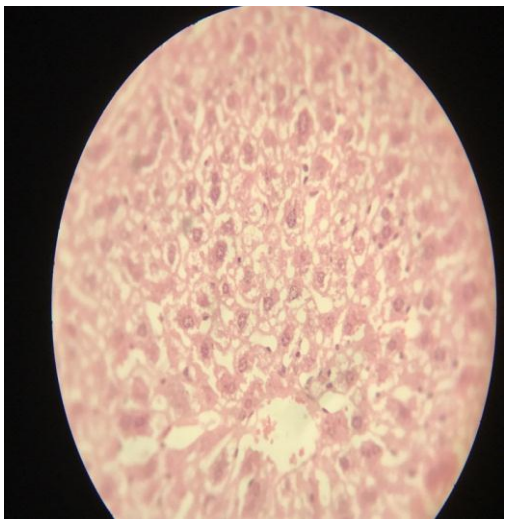

Fig. 2: Liver tissue specimen of the group 2
shows hydropic degeneration.

Experimental group3: Histological examination of liver in group 3, received 20mg/kg which shows hydropic degeneration, focal and hepatocytic necrosis as it is illustrated by the photomicrgraph in figure 3.

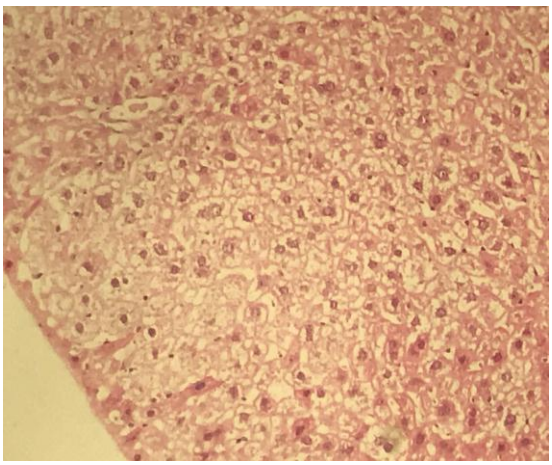

Fig. 3: Liver tissue specimen of the group 3 shows
hydropic degeneration, focal and hepatocytic necrosis

Experimental group 4: Histological examination of liver in group 4, received 40mg/kg shows irregular area of hepatocytes with condensed pyknotic nuclei (hepatocyte necrosis) as it is illustrated by the photomicrgraph in figure 4.

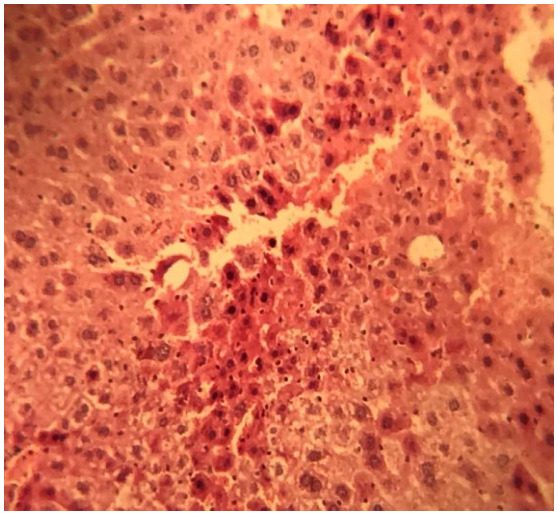

Fig. 4: Liver tissue specimen of the group 4 shows irregular area of hepatocytes with condensed pyknotic nuclei

The kidney tissue specimens:

Experimental group 1: Histological examination of kidney in the Control group showing morphology of renal unit composed of glomeruli and tubules as it is illustrated by the photomicrgraph in figure 5.

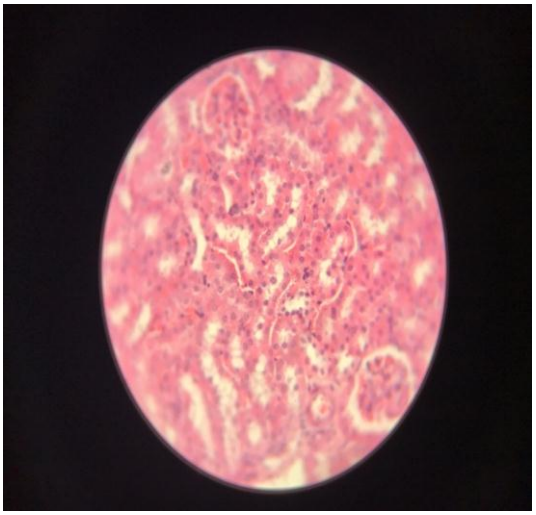

Fig. 5: Kidney tissue specimen of the control group showsmorphology of renal unit composed of glomeruli and tubules

Experimental group 2: Histological examination of kidney in group 2, received 10mg/kg which demostrated mild cloudy swelling (reversible hydropic degeneration) as it is illustrated by the photomicrgraph in figure 6.

