Protective efficacy of antioxidants on cisplatin-induced tissue damage caused in *Leishmania donovani* infected BALB/c mice against murine visceral leishmaniasis

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**Abstract**

**Objective:** Therapeutic interventions against visceral leishmaniasis (VL) are limited and facing serious concerns of toxicity, high cost and emerging resistance, there is a greater interest in new drug developments which are cost effective, efficient and easily available to people suffering from leishmaniasis. Cisplatin (cis-diamminedichloroplatinum II; CDDP) has been found to have antileishmanial activity *in vitro* and *in vivo* which lead towards an apoptosis like cell death of both promastigotes and amastigotes and a significant reduction in parasite load and enhanced DTH responses which suggested the generation of protective cell-mediated immune responses. But, at higher doses it causes nephrotoxicity-a major side effect.

The present study was designed to evaluate the protective efficacy of antioxidants on cisplatin induced tissue damage in *Leishmania donovani* infected BALB/c mice.

**Methods:** *L. donovani* infected and uninfected animals were treated with higher doses (5 and 2.5 mg/kg body weight) of cisplatin alone and in combination with antioxidants (vitamin C, vitamin E and silibinin) for 5 days. 6 mice from each group were examined for the protective effects of antioxidants on cisplatin induced tissue damage by DNA fragmentation and histological studies of kidneys, liver and spleen.

**Results:** The damage caused by cisplatin was ameliorated after the supplementation of antioxidants showing a marked reduction in the extent of tubular damage, the focal reaction changes in liver were reversed and no signs of toxicity in the spleen were reported. Moreover, no DNA damage was observed in animals treated with cisplatin along with various antioxidants.

**Conclusions:** The present results showed that antioxidants helped in the amelioration of drug induced toxic effects against murine visceral leishmaniasis, making the combination a potential anti-leishmanial therapy.

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**INTRODUCTION**

Visceral leishmaniasis is responsible for significant morbidity and mortality in the developing world [1] and patients with active disease exhibit marked immunosuppression and often succumb to secondary infections [2]. Untreated visceral leishmaniasis carries a mortality of 75-79% [3]. Visceral leishmaniasis targets the poorest communities; and children, young adults, and women are disproportionately affected [4,5]. An effective vaccine/drug could be integrated into a control strategy that would ideally comprise effective and well-tolerated treatment, vector control, and personal protection from vectors [6]. Thus, active research is underway in many laboratories to find alternative, effective, preventive and curative treatments.

Current therapeutic modalities available for the patients are ridden with unacceptable toxicity and/or prohibitive costs. In addition, the emergence of drug resistance is a major hurdle in the control of this disease. Due to the problems with the existing chemotherapy, it necessitates the discovery of new and more effective antileishmanial compounds. Cisplatin (cis-diamminedichloroplatinum (II)) is a water-soluble planar member of the platinum coordination complex class of anticancer drugs [7]. The *in vitro* and *in vivo*
studies have reported its antileishmanial activity with apoptosis like cell death of both promastigotes and amastigotes [8]. A significant reduction in parasite load and enhanced DTH responses in *Leishmania donovani* infected BALB/c mice have been observed [9,10]. However, its antileishmanial activity is often limited by the development of nephrotoxicity (a major side effect), which is evident in various animal species [11,10]. Biochemical investigations (liver and kidney function tests) of low and higher dosage of cisplatin have shown changes in certain enzymes and electrolyte levels during murine visceral leishmaniasis [9,10] but histological changes induced by cisplatin have not been reported. As cisplatin causes nephrotoxicity, it has been suggested that the toxic effects of cisplatin may be related to free radical induced damage that can be reduced by the supplementation of antioxidants [12,10]. Studies have focused on the role of antioxidants in altering cisplatin toxicities by acting as free radical quenchers that are produced by cisplatin and help in the protection against cisplatin toxicities. The present study investigated experimentally cisplatin-induced damage caused to kidneys, liver and spleen of *L. donovani* infected BALB/c mice and its prevention by administering antioxidants along with cisplatin.

