

Evaluation activity Cisplatin infection by leishmaniasis *in vivo* and *in vitro* and study of the associated histological changes in mice balb / C

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ABSTRACT

The current study included evaluation of the effectiveness of the drug Cisplatin vital phase promastigote leishmaniasis parasite inside and outside the living body, and its effects on the liver and kidney in laboratory mice balb/c. This study proved that the drug Cisplatin highly effective in killing phase promastigote in vitro, which reached a maximum of 100% on the fourth day when the drug concentration (0. 4 mg / mL). Histological study showed that infection caused histologic changes different intensity of the liver while changes were less severe in mice infected and drug treatment as well as with the kidney.

INTRODUCTION

Disease Leishmaniasis a group of diseases caused by parasites *Leishmania* parasite as it enters cells of the immune system after the bite of the sand fly insect *Phlebotomus* sp The disease spreads by type of parasite transmitted either skin, causing deformities and skin ulcers (cutaneous leishmaniasis) or to internal organs such as the liver and spleen, causing fatal injuries, especially in children (visceral leishmaniasis)[1].Interested many researchers to produce chemical treatment for the disease, but their attempts are still in the early stages was used Pentostam and Glucantime in the treatment of most cases of visceral leishmaniasis, is the first therapeutic line while longer Pentamidine Amphotericine B Of medicine used second-line treatment [2].The drug cisplatin of chemical drugs used in the treatment of cancer diseases as observed in the early sixties that some platinum compounds ability to inhibit cancer cells [3].Mechanical work cisplatin compound known as linked DNA and this is the key step in the treatment of cancer and diseases done depends on the decomposition reactions by replacing a chlorine molecule water and add a positive charge on the molecule [4].As well as studies have shown that this drug effective anti-parasites especially parasite *Echinococcus granulosus* , as indicated [5] that the drug Cisplatin highly effective in killing protoscolices for this parasite and proved its effect on the number of cyst also cause complete analyze the entire layer generated consisting of hydatid cyst. AS wellas explain study [6] that the drug effect on the parasite *Trypanosoma rhodesiense* and *Trypanosoma brucei* and *Trypanosoma cruzi* to note that the drug lead to her death through the inhibition of DNA building.Because of the health risks and economic costs of injury parasite *Leishmania* and lack of cure against injury so came this study to determine the effectiveness of the use of drug the Cisplatin treatment for injury and test its ability to kill phase promastigote in vitro and its effect on liver tissue and the kidney on the premise that it infected resistant to antibiotics.

MATERIALS AND METHODS

Was obtained isolation parasite *L.donovani* Center Leishmaniasis of the University of nahrain and planted on the central NNN-Medium proportion to Novy-MacNeal-Nicolle biphasic, and attended middle liquid solution (Lock) by way [7] also attended the middle soil by way [8]

Divided bottles solid part to 10 groups, added 1 ml of solution Lock each bottle of the solid part, and after several hours the transfer of the parasite each vial containing circles in both and then incubated circles degree $26\text{ m}^{\circ} \pm 2$, After 5 days I took a drop fluid from groups, the middle of each bottle aggregates and placed on a glass slide and covered with a lid slide and examined using optical microscope unusual for detecting the presence of the parasite and then added drug cisplatin groups (1,2,3,4) different concentrations (0. 1,0. 2,0. 3,0. 4) mg / ml respectively, while the other groups were treated with taking control sample contain any property by 3 replications for each group. and 24 hours after parasite numbers were calculated daily for four days. leaving aggregates (6,7,8,9,10) for the purpose of injury in vivo.

The results were analyzed using ANOVA analysis of variance table [9]

Histologic study

injured 16 mice laboratory strain Balb \ C 107 cell parasite *L.donovani* under albritton and left animals for a month divided animals into two groups each group contains 8 mice and treated one (100) mg / m² of cisplatin intravenous single dose a week for four weeks and then explained infected animals treatment and non-treatment a week after the last dose and took parts of the liver, kidney, and set with 10% formalin for histological study

The method adopted [10] in histological preparations as set pieces taken from different organs of an animal experiment appropriate quantity of 10% formalin for 24 hours, then washed models using running water and pull water upward series of absolute ethanol (90-70-50)% two hours for each concentration and then transferred to ethyl alcohol absolute 100% twice (hours per concentration), than roukt models chloroform for (24-12) hours and drank paraffin wax pure putting vessels special metal as left on the plate warm degree 60 m, and buried after putting them in templates and added to molten paraffin wax Highly pure 60 m taking into account the guidance models towards cutting required and left templates to harden at room temperature, and cut models thickness (6-5) Micron using Rotary Microtome (Reichert-Jung) then passages to a water bath degree Heat 45 m for the purpose brushes sections and then picked up by slides coated with albumin Mayers Albumine and place on a plate warm type Fisher slide warmer temperature of 50 m for 24 hours, and stained sections, Eosin-Haematoxylin and carried DPX material and then put the cover slide and examined and photographed compound microscope imaging type Nikon.