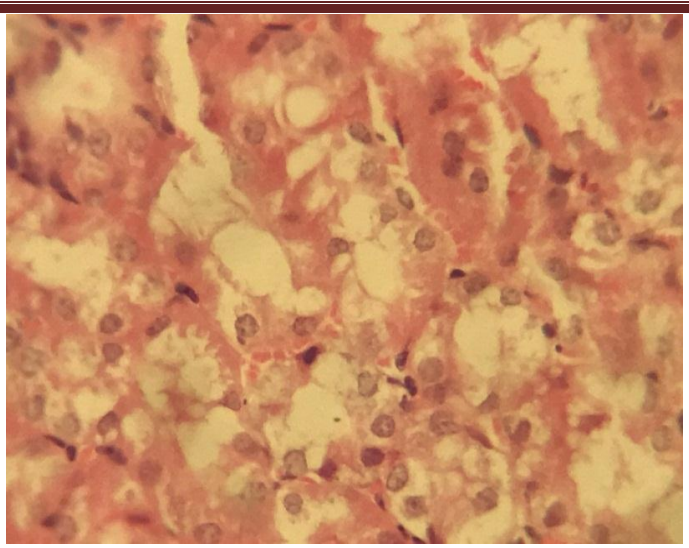

Fig. 6: Kidney tissue specimen of the group 2 shows mild cloudy swelling

Experimental group 3: Histological examination of kidney in group 3, received 20mg/kg showed stromal aggregates of inflammatory cells (nephriris) as it is illustrated by the photomicrgraph in figure 7.

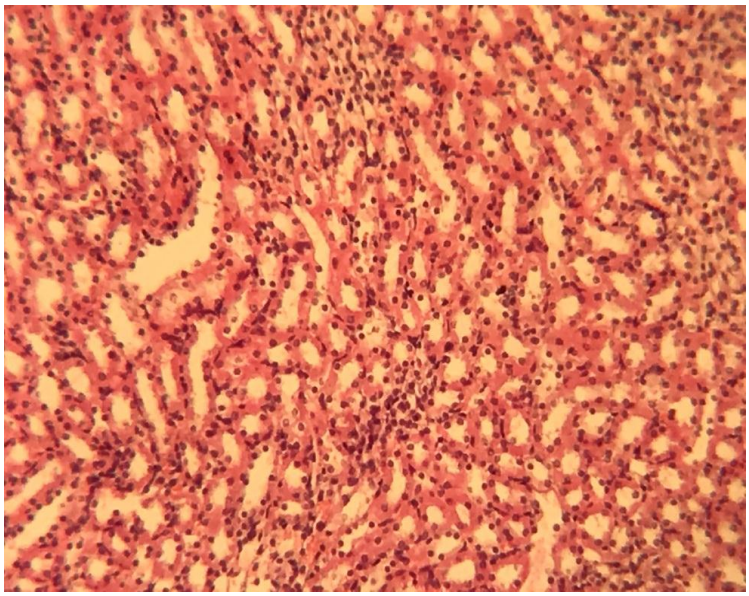

Fig. 7: Kidney tissue specimen of the group 2 shows inflammatory cell infiltrate (nephritis)

Experimental group 4: Histological examination of kidney in group 4, received 40mg/kg showed renal tubule casts and necrosis as it is illustrated by the photomicrgraph in figure 8.