**MATERIALS AND METHODS**

**Parasite:**

*Leishmania donovani* promastigotes of strain MHOM/IN/80/Dd8, originally obtained from the London School of Hygiene and Tropical Medicine, London, were used for the present study and maintained *in vitro* at 22 ± 1 °C in modified NNN medium by serial subcultures after every 48–72 h. The promastigote culture was examined by wet mount preparation and the promastigotes were seen as motile, spindle shaped, fast swimming organisms with long anterior flagellum.

**Drugs and antioxidants:**

Cis-diaminedichloroplatinum (II) dichloride (cisplatin, CP), vitamin C (ascorbic acid), vitamin E [(±)-α-tocopherol] and silibinin were purchased from Sigma Aldrich Co., USA. Sodium stibogluconate (SSG) was purchased from Wellcome Research Laboratories, U.K.

**Animals:**

Inbred BALB/c mice (both sex), 5-6 weeks old weighing 20-25 gm were procured from IMTECH and Central Animal House of Panjab University, Chandigarh. They were fed with water and mouse feed *ad libitum*. All animal procedures have been reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the Panjab University, Chandigarh (Approval No. 1334-50/CAH/3.09.2008).

**Infection and treatment:**

Animals were infected with 10⁷ promastigotes of *L. donovani* and after 30 post infection days (p.i.d.), cisplatin (5 mg/kg b.wt. and 2.5 mg/kg b.wt., intraperitoneally) and its combination with various antioxidants (200 mg/kg b.wt. of vitamin C, orally; 100 mg/100 gm b.wt. of vitamin E and 200 mg/kg b.wt. of silibinin) was administered to all groups of animals daily for 5 days. Vitamin C and vitamin E were given orally while silibinin was injected intraperitoneally into mice daily for 5 days. Both Vitamin E and C were given 30 minutes before the drug treatment and silibinin was given 1 h before drug treatment [10].

**Groups of animals:**

The animals were divided into 7 groups as follows:

- **Group 1** Infected+Cisplatin treated
- **Group 2** Infected+Cisplatin+Silibinin
- **Group 3** Infected+Cisplatin+Vitamin C+Vitamin E
- **Group 4** Infected+Cisplatin+Silibinin+Vitamin C+Vitamin E
- **Group 5** Infected+Sodium stibogluconate (SSG) (Positive control)
- **Group 6** Infected control
- **Group 7** Normal control (negative control)

6 mice were assigned for each group. Groups 1-4 were further divided into two groups (A and B). Group A was treated with cisplatin at a dose of 2.5 mg/kg b.wt., intraperitoneally daily for 5 days and group B was treated with cisplatin at a dose of 5 mg/kg b.wt. intraperitoneally daily for 5 days. Twenty uninfected animals each from group A and group B were also treated with cisplatin, various antioxidants at the same dose and served as controls for infected animals treated with cisplatin and respective antioxidants.

**DNA isolation:**

Kidneys and spleen from different groups of animals were removed and used for isolation of DNA by using commercially available kits (ZR Genomic DNA II Kit). Nucleic acids were quantitated using ND-1000 Spectrophotometer (Nanodrop®, UK).

**Polymerase chain reaction (PCR) for the detection of antigen:**

The 792-bp *L. donovani* kinetoplast mini-circle sequence was amplified by using PCR. In brief, the reaction mixture (50 µl) was prepared which contained standard 10 X PCR buffer (2 µl), 1.5 mM MgCl₂ (1.5 µl), deoxynucleotide solution mix (10 mM) (1.5 µl), 1