RESULTS

Test the direct effect of the drug cisplatin on promastigote phase of *L.donovani* invitro Proved the results current study below the level of statistical analysis ($P < 0.05$) the direct influence of the drug the cisplatin on stage promastigote in vitro and effectiveness high in low numbers Promastigote live with increasing concentration of the substance as average numbers 1825 when concentration 0. 1 mg / ml of the fourth day, and decreased numbers of live increase the concentration of up to 100% kill rate at concentration 0. 4 mg / ml for the same period. (Table 1)

Table (1) the effect of drug cisplatin on rates number of phase promastigote live in vitro

Drug cisplatin				concentration mg / mL
After 4days	After 3 days	After 2 days	After 1 day	
1825	2175	2850	3500	0. 1
950	1300	1800	2350	0. 2
125	250	575	925	0. 3
0	25	100	200	0. 4
25250	16350	7800	5475	Control

RLSD concentrations = 1196.3

RLSD days = 1070.0

Histologic study

Liver

Explained tissue sections of livers animals control the liver tissue consists of lobules hexagonal almost mediates each lobule branch of hepatic vein called central vein and running each lobule Hepatocytes ranked shaped ropes radial and notes including make way called hepatic Sinusoids (Picture 1)

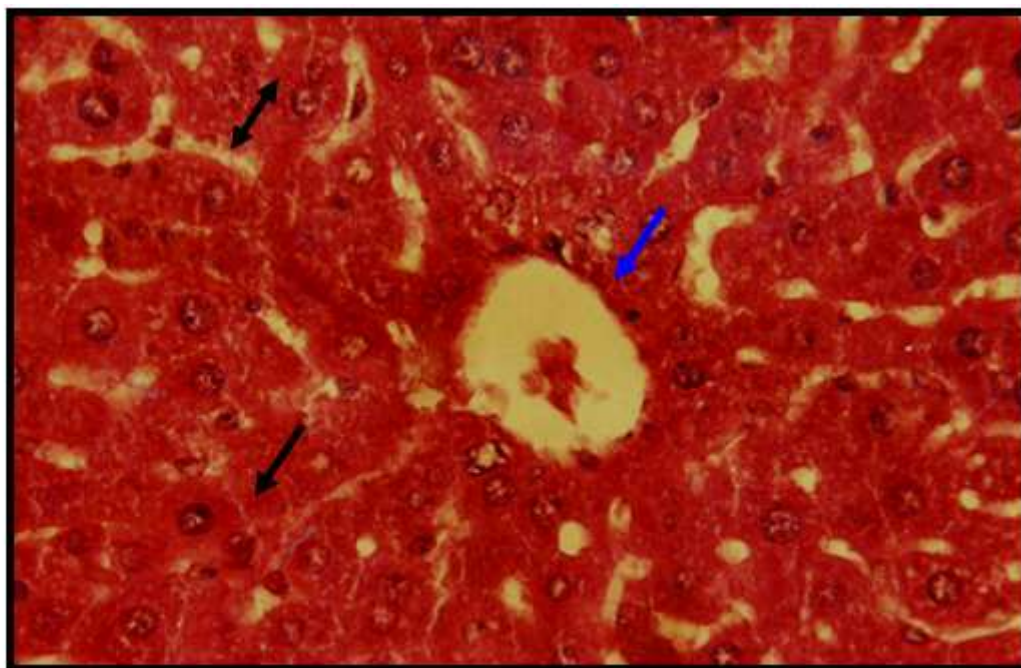


Image (1): section in mice liver healthy showing central vein (←) and hepatocytes (←) and the liver sinusoids (↔). Dye (H.E). 475 X

