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Effect of Human Menopausal Gonadotropin on Rat Testes Damaged by Cisplatin Treatment

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ABSTRACT: Cisplatin is an established chemotherapeutic substance commonly used in the treatment of cancer. This study was conducted to examine the possible counter effects of Human Menopausal Gonadotropins (HMG) against testicular toxicity induced by Cisplatin in male rats. 20 adult male rats were equally divided into four groups; A, B, C and D which were the control, Cisplatin, HMG and Cisplatin+HMG groups respectively. Testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) while the testes and epididymis were dissected out and fixed in Bouin's fluid for histological preparation. A decrease in Testosterone, LH and FSH was observed in the (Cisplatin) group and group D (Cisplatin and HMG treatment) when compared to that of the control. Histological examinations in epidydimis and testes were further indication of significant damage to sertoli cells, leydig cells and germ cells populations induced by cisplatin. The adverse effect of Cisplatin was countered by the administration of HMG. In conclusion, Cisplatin-induced testicular toxicity was ameliorated by the administration of HMG.

Keywords: Cisplatin, Human menopausal gonadotropins, Reproductive hormones, Testes, Epidydimis

Introduction

Infertility has been reported to affect about 15% of reproductively aged couples (WHO, 1999). The male partner is involved in 40-50% of infertility cases (Lund and Larsen, 1998). Many drugs have been proposed for infertility in association with surgery. Gonadotropin therapy against infertility has been available for over four decades (Gemzell and Kjessler, 1964; MacLeod *et al.,* 1964). Human Menopausal Gonadotropin (HMG) is a natural hormone necessary for human reproduction. This hormone is a leading treatment of infertility as it contains equal amount of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). In men, HMG promotes sperm formation if testosterone level and FSH levels are low. The LH stimulates the production of testosterone and FSH promotes the formation of sperm (Vollenhoven, 1996).

Cisplatin is an established chemotherapeutic substance commonly used in the treatment of cancer (Autunes *et al.,* 2001). Researchers reported that chemotherapy with antineoplatic drugs caused significant toxic effect on genital epithelial cells most especially spermatogonium and spermatocytes which are involve in high mitotic activity, this makes them susceptible to most of the cytotoxic chemotherapeutics. Cisplatin treatment was found to cause testicular damage due to its harmful attack on spermatogenic cells and sertoli cells (Autunes *et al.,* 2001; Hu *et al.,* 1997; Seaman *et al.,* 2003; Zhang *et al.,* 2001).

It has also been reported to cause functional disorders in leydig cells and induced infertility because of the irreparable damage caused to the stem cell colony (Seaman *et al.,* 2003). However, mechanism through which Cisplatin induced harm to testicular cell is yet to be fully elucidated (Seaman *et al.,* 2003; Sawhney *et al.,* 2005). In this study, we investigated the protective effect of HMG on rat testes damaged by Cisplatin treatment.

Materials and Methods

Experimental animals and protocol: A total of 20 adult male albino rats 4 months; weighing between 200 and 320g were used in this study. Experimental animals (5 rats each) were divided into 4 groups; control, Cisplatin, HMG and Cisplatin + HMG groups. In all cases drug dose was adjusted according to body weight of rats and injected intraperitoneally. Animals in Cisplatin treatment group received a single dose of 2.5 mg/kg Cisplatin. HMG group was given 3.1 IU/kg. Rats in the combined Cisplatin + HMG treatment group received a single dose of 2.5 mg/kg Cisplatin followed by 3.1 IU/kg HMG treatment. The experiment lasted for 56 days.

Histology procedure: The testis was fixed overnight in Bouin's fluid, dehydrated in ethanol, and embedded in paraffin. Tissue sections (6 μm thick) were cut on a microtome, mounted on glass slide. Staining of section was with hematoxylin and eosin (HE).

Biochemical assay: Enzyme linked immunosorbent assay (ELISA) method on micro plate format were used for the estimation of serum total testosterone, FSH and LH. The kit used was a product of Dialab, Austral, Germany. The manufacturer's analytical procedures were followed and adhered to strictly. Replicates determination of each sample were made and the mean used for the group statistical analysis. The intra and inter assay variations were 2 % and 4 % respectively.

Statistical analysis: All values were presented as mean ± standard error of the mean for all groups. The significance of the difference in the means of all parameters was determined using one-way analysis of variance (95% confidence interval). Least Square difference, *post-hoc* tests was carried out for all groups with control and comparison of all pairs of groups respectively. All statistical analysis was carried out using statistical package for Social Sciences (SPSS) (version 20).