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μl of each primer, DNA template (genomic DNA from spleen cells) (1.5 μl), 1.25 U/50 μl PCR mix of *Taq* DNA polymerase (1 μl) and nuclease free water (brought to 50 μl). The reaction mixture was amplified in a thermal cycler (Biometra, UK) programmed for 35 cycles of denaturation at 94°C for 3 sec, annealing (56-60°C) for 40 sec, and extension at 72°C for 40 sec, preceded by an initial denaturation of 2 min at 94°C. Final extension was for 4 min at 72°C. Gene-specific primers used were 5'-AAATCGGCTCCGAGGCAGGAAAC-3' (Forward) and 5'-GGTACACTCTATCAGTAGCAC-3' (Reverse) as per Salotra et al. [13]. Amplified products were resolved on 0.8% agarose gel containing ethidium bromide at 100 V for 30–40 min using TBE buffer; visualized and analysed for DNA fragmentation in the form of classical ladder or smear under UV light transilluminator (Amersham, USA) and photographed by using gel doc (GE Healthcare, USA).

DNA fragmentation assay:

Extracted genomic DNA samples (spleen and kidney cells) were resolved on agarose gel containing ethidium bromide at 100 V for 30–40 min using TBE buffer; visualized and analysed for DNA fragmentation in the form of classical ladder or smear under UV light transilluminator (Amersham, USA) and photographed by using gel doc (GE Healthcare, USA).

Histological studies:

Kidneys, liver and spleen from each group of animals were fixed in Bouin's fixative [14] and the sections were stretched in hot water on albumin coated slides and stained with Delafield's Hematoxylin&Eosin Technique (H&E) to study histology [15]. The DPX mounted sections were observed under light microscope and photography was done by using Phase contrast microscope (Nikon) fitted with a digital camera (ProRes, Jenoptik-Germany).

RESULTS

Detection of parasite antigen in the spleen cells of infected and treated BALB/c mice by PCR: The identical product of 792-bp was observed in PCR of DNA samples (spleen cells) in all group of animals (Fig. 1A-B). The specificity of 100% was observed by PCR.

DNA fragmentation assay: In normal control where no infection/drug was given, DNA fragmentation was not observed in any of the tissues. In infected controls, DNA fragmentation was observed in the spleen tissue but no DNA damage was observed in kidney tissues. On the contrary, infected+cisplatin treated animals showed DNA fragmentation in the form of smear or faint ladder in both the tissues. No DNA damage was observed in animals treated with cisplatin along with various antioxidants (Fig. 2A-B, 3A-B).

Histological studies of kidneys, liver and spleen:

Light microscopic observation of kidneys of normal animals revealed the presence of tubules, distinct nucleus and renal corpuscles comprised of glomerulus and Bowman’s capsule supported by connective tissue. Brush border cells distinguished into proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) were observed. Medulla comprised of a number of renal pyramids, whose base was found to be adjacent to cortex and apex inwards (Fig 4A). In infected animals, many foci of lymphocytic infiltration in the interstitium were seen which depict focal interstitial nephritis (Fig 4B).

Cisplatin treatment at both doses induced mild vascular and inflammatory changes with signs of vascular congestion and exhibited contracted glomerulus which is a degenerative phenomenon. The kidney showed unknown distinct cell like bodies within the tubules which were of the size of a tissue monocyte, round and appear granular and nucleated. In addition there were larger single granular bodies, one per tubule. Tubular lumen filled with cellular debris and elevated tissue in interstitium indicative of necrotic cell was also observed after cisplatin treatment. Occasional focus of lymphocytes was present in the interstitium. Thus, kidney showed intratubular parasitic bodies with focal interstitial inflammation and showed many foci of lymphocytic infiltration in the interstitium, showing focal interstitial nephritis. Brush border epithelial damage of tubules and pyknotic nucleus was also observed (Fig. 4E-H). In contrast, animals treated with SSG did not show any of the above changes in kidney sections, however, swelling, desquamation and congestion in dilated glomeruli were observed in infected and SSG treated animals (Fig 4C-D).