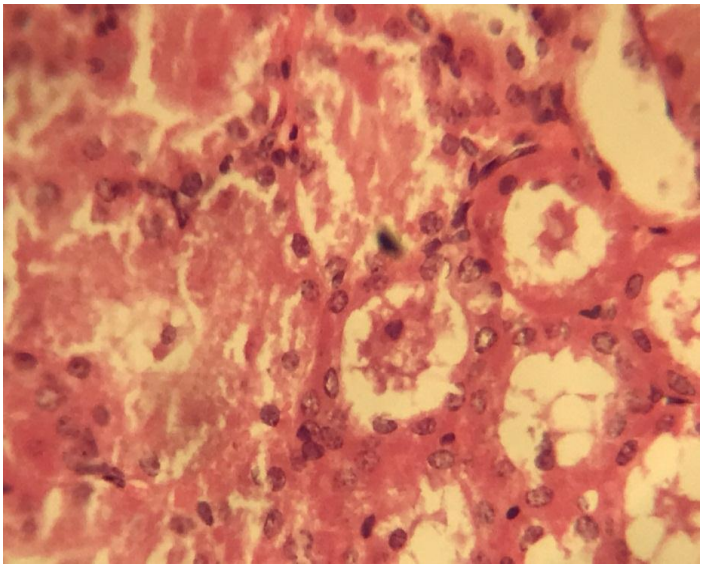

Fig. 8: Kidney tissue specimen of the group2
shows renal tubule casts and necrosis

Discussion:

As an interested parasite, *Leishmania* has an intricate life cycle, and one of it is developmental forms, the amastigote, dwells inside the host immunological cells, which adds to the challenge of accessing this parasites with specific drugs. Nevertheless, the used chemotherapy should kill the intracellular parasites (Garc et al., 2012). Therefore, the antileishmanial chemotherapy remains the best means available to cure the disease and it should contain toxic chemicals to kill the intracellular Leishmanial parasites. However, the liver is well known target organ of the toxic impact regarding its function in biotransformation and excertion of xenobiotics. After entering uptake, liver is the first organ to be exposed by portal circulation (Roganovic-Zafirova & Jordanova, 1998). Hepatotoxicity is toxicity to the liver, bile duct and gall bladder. However, the liver is particularly susceptible to xenobiotics due to a large blood supply and its role in metabolism (Afshar et al, 2008). Consequently, the findings of the histological examination of liver tissue of the male BALB/c wild mice in this study were ranged between demostrating cytoplasmic vacuoles (hydropic degeneration) in 10mg/kg pentostam group, to hydropic degeneration, focal and hepatocytic necrosis in 20mg/kg pentostam group, and lastlly irregular area of hepatocytes with condensed pyknotic nuclei (hepatocyte necrosis) in 40mg/kg pentostam group as it is illustrted in figures 2,3 and 4. These findings are in agreement with the fact that the liver is highly susceptible to be affected by the toxic chemicals (Afshar et al, 2008). Since long time, Ludwig et al. (1994) clarified that in less acute presentations, liver histological changes can be very varied, as changes progress, appearances may mimic chronic hepatitis with portal inflammation, interface hepatitis and fibrosis. In addition to that, acute hepatitis usually causes a lobular pattern of inflammation. The inflammation can be mild with minor infiltrates and spotty necrosis of single hepatocytes, or in severe cases cause widespread necrosis with architectural disturbance (lobular disarray) or collapse (Torbenson, 2014). However, it seems that accumulation of Pentostan is directly toxic to hepatocytes, this hepatocyte toxicity is increased by increasing the Pentostan dose. Al-Jahdali, et al. (2007) concluded that the liver syndrome's intensity correlated with the increase in dose and duration time.

In the same line, the kidney is a vital organ of body and proper kidney functioning is important to maintain the homeostasis. Kidney is not only involved in removal of wastes from

blood but it is also responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids, erythropoieses and help in regulating blood pressure by producing the enzyme rennin. Kidney is one of those organs, which are severely affected by different toxic chemicals (Furhan et al., 2004; Hole, 1992). Unfortunately, kidneys are highly susceptible to toxicants for two reasons:

i. a high volume of blood flows through it.

ii. ii. they filtrates large amount of toxins which can be concentrate in their tubules.