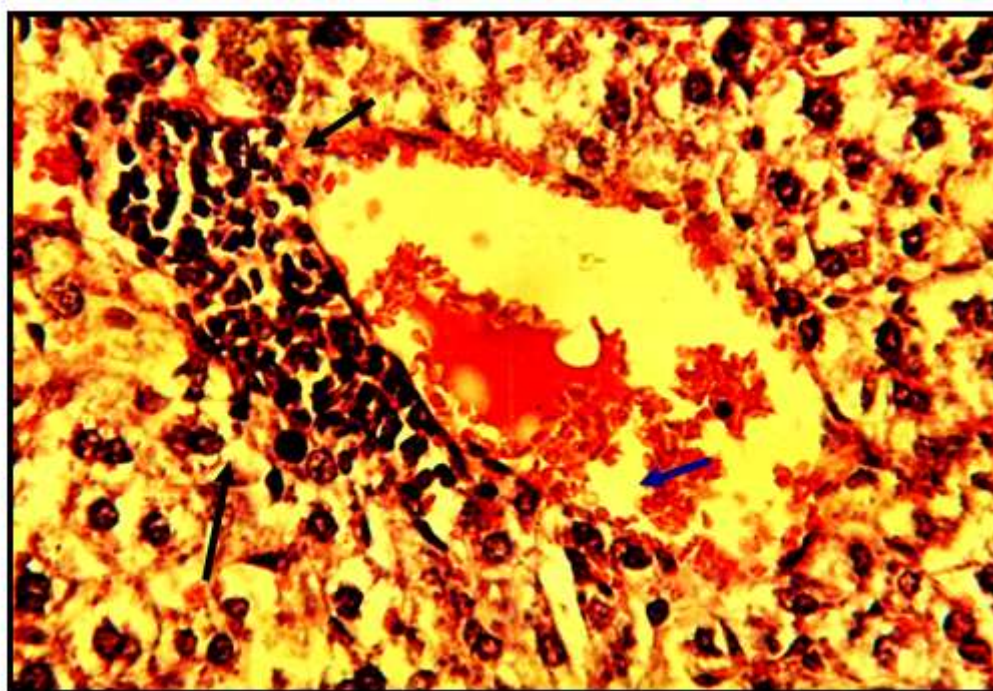


Image (2): section in mice infected liver *Leishmania* shows which combine defensive cells (←) Around the blood vessels with vascular congestion (←) dye (HE). 475 X

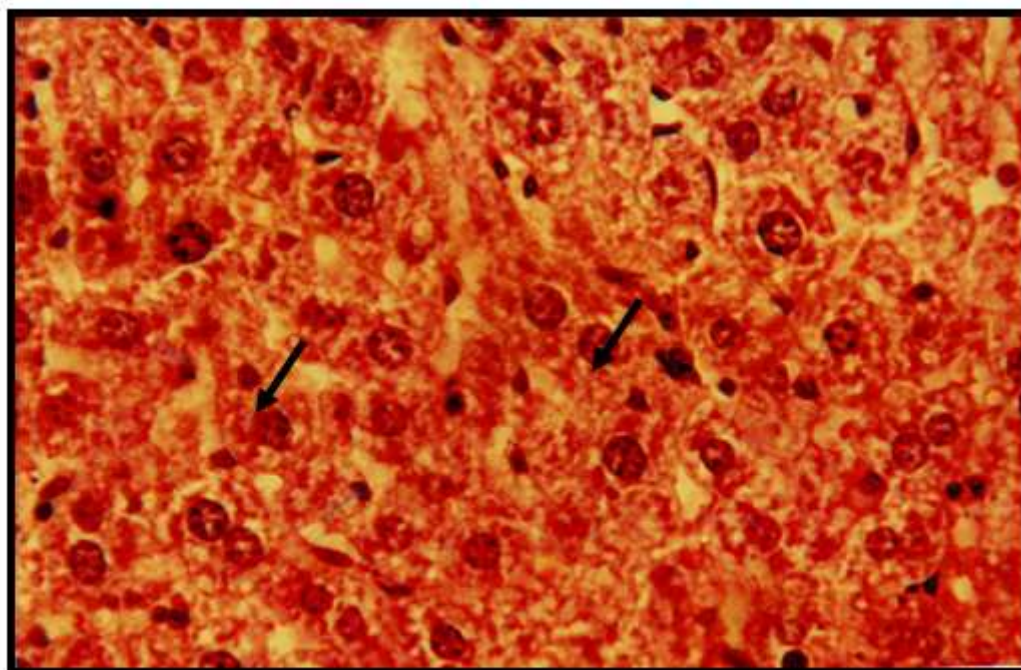



Image (3): section in mice infected liver *Leishmania* and drug labs showing necrosis in some liver cells () dye (HE). 475 X