Moreover, the damage caused by cisplatin was ameliorated after the supplementation of antioxidants, and a marked reduction in the extent of tubular damage was observed. The kidney glomeruli and other tubules were observed to be normal. The nucleus attained a normal structure with no signs of pyknosis. The Bowman’s capsule was found to be normal having two distinct visible layers i.e. parietal and visceral layer. The lumen of the tubules was clearly visible and nuclei were clearly distinct. Proximal and distal convoluted tubules were distinguishable with proximal convoluted tubules having small lumen as compared to distal convoluted tubules (Fig 5A-F).

Light microscopic observations of liver tissue of normal control revealed normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in between the hepatic cords with fine arrangement of Kupffer cells.
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Figure 1. Agarose gel electrophoresis of amplified product of 792-bp fragment of gene encoding kinetoplast mini-circle DNA

A: 
Lane 1—DNA ladder (100-1000 bp), 
Lane 2—Normal control, 
Lane 3—Infected control, 
Lane 4—Infected+SSG, 
Lane 5—Infected+5mg CP, 
Lane 6—Infected+2.5mg CP

B: 
Lane 1—DNA ladder (100-1000 bp), 
Lane 2—Normal control, 
Lane 3—Infected+5mg CP+silibinin, 
Lane 4—Infected+2.5mg CP+silibinin, 
Lane 5—Infected+5mg CP+vitC+vitE, 
Lane 6—Infected+2.5mg CP+vitC+vitE, 
Lane 7—Infected+5mg

Figure 2. DNA fragmentation assay in kidney cells of infected and treated BALB/c

A: 
Lane 1—DNA ladder (100-1000 bp), 
Lane 2—Normal control, 
Lane 3—Infected control, 
Lane 4—Infected+SSG, 
Lane 5—Infected+5mg CP, 
Lane 6—Infected+2.5mg CP

B: 
Lane 1—DNA ladder (100-1000 bp), 
Lane 2—Infected+5mg CP+silibinin, 
Lane 3—Infected+2.5mg CP+silibinin, 
Lane 4—Infected+5mg CP+vitC+vitE, 
Lane 5—Infected+2.5mg CP+vitC+vitE, 
Lane 6—Infected+5mg CP+vitC+vitE+silibinin, 
Lane 7—Infected+2.5mg CP+vitC+vitE+silibinin
Figure 3. DNA fragmentation assay in spleen cells of infected and treated BALB/c

A:
Lane 1 — DNA ladder (100-1000 bp),
Lane 2 — Normal control,
Lane 3 — Infected control,
Lane 4 — Infected+SSG,
Lane 5 — Infected+5mg CP,
Lane 6 — Infected+2.5mg CP

B:
Lane 1 — DNA ladder (100-1000 bp),
Lane 2 — Infected+5mg CP+silibinin,
Lane 3 — Infected+2.5mg CP+silibinin,
Lane 4 — Infected+5mg CP+vitC+vitE,
Lane 5 — Infected+2.5mg CP+vitC+vitE,
Lane 6 — Infected+5mg CP+vitC+vitE+silbinin,
Lane 7 — Infected+2.5mg CP+vitC+vitE+silbinin
Figure 4. Transverse sections of kidney of infected and treated BALB/c. A. Normal control, B. Infected control, C. Infected+SSG, D. SSG alone, E. Infected+5mg CP, F. 5mg alone, G. Infected+2.5mg CP, H. 2.5mg alone (Hematoxylin and Eosin, x400).

(Abbreviations: G—glomerulus, BC—Bowman’s capsule, AP—apical pole, DCT—distal convoluted tubule, PCT—proximal convoluted tubule, N—nucleus, A—arteriole, B—bodies, PN—pyknotic nucleus, CG—contracted glomerulus, circle—focal interstitial nephritis, arrow—brush border epithelial damage of tubules)
Cytoplasm of hepatocyte was found to be eosinophilic (Fig 6A). In contrast, Hematoxylin&Eosin stained tranverse section of liver of infected (Fig 6B) and infected and drug treated animals (Fig 6C-H) showed tiny focus of lymphocyte aggregate depicting focal reaction changes in liver and mild kupffer cell hyperplasia was observed. Fatty change in the cytoplasm and pyknotic nuclei was observed. The accumulation of red blood cells around the draining pathways of the central vein suggesting backflow congestion was also observed after the cisplatin treatment.