Results

Changes in some reproductive hormones measured in control and experimental animals: There was a significant ($p<0.05$) decrease in serum concentration of Follicle stimulating hormone, Luteinizing hormone and Testosterone of animals in Group B (treated with cisplatin only) compared to that of the control as shown in Figs. 1, 2 and 3. Serum concentration of Follicle stimulating hormone, Luteinizing hormone and Testosterone in Group C (HMG only) animals were not significantly different from Group A (control). The concentration of Follicle stimulating Hormone and Testosterone were significantly reduced in Group D (treated with cisplatin and HMG) ($p<0.05$) compared to that of the control group while Luteinizing hormone concentration was not significantly ($p<0.05$) different from Group A (control).

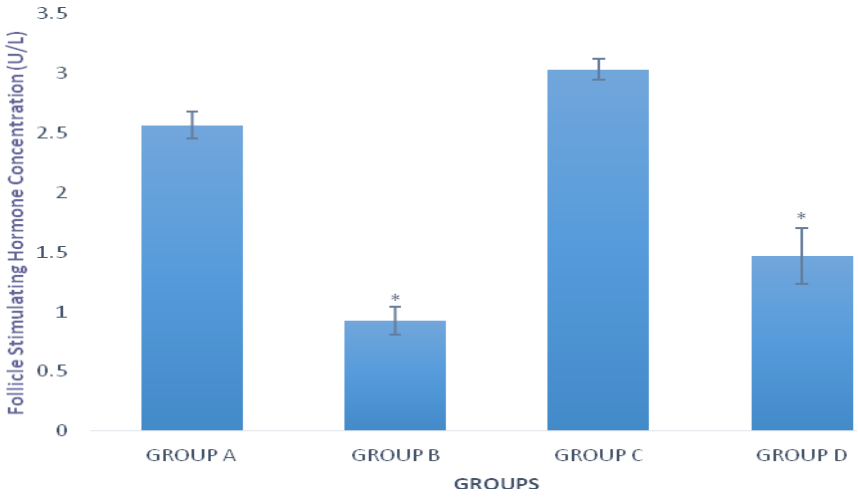

Figure 1**:** Changes in serum concentration of Follicle Stimulating Hormone in the experimental groups

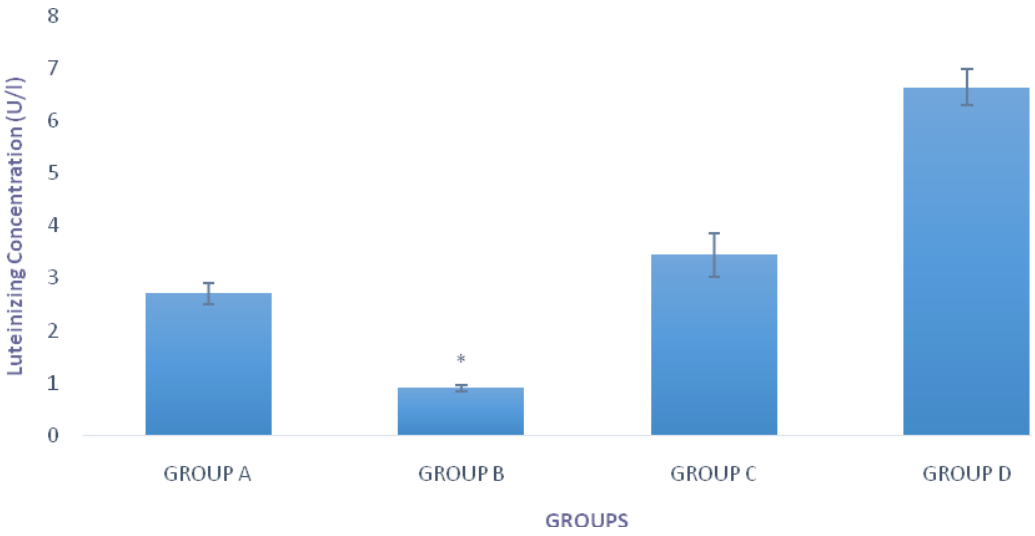

Figure 2: Changes in serum concentration of Luteinizing Hormone in the experimental groups.