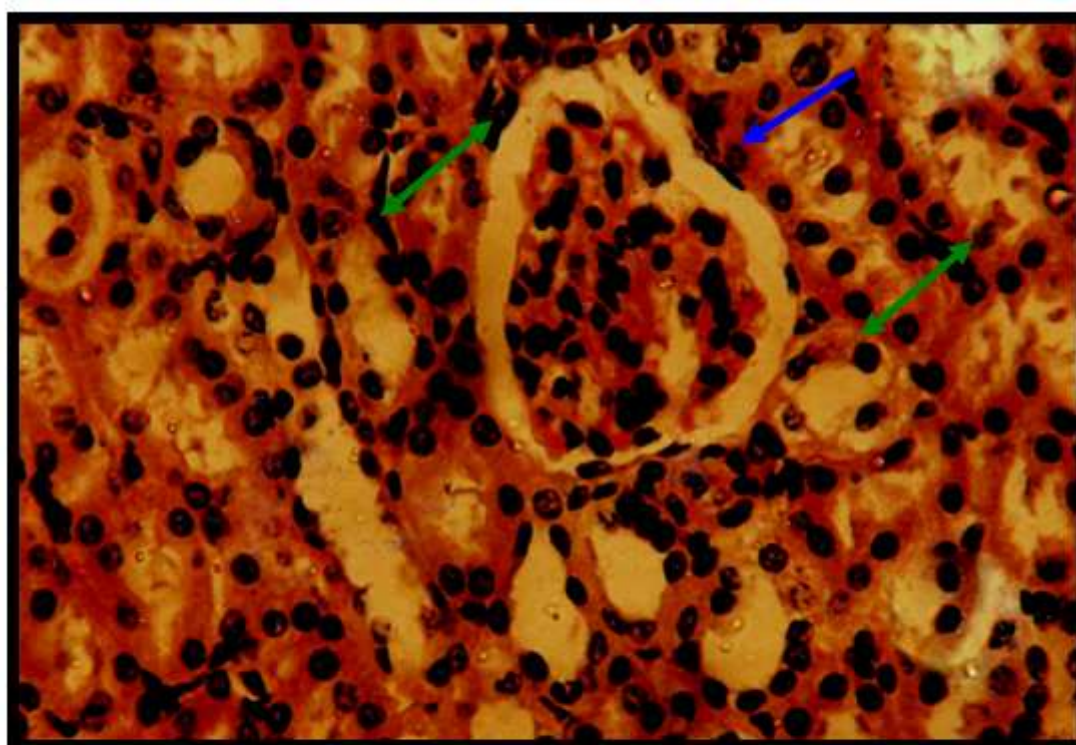




Image (4): section in mice kidney healthy showing renal glomerulus () and renal tubules () dye (HE). 475 X