Figure 5. Transverse sections of kidney of infected and treated BALB/c. A. Infected+5mg CP+silibinin, B. Infected+2.5mg CP+silibinin, C. Infected+5mg CP+vitC+vitE, D. Infected+2.5mg CP+vitC+vitE, E. Infected+5mg CP+vitC+vitE+silibinin, F. Infected+2.5mg CP+vitC+vitE+silibinin (Hematoxylin and Eosin, x400).

(Abbreviations: G —glomerulus, BC —Bowman’s capsule, DCT—distal convoluted tubule, PCT— proximal convoluted tubule, N— nucleus)
Figure 6. Transverse sections of liver of infected and treated BALB/c. A. Normal control, B. Infected control, C. Infected+SSG, D. SSG alone, E. Infected+5mg CP, F. 5mg alone, G. Infected+2.5mg CP, H. 2.5mg alone (Hematoxylin and Eosin, x200).
(Abbreviations: H —hepatocytes, S —sinusoids, KC —Kupffer cell, PV —portal vein, KH —Kupffer cell hyperplasia, RBC—red blood cells, circle—focal reaction changes)
After the supplementation of antioxidants along with cisplatin, the focal reaction changes were reversed and liver attained a normal structure. Hepatocytes and portal tracts were found to be normal in shape. Treatment with antioxidants reduced the pathological lesions induced by cisplatin. The portal vein, portal triad and central veins were found to be dilated at certain foci with a moderate number of Kupffer cells (Fig 7A-F).

Light microscopic observation of spleen tissue of normal controls revealed normal morphology including the red and white pulp areas, separated by marginal zone which were clearly visible (Fig 8A). In infected controls, the spleen showed the reactive enlargement of follicles. Brown pigment (hemozoin) within the cells was observed indicating intravascular hemolysis which may be due to the presence of *Leishmania* in the spleen tissue. Both red pulp and white pulp were undistinguished and proliferation of marginal zone was observed (Fig 8B). In cisplatin treated animals, spleen showed the expansion of marginal zone and white pulp with occasional focus of acute abscess containing neutrophils. There was wide spread capillary and venule thrombosis. The excess of megakaryocytes was also observed. The above changes showed that the spleen is reactive suggesting septicemia (Fig 8E-H). In SSG treated animals, the spleen appeared normal, some lymphoid follicles suggested a mild expansion of mantle zone around the follicles (Fig 8C-D). There were no signs of toxicity in the spleen in the groups of animals treated with cisplatin along with various antioxidants. Red and white pulp areas were clearly demarcated. Trabeculae emanating into splenic parenchyma were clearly visible. Hemozoin pigment depositions were not observed (Fig 9A-F).

![Figure 7. Transverse sections of liver of infected and treated BALB/c. A. Infected+5mg CP+silibinin, B. Infected+2.5mg CP+silibinin, C. Infected+5mg CP+vitC+vitE, D. Infected+2.5mg CP+vitC+vitE, E. Infected+5mg CP+vitC+vitE+silibinin, F. Infected+2.5mg CP+vitC+vitE+silibinin (Hematoxylin and Eosin, x200). (Abbreviations: H —hepatocytes, S —sinusoids, KC—Kupffer cell, PV—portal vein, CV —central vein, PT —portal triad, EL — endothelial layer)](image-url)
Figure 8. Transverse sections of spleen of infected and treated BALB/c. A. Normal control, B. Infected control, C. Infected +SSG, D. SSG alone, E. Infected +5mg CP, F. 5mg alone, G. Infected +2.5mg CP, H. 2.5mg alone (Hematoxylin and Eosin, x400 for A and B panel, x200 for other panels)

