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Short Communication

Histological Evaluation of Cisplatin-Induced Gonadotoxicity against Murine Visceral Leishmaniasis

Meenakshi Sharma and *Sukhbir Kaur

Department of Zoology, Panjab University, Chandigarh-160014, India

Corresponding Author: Sukhbir Kaur

Abstract

Cisplatin had shown anti-leishmanial effect both in vitro and in vivo against murine visceral leishmaniasis. The present study was designed to evaluate the effect of cisplatin on gonads of Leishmania donovani infected BALB/c mice. In drug treated animals, mild reduced spermatogenesis was observed but when antioxidants were supplemented along with cisplatin, the morphological characteristics of testes were comparable to those in control groups. Moreover, the ovary was also found to be normal in the oestrus phase with development of corpus luteum. The above findings showed that damage caused by cisplatin to gonads was ameliorated by administration of antioxidants.

Keywords: *Leishmania,* cisplatin, gonads, antioxidants

Introduction

Cisplatin has shown good promise as an antileishmanial drug as evidenced by *in vitro* and *in vivo* studies against visceral leishmaniasis (Tavares et al., 2007; Kaur et al., 2010; Sharma et al., 2012). However, it has been reported to cause cumulative toxicity to kidneys (Kaur et al., 2010), but its effect on the reproductive organs has not yet been reported. The present studies deals with *in vivo* evaluation of cisplatin induced damage to testes and ovaries. In addition, the protective efficacy of the antioxidants (vitamin C, vitamin E and silibinin) when administered along with cisplatin has been studied in *Leishmania donovani* infected BALB/c mice.

Inbred BALB/c mice (5-6 weeks old weighing 20-25 gm) fed with water and mouse feed *ad libitum* were used for the study. The ethical clearance for conducting various experiments was taken from Institutional Animal Ethics Committee (IAEC) of the Panjab University, Chandigarh.

The animals were infected with 10^7 promastigotes of *L. donovani* intracardially 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, orally and 200 mg/kg b.wt. of silibinin, intraperitoneally) was administered to all groups of animals daily for 5 days. All groups of animals (1-3) were divided into two groups (A and B). Group A was treated with cisplatin at a dose of 5 mg/kg b.wt. and group B was treated with cisplatin at a dose of 2.5 mg/kg b.wt. (Table I). Testes and ovaries from each group of animals were fixed in Bouin's fixative 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.

Results and Discussion

The hematoxylin/Eosin stained transverse sections of testes of normal animals showed normal testicular morphology with regular spermatogenesis and normal sertoli cells. Mature spermatozoa were found in the cavity of seminiferous tubules (Fig. 1A). In infected animals (Fig. 1B), reduced spermatogenesis was observed showing maturation arrest. Decreased number of spermatogenic cells was found in accordance with the descriptions of Rioux et al. (1971). In contrast, Gonzalez et al. (1983) found that experimental infection of hamsters with *L. donovani* causes testicular amyloidosis and a gradual development of inflammatory and degenerative changes in the testes, leading to total azoospermia. No *Leishmania* amastigotes could be detected in testicular tissue (Eissa et al., 2011), which justifies our findings. In cisplatin treated animals (Fig. 1C-F), the testes is more or less normal, there is possibly reduced spermatogenesis in the final step of maturation. Reduced seminiferous epithelial layers were found in numerous tubules, and irregular and diminished tubules containing a few germ cells were also seen. The effects of cisplatin on testes may be due to its specific toxic effects on the target organ and not due to its general toxicity. In studies conducted by Llbey et al. (2009) all rats treated with cisplatin alone were characterized by a depletion of germ cells, irregular seminiferous tubules exhibiting sertoli cell and a few spermatogonia. Significant maturation arrest was also observed in the group which received cisplatin alone compared with the control groups. In contrast to our study in the rats receiving only cisplatin, mild perivascular fibrosis

and hyalinization of intertubular tissue was observed (Llbey et al., 2009). In animals treated with cisplatin along with various antioxidants, the morphological characteristics of testes as comparable to those in control group, confirming previous reports that curcumin (antioxidant) has a strong potential for use as a therapeutic adjuvant in cisplatin gonadotoxicity. The improvement that was observed in spermatogenesis among antioxidant delivered animals may be associated with the antioxidant and free radical scavenger properties of antioxidants.

The hematoxylin/Eosin stained transverse section of ovary of normal and infected controls (Fig. 2A, 2B) showed many rounded or oval bodies called ovarian or graaffian follicles at various stages of development. Each follicle contains a large ovum surrounded by many layers of follicle cells. In another study also C57BL/6 mouse showed a diffuse amyloid deposition in the ovary with no significant damage in the ovary was (Elizabeth et al., 1984), which is in consistence to our study. According to Reithinger et al. (2002), detection of *Leishmania* parasites in the ova has been reported in naturally infected dog which contradicts our study where no parasite was reported in the ovary. In drug treated animals and animals treated with cisplatin along with various antioxidants, the ovary is normal in the oestrus phase with development of corpus luteum. Preventive ova as well as primordial follicles are normal. In contrast to our study, Singh et al. (2005) found that in humans, cisplatin has been known to cause damage to the ovary as well, leading to premature ovarian failure in approximately 40% of female patients who undergo chemotherapy. Yeh et al. (2011) found that the administration of antioxidant sodium 2-mercaptoethanesulfonate immediately prior to treatment with cisplatin modulated the reproductive loss in animal 28 to cisplatin which was in accordance to our stu administration of antioxidants showed normal ovarian histology.