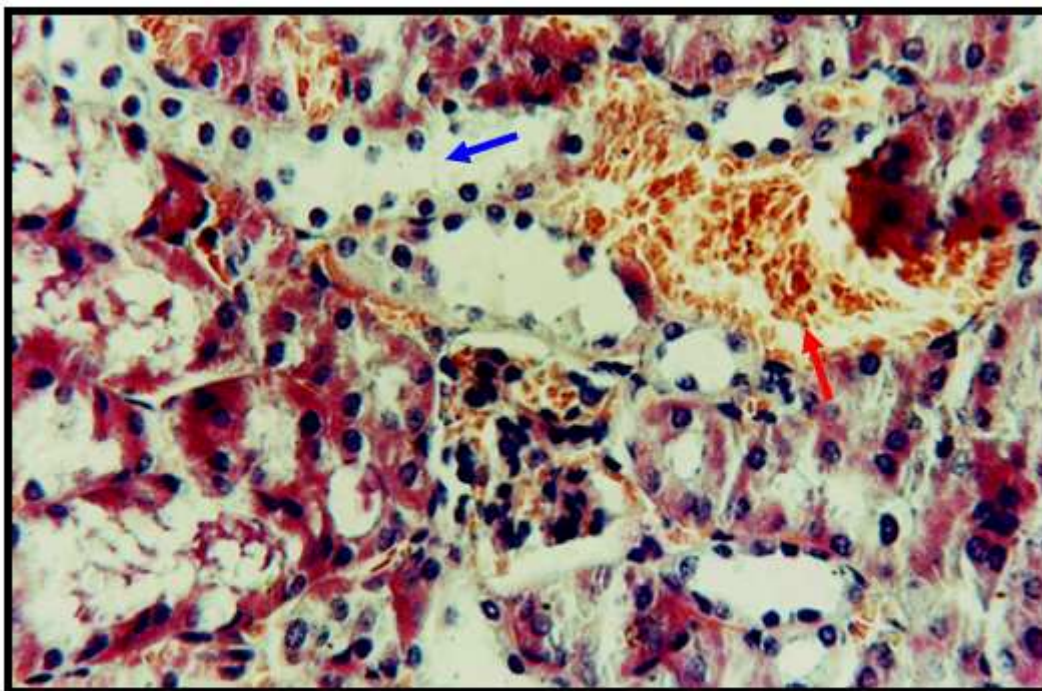




Image (5): section in mice kidney infected showing dissolution of renal tubules () and bleeding () dye (HE), 475 X

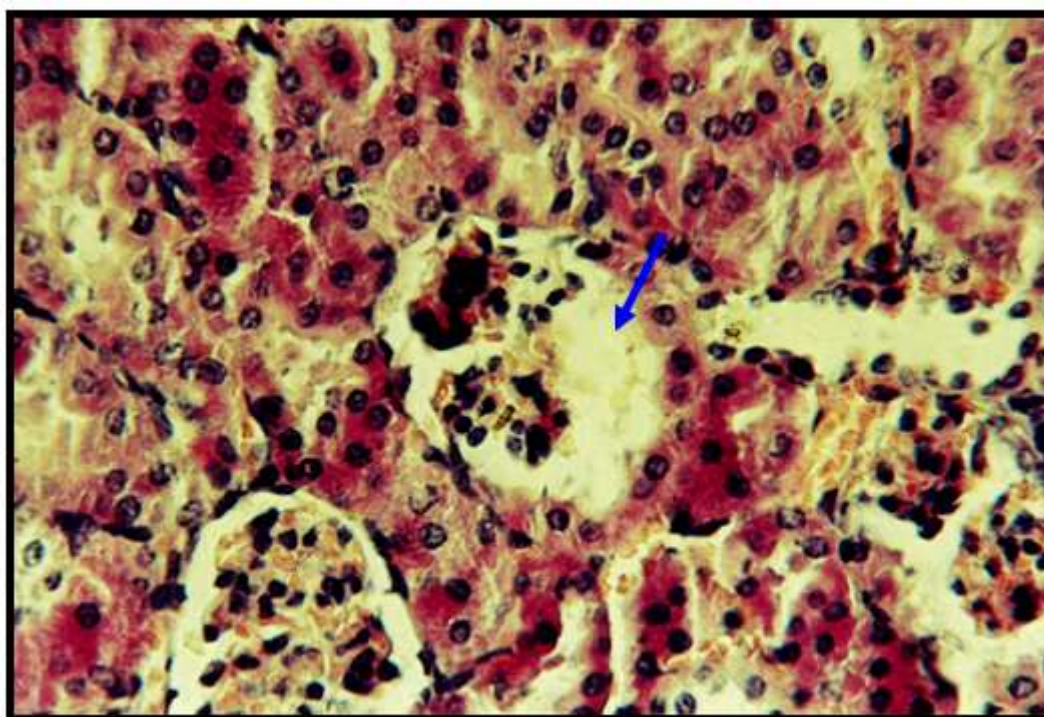



Image (6): section in mice kidney infected showing dissolution of glomeruli () dye (HE). 475 X