(Abbreviations: WP —white pulp, RP —red pulp, MK —megakaryocytes, BP —brown pigment, LC —lymphoid cells, T —trabecula, GC —germinal centre, MZ —marginal zone, CA —central artery, C —capsule, N —neutrophils, arrow —pigment contained in macrophages)
DISCUSSION

PCR has been proved to be a useful non-invasive tool for the diagnosis of visceral leishmaniasis with great accuracy. In the present study, the PCR assay was able to detect parasite DNA from the spleen cells of infected BALB/c mice, animals treated with cisplatin and animals treated with cisplatin along with various antioxidants, indicating that it is a very sensitive assay detecting infections that may be missed by conventional smear staining techniques. The detection of kDNA from various samples also proved that cisplatin is not able to completely eliminate the parasite from the *L. donovani* infected BALB/c mice and confirmed the presence of visceral infection. From the present study, it can be concluded that cisplatin treatment is effective for treatment of visceral leishmaniasis and reduces the parasite burden [10] but does not completely eliminate the parasite at the present dose.
In the present work, we evaluated the occurrence of DNA fragmentation in *Leishmania donovani* infected and drug treated BALB/c mice in the spleen and kidney tissue. DNA damage was observed in spleen cells of infected controls. In an earlier study also Kaur et al. [16] observed that DNA from infected mice showed the characteristic ladder pattern, while an intact genomic DNA was observed in control animals. Treatment of *Leishmania infantum* amastigotes with Sb (III) at low concentrations was found to induce DNA fragmentation, suggesting the appearance of late events of programmed cell death (apoptosis) [17] which is found to be in accordance to our study where DNA damage was observed in spleen cells. It has already been reported that DNA of cisplatin treated mice form DNA adducts [18]. In vitro effects of cisplatin on DNA shows that the formation of platinum adducts block DNA replication and consequently growth arrest. DNA damage was also detected in case of amastigotes at a dose of 0.25-64 µM of cisplatin [8]. The above findings are in accordance to our study where DNA fragmentation was observed in spleen cells of animals treated with both dosages of cisplatin. Reduction in cell viability as well as increase in the number of cells with fragmented nuclei correlated with cisplatin exposure in a dose-dependent manner. Apoptosis or programmed cell death is characterized by distinct morphologic changes consisting of cell shrinkage, nuclear condensation, and internucleosomal DNA fragmentation. Renal tubule cell apoptosis has recently been observed in an increasing array of renal disorders [19] and is emerging as a final common pathway in response to a wide variety of cellular stresses applied at intensity below the threshold for necrosis. This observation also holds true for cisplatin nephrotoxicity, in which necrotic cell death is encountered with higher doses whereas lower concentrations induce apoptosis [20,21]. Our findings also found DNA damage in renal tissue after the administration of cisplatin which may be due to oxidative stress injury which is actively involved in the pathogenesis of cisplatin-induced acute kidney injury. Reactive oxygen species (ROS) directly act on cell components, including lipids, proteins, DNA, and destroy their structure. The antioxidant supplementation is essential to impair the antioxidant-oxidant imbalance which helps in the reduction in damage caused by cisplatin. Antioxidants melatonin [22], vitamin C [23], and vitamin E [24] have been shown to prevent cisplatin-induced acute nephrotoxicity. In the present study also when antioxidants were given along with cisplatin, no DNA damage was reported in any of the tissues suggesting the amelioration of the side effects caused by cisplatin and gave significant protection against cisplatin induced nephrotoxicity.