Hence, we establish that higher dosage of cisplatin cause damage to gonads and it is further recommended that higher dose should be used in combination with antioxidants which help in suppression of drug-induced toxic effects. The results presented here are promising with respect to the amelioration of cisplatin-induced gonadotoxicity by antioxidants against murine visceral leishmaniasis.

Acknowledgements

The authors hereby declare that the experiments comply with the current laws in India.

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Sr. No.	1	2	3	4	5
Groups	Cisplatin+Silibinin	Cisplatin+Vitamin C+ Vitamin E	Cisplatin+Silibinin+ Vitamin C+Vitamin E	Infected control	Normal control

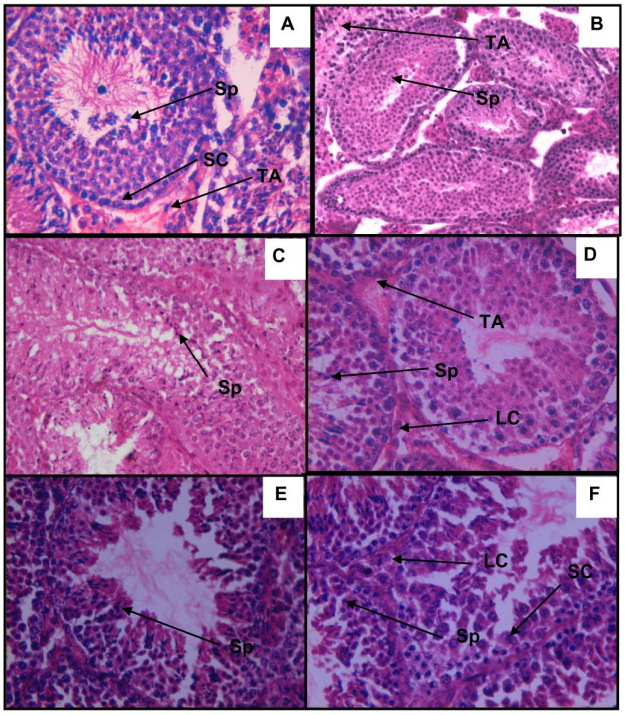

Figure 1 Transverse section of testes of infected and treated BALB/c stained with hematoxylin and eosin stain (400X). A —Normal control, B —Infected control, C —Infected+5mg CP, D—5mg alone, E —Infected+2.5mg CP, F—2.5mg alone (Abbreviations: Sp —spermatids, SC —sertoli cells, LC —leydig cells, TA —tunica albuginea)

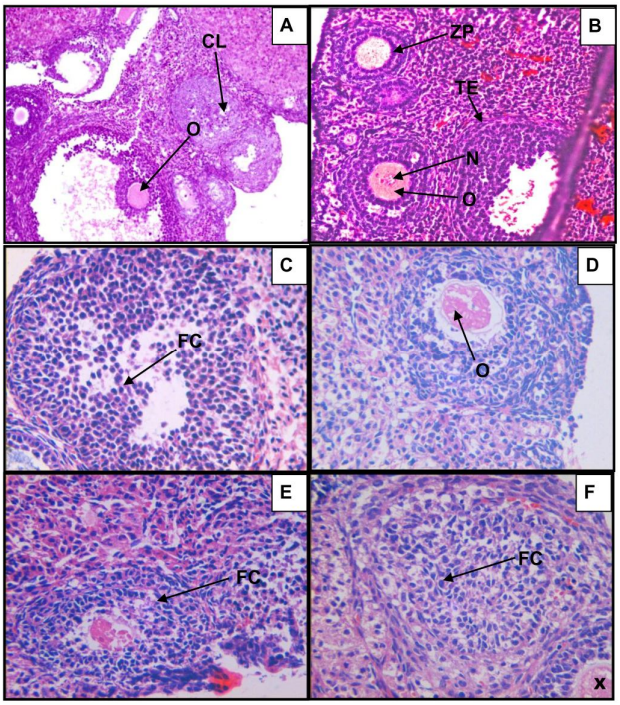

Figure 2 Transverse section of ovary of infected and treated BALB/c stained with hematoxylin and eosin stain (400X). A —Normal control, B —Infected control, C —Infected+5mg CP, D—5mg alone, E —Infected+2.5mg CP, F—2.5mg alone (Abbreviations: O —oocyte, FC —follicle cells, GE —germinal epithelium, N —nucleus, CL —corpus luteum, TE —theca externa, X—stained cytoplasm)