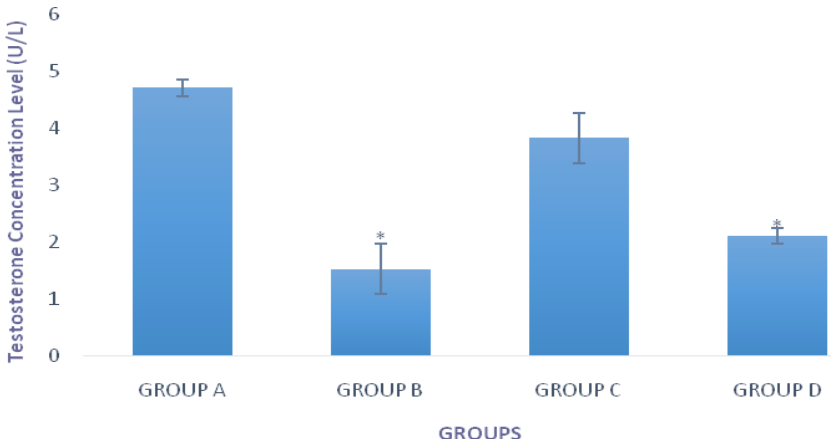

Figure 3: Changes in serum concentration of Testosterone in the experimental groups.

Histology of testes and epididymis: Testicular section of rats in Group B showed the disruption of cells of the spermatogenic series, wide lumen and reduced interstitial cells of leydig. Cellular debris from necrosed spermatozoa and degenerative changes in the principal cells of the epididymis were observed. These negative effects where abrogated by the administration of HMG as shown in Group D.

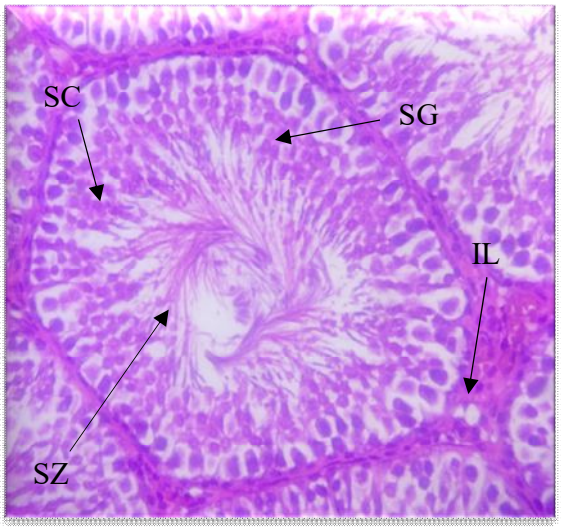

Plate 1: A photomicrograph of testicular section from a rat in Group A (control) showing normal spermatogenic series comprising spermatogonia (SG), spermatocytes (SC), spermatozoa in the lumen of the seminiferous tubules (SZ) and the interstitial cells of leydig in a well-vascularised interstitial space (IL) x 400

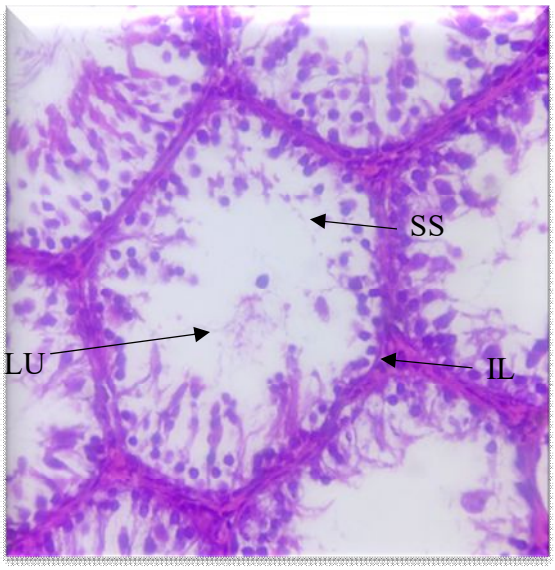

Plate 2: A photomicrograph of testicular section from a rat in Group B (treated with 2.5 mg/kg body weight Cisplatin) showing the disruption of the cells of the spermatogenic series (SS), wide lumen (LU) and reduced interstitial cells of leydig (IL) x 400

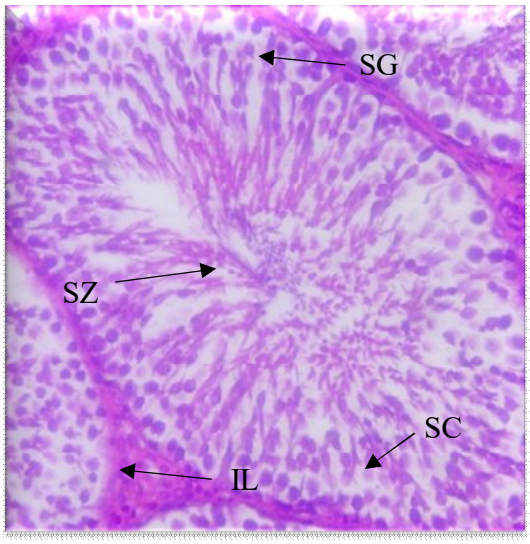

Plate 3: A photomicrograph of testicular section from a rat in Group C (treated with 3.1 IU/kg body weight of HMG) showing the normal spermatogenic series comprising spermatogonia (SG), spermatocytes (SC), spermatozoa in the lumen of the seminiferous tubules (SZ) and the insterstitial cells of leydig in a well-vascularised interstitial space (IL) x 400