The histological changes in the kidneys, liver and spleen were evaluated in different groups of animals. Infected animals showed many foci of lymphocytic infiltration in the intestitium which depict focal interstitial nephritis which were found to be similar to studies of Dutra et al. [25] where acute interstitial nephritis was reported in patients with leishmaniasis. Acute glomerulonephritis [26], proliferative glomerulonephritis [25], collapsing focal segmental glomerulosclerosis [27] and tubular cell necrosis and tubulitis [28] have all been described in patients with leishmaniasis. Extensive use of cisplatin is usually accompanied by long term non-hematological toxicity such as nephrotoxicity [29]. Several lines of evidence reported so far state that reactive oxygen species play a deleterious role thereby causing nephrotoxicity [11]. It has been recognized that the nephrotoxic effect of cisplatin has been witnessed by the spectrum of cytotoxic injury ranging from mild sublethal changes to a catastrophic necrotic death which leads to an inflammatory response [30]. In the present study, the overall external appearance of kidney looked weathered and discoloured. Histological changes of BALB/c kidney after cisplatin treatment revealed intoxication which showed a severe atrophy of glomerulus, which was apparent due to the reduction in its size. This is in line with the earlier reports by DeviPriya and Shyamala [31]. Marked dilatation of proximal convoluted tubules with slogging of almost entire epithelium due to desquamation of tubular epithelium was evident. Cellular debris in the tubular lumen and increased tissue in the interstitium is also an indication of cisplatin-induced renal necrosis. The changes obtained in the present study run parallel with the report documented by Shirwaikar et al. [32] where the investigators had demonstrated cisplatin induced acute renal necrosis with marked congestion of the glomeruli and glomerular atrophy. Cisplatin treatment also showed desquamation of the tubular epithelial cells with casts in the tubular lumen and infiltration of inflammatory cells in the interstitium [29]. Goldstein and Mayor [33] have observed that cisplatin toxicity primarily caused degenerative changes in the proximal tubules [34] which were in accordance with our study. In an earlier study from our laboratory with low dose of cisplatin, it was found that cisplatin (1 mg/kg b.wt.) treated kidney exhibited contracted glomerulus, decreased lumen and damaged brush border. However, animals treated with 0.5 mg/kg b.wt. of cisplatin also revealed some tubules lined with flattened cells having diminished cell size and reduced cytoplasmic volume [9]. It has been suggested that the pathogenesis of cisplatin nephrotoxicity has been associated with oxidative stress, DNA damage and apoptosis [35,36]. Reactive oxygen species (ROS) have been considered to play a central role in injury caused to kidneys [37,38,39]. Various experimental studies have indicated that these
vitamins are effective in preventing the oxidative renal damage [40,41] and stress induced brain damage in animals [42]. A large number of studies have been accumulated documenting the beneficial effects of a variety of antioxidants in cisplatin induced nephrotoxicity [43]. In the present study, different antioxidants have been supplemented along with cisplatin to reduce the side effects caused by cisplatin. The histological evaluation of the kidney preparation in antioxidant supplemented groups revealed a decrease in cisplatin-induced damage caused to kidney tissue. The kidney glomeruli, PCT, DCT and Bowman’s capsule appeared to be normal. The nucleus attained a normal structure showing no signs of pyknosis. The lumen of the tubules was clearly visible and nuclei were clearly distinct. Proximal and distal convoluted tubules were distinguishable, proximal convoluted tubules having small lumen as compared to distal convoluted tubules. The present study is in accordance to the studies conducted by Atessahin et al. [44] where pre treatment with lycopene showed protective effect against cisplatin induced nephrotoxicity. The results were also found to be in consistent with the studies of Annie et al. [45] where Cassia auriculata at a dosage of 600 mg/kg b.wt. normalized the damage induced by cisplatin.