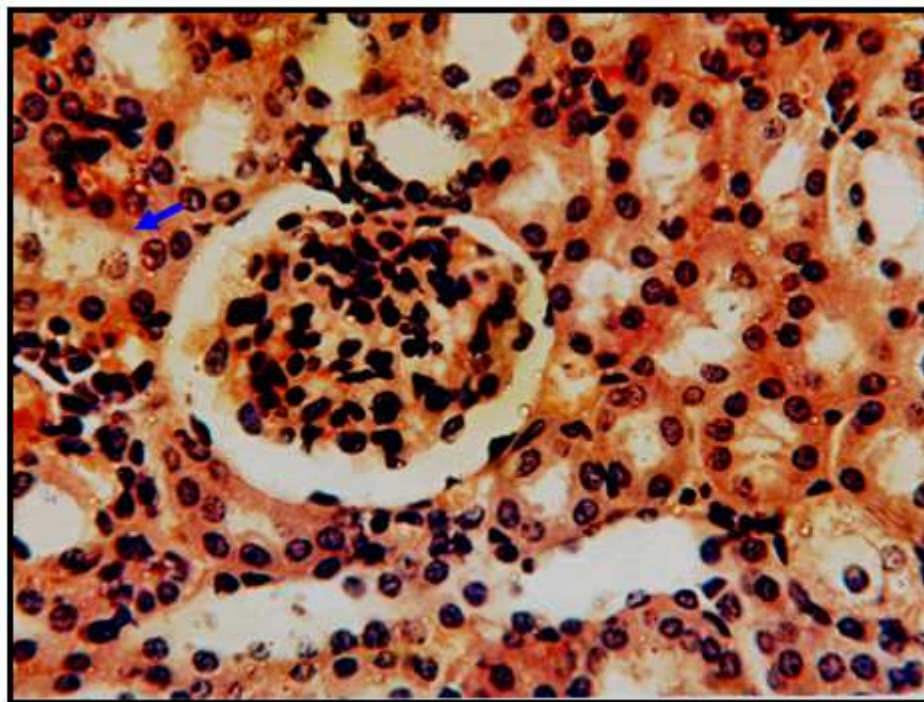


Image (7): section in mice kidney infected and drug labs showing dissolution of some renal tubules (←) dye (HE). 475

Caused injury mice infested with severe liver changes was gathering defensive cells around blood vessels and vascular congestion (Picture 2)

The results of histological examination showed the livers of infected animals and Cisplatin treatment during the probation period and a small changes on the livers was necrosis in some liver cells (Picture 3)

Kidney

Showed histologic sections of the control animals contain kidneys cortex tissue kidney Bowmans Capsule in each portfolio circuitous network of capillary blood vessels, which represents Glomerulus There are also many renal tubules that appear in different sections (Picture 4)

The results of a microscopic examination of kidneys of animals infected with the parasite during the period of the experiment the presence of pathological changes was the dissolution of renal tubules with bleeding (Picture 5) injury also caused the dissolution of some renal glomeruli (Picture 6)

While the kidneys of infected animals and drug treatment less severe changes dissolution was to some urinary tubules (Picture 7)

DISCUSSION

Test the direct effect of the drug Cisplatin on promastigote phase in vitro caused the treatment phase promastigote drug cisplatin in vitro low phase promastigote live numbers with increasing concentration of the substance as rates were prepared live (3500, 2350, 925, 200) at concentrations (0. 1, 0.2, 0.3, 0.4) mg / mL, respectively, in the first day, and decreased numbers increase concentration of the substance to reach the fourth day (1825, 950, 125.0) for the same concentrations, can be explained by the Cisplatin effect by stopping the synthesis of proteins necessary for the survival of the parasite as well as the destruction of nuclear material of promastigote phase led to her death early as characterized by drug cisplatin ability to stop the process of protein synthesis through its effect on DNA [11]. In other studies observed of this drug a fatal effect on parasite has been found to [12] that the drug is doing fought on several strains of the parasite *Trypanosoma cruzi*

Histological study

Demonstrated by the study was that the injury parasite caused analyzed in liver tissue and combines defensive cells around the blood vessel and this explains the ability of the parasite to multiply inside macrophages and explode inside the organs effected injury and the immune stimulation from the body against infection and this is consistent with the sentiments, [13]. The injury caused severe infiltration of inflammatory cells on blood vessel also found [14] after examining the tissue sections collecting and infiltration of inflammatory cells, especially blood cells only and roled this to its immune system against infection [15]. It was noted the effect of drug in the sections of the liver through the dissolution of the cytoplasm of cells may be due to the drug of Cisplatin ability to stop the process of protein synthesis through its association with DNA and prevent the process of reproduction [16].as associated with a platinum Alkuanin GPG or associated with adenine and Alkuanin mechanical depends compound in eliminating malignant diseases [17].The histologic changes severe kidney infected animals represented decomposition glomerulus and urinary tubules where immune complexes caused sediment nephritis [18].The present study indicated to cases bleeding and possibly due to get interaction between products parasite antibodies and its effects on the lining of blood vessels and this is consistent with [19]. when interpreted if bleeding inflammation of the lining of the blood vessels as putout red blood cells through the holes between endothelial cell. While changes were less treatment with the drug samples may be due to the drug inhibitory effect of the immune system [20]

CONCLUSION

cisplatin good drug in the treatment of leishmaniasis

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