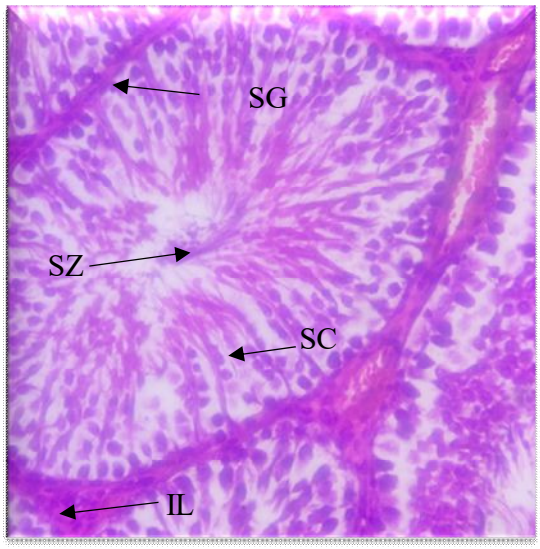

Plate 4: A photomicrograph of testicular section from a rat in Group D (treated with mg/kg body weight of HMG and 2.5mg/kg body weight of cisplatin) showing the normal spermatogenic series comprising spermatogonia (SG), spermatocytes (SC), spermatozoa in the lumen of the seminiferous tubules (SZ) and the interstitial cells of leydig in a well-vascularised interstitial space (IL) H&E x 400

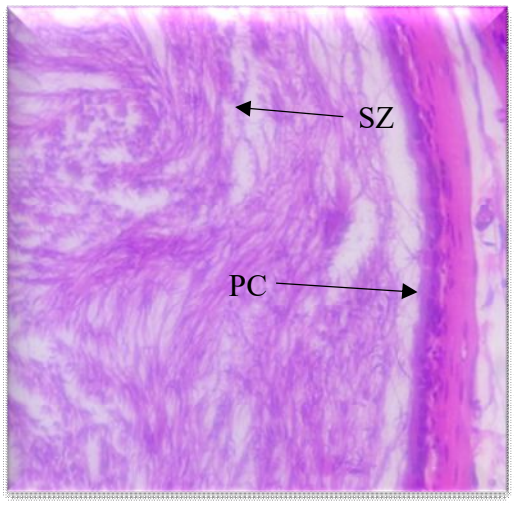

Plate 5: A photomicrograph of epididymis section from a rat in Group A (control) showing matured spermatozoa (SZ) and well-arranged principal cells of the epididymis (PC) H&E x 400

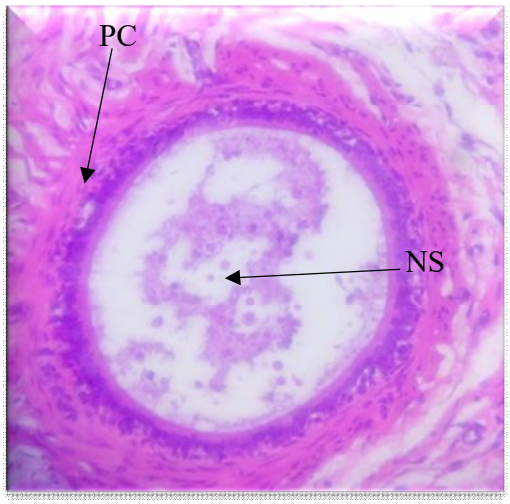

Plate 6: A photomicrograph of epididymis from a rat in Group B (treated with 2.5 mg/kg body weight of Cisplatin) showing cellular debris from necrosed spermatozoa (SZ) and degenerative changes in the principal cells of the epididymis (NS) H&E x 400