The liver is considered as a monitor organ, being able to represent in its ultrastructure the biological responses to environmental alterations [46,47,48]. Histological studies were carried out in normal controls, infected controls, infected plus drug treated and infected plus drug plus antioxidant treated animals. In infected controls, the liver showed focus of lymphocyte aggregate depicting focal reaction changes and mild kupffer cell hyperplasia. The hypertrophy and hyperplasia of kupffer cells was also found in naturally and experimentally L. chagasi infected animals [49]. Increased iron content in hepatocytes and kupffer cells was seen in all patients with visceral leishmaniasis before and after treatment. The inflammation consisted of hyperplastic and hypertrophic kupffer cells and sinusoidal inflammatory mononuclear cells [50]. In cisplatin treated animals, tiny focus of lymphocyte aggregate depicting focal reaction changes in liver and mild kupffer cell hyperplasia was observed. The accumulation of red blood cells around the draining pathways of the central vein suggesting backflow congestion was observed after the cisplatin treatment. Liver damage characterized by hepatocellular vacuolizations around the central vein and clusters of inflammatory cells (mostly plasma cells and lymphocyte) surrounding portal area was also observed by Yu et al. [51]. The depletion of GSH and decreased activities of antioxidant enzymes seemed to be an early and key event during cisplatin-induced lipid peroxidation and subsequent toxicity [52]. The supplementation of antioxidants ameliorated the side effects induced by cisplatin which was confirmed by reversal of the focal reaction changes and reduction in the pathological lesions induced by cisplatin. In findings of Yu et al. [51], pre-treatment of bicyclol offered a significant protection from the cisplatin-induced hepatotoxicity. The histological injuries such as hepatocellular vacuolizations and clusters of inflammatory cells surrounding the portal area induced by cisplatin were also markedly improved by bicyclol which further justifies our study. Our findings are also in accordance to the studies of Abdelmeguid et al. [53], where pre treatment with silymarin 2 hr before cisplatin significantly decreased the pathological changes and was highly protective.

Histological studies of normal spleen revealed well demarcated red and white pulp areas, separated by marginal zone. In infected controls, the spleen showed the reactive enlargement of follicles. Brown pigment (haemozoin) within the cells has been observed indicating intravascular hemolysis which may be due to the presence of Leishmania in the spleen tissue. Both red pulp and white pulp were undistinguished and proliferation of marginal zone was observed. Similarly, Tafuri et al. [49] also reported changes in the spleen tissue of infected animals where profound modifications were observed in red pulp due to the considerable increase in cellular proliferation. The intense proliferation of the marginal zone consisting of small cells with dense nuclei and sparse cytoplasm (lymphocytes) and larger cells with weak nucleus and ample cytoplasm (macrophages) was observed. In cisplatin treated animals, spleen showed the expansion of marginal zone and white pulp with occasional focus of acute abscess containing neutrophils. There was wide spread capillary and venule thrombosis and excess of megakaryocytes. The above changes suggest that the spleen is reactive suggesting septicemia. Our results were found to be in accordance to the studies by Milicevic et al. [54] where cisplatin (6 mg/kg b.wt.) showed histological changes in the spleen. Volume density of the marginal zone was significantly reduced and significant increase of volume density of connective tissue was observed. The reduction in number of follicles per mm2 of spleen section area, the numerical density of the follicles in the spleen, and the total number of the follicles was observed. Similarly, Endo et al. [55], reported changes in the spleen after treatment with cisplatin (5 to 10 mg/kg) which includes karyorrhexis of lymph follicles of the spleen of cisplatin-treated ferrets. On administration of antioxidants, the spleen damage reversed and showed protection against cisplatin induced damage to spleen which was found to be in accordance to the studies by Atroshi et al. [56] where pretreatment with coenzyme Q10, L-carnitine, α-tocopherol and selenium showed protection against Fumonisin B1 induced damage to
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spleen. Hence, we establish that higher doses of cisplatin should be used in combination with antioxidants as they act as antagonists of the toxicity and helped in the reversal of the drug induced toxic effects.

The research work therefore concludes that cisplatin when used in combination with antioxidants can be used as an alternative antileishmanial therapy. Further refinements are needed in the optimization of cisplatin by combination with immunomodulators/herbal extracts which may act as better rivals of toxicity.

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