Nephrotoxicity is toxic to the kidneys which may result in systemic toxicity causing decrease ability to excrete the body wastes, inability to maintain the body fluid and electrolyte balance and decreased synthesis of essential hormones (Fin, 1977; Afshar et al, 2008). Nephrotoxicity induced by drugs is common in children and underlying renal disease and cardiovascular disease. Drugs can cause acute renal injury, intra renal obstruction, nephrotic syndrome, interstitial nephritis. Certain drugs can cause alteration in intraglomerular hemodynamics, inflammatory changes in renal tubular cells leading to acute kidney injury (Shahrbaf&Assadi, 2015). Consequently, the findings of the histological examination of kidney tissue of the male BALB/c wild mice in this study were ranged between demostrating mild cloudy swelling (reversible hydropic degeneration) in 10mg/kg pentostam group, to showed stromal aggregates of inflammatory cells (nephritis) in 20mg/kg pentostam group, and lastlly showed renal tubule casts and necrosis in 40mg/kg pentostam group. These findings are in agreement with that observations obtained by Furhan et al. (2004), as he discriped that the necrosis of hematopoietic tissue, vaculoation of tubule cells, dilation of glomerular capillaries and degeneration of epithelial cell lining are some of the pathological changes observed in kidney of various toxins (Furhan et al., 2004).

Finally, histological analysis of liver and kidney tissue seems likely to remain an important investigation to determine the drug toxicity and effectivity. As a final point, it is worth remembering that it is imperative to include information on drug toxicity, which is essential in designing therapeutic guidelines. After all, In this study, after 28 i.p. pentostam therapeutic days, which administrated in different doses (10, 20 and 40 mg/kg), a clear histopathological changes were observed in the mice's liver and kidney tissue compared to the control group. In addition, these histological changes were clearly related to the pentostam dose. In the meaning of that, the histological changes were more significant by increasing the pentostam dose (dose dependent changes). However, this study revealed that exposure to pentostam for about one month can cause histological changes in the male of BALB/c wild mice liver and kidneys even within the normal therapeutic dose.

Conclusion:

Histological analysis of liver and kidney tissue still an important role to evaluate the drug toxicity. However, in this study we found a clear histological changes in liver and kidneys which are related to the pentostam dosage (dose dependent changes). Therefore, it well be of an important to investigate the efficacy of the anti-leshmanial drug "pentostam" on the biological function of the liver and kidneys in the male of BALB/c wild mice by using the same different doses which have been used in this study.

مضاد الليشمانيا البنتوستام يحفز ظهور تغييرات نسيجية في الكبد والكلية عند فئران BALB/c-WT

*سالم رمضان السريتي **نوارة الأزيرق العماري

المستخلص: يعتبر مرض الليشمانيا الناتج عن طفيل Leishmania من الأمراض المعقدة المنتشرة في المناطق المدارية وشبه المدارية. و يعتبر البنتوستام من أهم العقاقير المستخدمة على المستوى الطبي في ليبيا. هذا العقار لديه تأثير جانبي حاد ومزمن، غير أنه من الممكن التقليل من شدة هذا التأثير. لذلك فإن هذه الورقة البحثية تهدف لدراسة التغير النسيجي في الكبد والكلية الناتج عن استخدام جرعات مختلفة من البنتوستام. قسمت ذكور فئران BALB/c-WT إلى أربعة مجموعات بواقع ستة أفراد للمجموعة. مجموعة ضابطة وثلاثة حقنت بتراكيز مختلفة من البنتوستام 10mg/kg و 20mg/kg و 40mg/kg. حقنت المجاميع i.p. لمدة 28 يوم. بعدها تم تجميع الأنسجة الكبدية والكلوية من أفراد التجربة وتمت إجراءات تجهيزها للفحص النسيجي. أظهرت نتائج هذه الدراسة تضررا واضحا في النسيج الكبدي تراوح هذا التضرر بين ظهور فجوات سيتوبلازمية وبؤر من الخلايا الكبدية المتليفة و وصولا إلى مساحات كبدية متليفة وغير منتظمة. أما نتائج النسيج الكلوي فقد تراوح تضرره بين تورمات متوسطة القتامة إلى ظهور تكتل من الخلايا الضامة الملتهبة و وصولا إلى تليف و تحجر الأنيبيبات الكلوية. و الاستنتاج النهائي لهذه الدراسة هو ظهور تغيرات نسيجية واضحة على الكلى والكبد ترتبط هذه التغيرات بزيادة جرعة البنتوستام المستخدمة.

الكلمات المفتاحية: مرض الليشمانيا – عقاقير ضد الليشمانيا – بنتوستام – اختبار – النسيج الكبدي – النسيج الكلوي.

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* قسم علوم الحياة، الأكاديمية الليبية، مصراتة

** قسم الميكروبيولوجي والباراسيتولوجي، كلية الط ب البشري، جامعة بنغازي

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