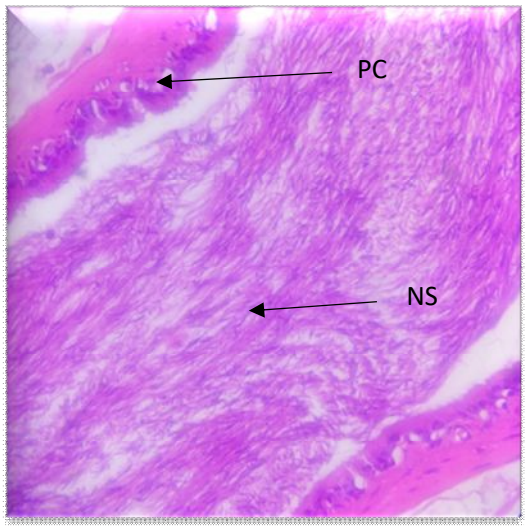

Plate 7: A photomicrograph of epididymis from a rat in Group C (treated with 3.1 IU/kg body weight of HMG) showing dense population of spermatozoa (SZ) with normal principal cells of the epididymis (NS) H&E x 400

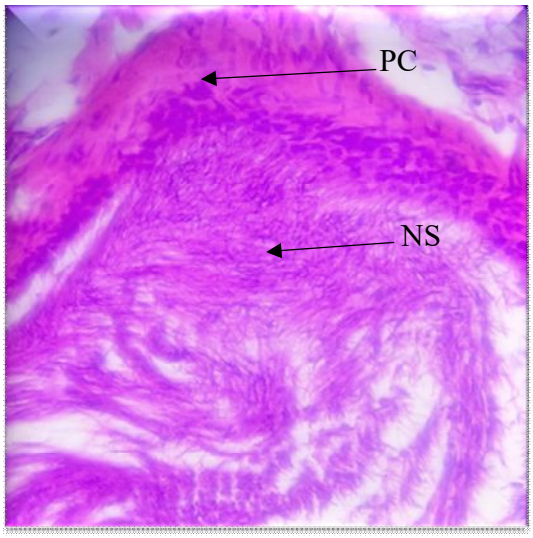

Plate 8: A photomicrograph of epididymis from a rat in Group D (treated with 3.1 IU/kg body weight of HMG and 2.5 mg/kg body weight of Cisplatin) showing dense population of spermatozoa (SZ) with patent principal cells of the epididymis (PC) H&E x 400.

Discussion

Cisplatin is one of the leading anticancer drugs in the chemotherapy treatment of variety of cancer types (Rebillard *et al.,* 2008; Park *et al.,* 2012). However, it intercalates the DNA backbone of rapidly growing cells and interferes with cell division (Sadowitz *et al.,*2002).Testicular dysfunction and infertility are serious complications that may impact the clinical effectiveness of cisplatin. Thus, it seems imperative to search for agents that can protect against testicular toxicity whenever cisplatin chemotherapy is employed. This study highlights the protective effects of HMG against cisplatin-induced testicular damage in rats as verified by the restoration of testicular architecture, enhancement of steriodogenetic hormones and preservation of spermatogenesis. In the current study, the hormonal assays showed that Cisplatin treatment led to significant reduction in Testosterone, Luteinizing hormone and Follicle stimulating hormone in the group treated with Cisplatin and then HMG while HMG treated group showed a restoration of the decreased hormones as shown in Fig. 1, 2 and 3. Reduction in hormone level may be due to severe damage exerted by cisplatin on Leydig and sertoli cells. This finding was in consonance with other studies. (Turk *et al.,* 2008; Beytur *et al.,* 2012), the administration of Cisplatin was for a relatively long period of time and this might have resulted to suppression of hypophysis-testes and reduction of testosterone which do not up regulate luteinizing hormone and follicle stimulating hormone. As reported by Pogach *et. al.,* (1989) that Cisplatin treatment on adult rats affected pituitary-testicular axis, the effects were manifested by decrease in level of sex hormones measured.

Histological examination in testes further indicate significant damage to sertoli cells, leydig cells and germ cells populations induced by Cisplatin (Cherry *et al.,* 2004). Cisplatin treatment induced a significant increase of germ cell apoptosis in mouse testes (Zhang *et al.,* 2001) which might contribute to decrease in spermatogonia, primary spermatocyte and spermatid. Cisplatin induced the histopathological changes in the testis by inducing the vacuoles in the seminiferous epithelium. The presence of vacuoles and sloughing of the seminiferous epithelium observed in rat treated with Cisplatin is an indicator of sertoli cell damage owing to microtubule distruption (Nakai *et al.,* 1994; Narayana *et al.,* 2005). The adverse effect of Cisplatin was corrected with the treatment of HMG as shown in group D rats. In conclusion, the male reproductive toxicity induced by Cisplatin administration would be augmented by decreased serum level of testosterone, luteinizing hormone and follicle stimulating hormone as well as causing sertoli cells damage, these were countered with the treatment of HMG.

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