*Y.V.Kishore Reddy et al. / Drug Invention Today 2010,2(1),42-48*

ISSN: 0975-7619

Drug Invention Today

**Available online through**

**www.ditonline.info**

**Research Article**

# Carboplatin-mediated changes in the activity levels of steroidogenic marker enzymes, serum hormones and testicular architecture in male rats.

**Y.V.Kishore Reddy[1]*, P.Sreenivasula Reddy[2], M.R.Shivalingam[1], B.Suvarchala Devi[1], CNNS Pravallika G[1], D.Naga Anusha[1]**

*[*1]Department of Biotechnology, Victoria College of Pharmacy,Guntur,Andhrapradesh.*

*[2]Department of Biotechnology, S.V.University, Tirupati, Andhrapradesh.India.*

**Received on: 10-07-2009; Revised on: 15-08- 2009; Accepted on:15-12-2009**

## ABSTRACT

Carboplatin (10 mg/ kg body weight) caused significant decrease in the activity levels of steroidogenic marker enzymes such as 3ß-hydroxy steroid dehydrogenase and 17ß-hydroxy steroid dehydrogenase in experimental rats when compared to that of the control rats. In addition, activity levels of serum hormones including LH, FSH and Testosterone were also altered significantly in experimental animals. Besides, causing significant alterations in the activity levels of steroidogenic marker enzymes, and serum hormones, carboplatin treatment also caused changes in testicular architecture in rats.

***Keywords:*** Carboplatin, Steroidogenic marker enzymes, LH, FSH, Testosterone, Testicular architecture, Rats.

## INTRODUCTION

Platinum-derived drugs are playing an increasing important role in the treatment of a variety of neoplasms [1]. Much understanding of the present platinum drugs has come from the studies with cisplatin. [2]. Even though a number of platinum coordination compounds exhibit antiviral and antitumor activities, cisplatin and its direct analog carboplatin are effective anticancer drugs currently approved for the treatment of several human carcinomas [3], [4].

The use of cisplatin, however, is limited by significant dose related toxicity, notably, nephrotoxicity, emesis, ototoxicity and peripheral neuropathy [5]. To improve the therapeutic index of platinum compounds, new analogs have been developed [6]. The second-generation platinum compound i.e., carboplatin entered the clinical trails in 1981 and showed a very similar activity profile to that of cisplatin, with a good response in ovarian, lung, head and neck and testicular cancers. It is currently the second most widely used platinum anticancer drug in the world [7].

The mechanism of action of carboplatin is very similar to that of cisplatin, forming preferential cross links with guanine in DNA, thus eventually causing cell death [8]. Carboplatin readily crosses the cell membrane and inside the cell, the ring structure is hydroxylated by water to form the active moiety. In the active form, it forms irreversible covalent bonds with DNA and inhibits DNA replication, RNA transcription and protein synthesis. Intra-stand cross links at the N-7 position of guanine are predominant binding sites of carboplatin. Carboplatin causes cell cycle arrest in the G2-phase and then induces programmed cell death or apoptosis [9].

Despite its effectiveness in the suppression of cancer cells, the administration of carboplatin is associated with a variety of side effects which include myelo-suppression, alopecia, rash, and other mild effects [10]. Moreover, carboplatin has also embryotoxic and teratogenic effects [11], [12]. However, only few studies have been carried out to investigate its anti-fertility effects. Hence, an attempt has been made in the present study to investigate the effect of carboplatin activity levels of steroidogenic marker enzymes, serum hormones and testicular architecture in male rats. The aim of this type of work has been to find analogues with reduced toxicity and /or wider spectrum of activity. One such analogous compound is carboplatin.

In the present study platinum based anti-cancer drug i.e., carboplatin was administered to male rats in order to investigate the possible interference of carboplatin in affecting male reproduction in rats. Because these observations may be helpful to understand the causes behind the reduction of male reproductive health of cancer patients who are under treatment with carboplatin. However many reports available on cisplatin caused reproductive toxicity, but very limited studies demonstrated interference of carboplatin in affecting male reproduction.

***Corresponding author.**

*Dr.Y.V.Kishore Reddy,*
*Department of Biotechnology,*
*Victoria College of Pharmacy,*
*Guntur,Andhra Pradesh.India*
*Tel.: + 91-0863-2596544,: 09703391836*
*Fax: +91-0863-2596530*
*E-mail:kishorey2@rediffmail.com*

## 2. MATERIALS & METHODS

### 2.1. **Animals**

Healthy adult male wistar rats of same age group (70±5 Days) were selected for the present study. Animals were housed in an air conditioned animal house facility at 26±1° C, with a relative humidity of 75%, under a controlled 12 h light/dark cycle. The rats were reared on a standard pellet diet (HLL Animal Feed, Bangalore, India) and tap water adlibitum.

### 2.2. **Test chemicals**

Carboplatin was purchased from Sigma chemicals, St.Louis Co., MO, USA. This compound was dissolved in 0.9% normal saline to obtain the final concentration of the 10 mg/kg body wt. of the animal.

### 2.3. **Experimental Design**

The rats were divided into two groups consisting of eight animals in each group. The rats in the first group were served as control and received 0.9% of normal saline only. The rats in the second group were received carboplatin (10 mg/kg body wt). Injections were given intra-peritoneally to rats on 1st, 3rd and 5th day of experimentation. On 45th day of experiment animals were sacrificed by cervical dislocation.

### 2.4. **Assay of testicular steroidogenic marker enzymes**

The testicular tissue was homogenized in ice-cold Tris-HCl buffer (pH 6.8). The microsomal fraction was separated and used as enzyme source. The activity levels of 3ß-hydroxy steroid dehydrogenase (3ß-HSD; EC.1.1.1.51) and 17ß-hydroxy steroid dehydrogenase (17ß-HSD; EC.1.1.1.64) were assayed by the method of Berg Meyer [13]. The enzyme assays were made under the conditions following zero order kinetics after preliminary standardization regarding linearity with respect to time of incubation and enzyme concentration.

The reaction mixture in a volume of 2.0 ml contained: 100 µ moles of sodium pyro phosphate buffer (pH 9.0), 0.5 µ moles of co-factor (NAD for 3ß-HSD and NADPH for 17ß-HSD), 0.08 µ moles of substrate (dehydro epi andro sterone for 3ß-HSD and androstenedione for 17ß-HSD) and 20 mg equivalent of microsomal protein as enzyme source.

The reactions were carried out in a quartz cuvette of 1.0 cm path at 23±1° C. The absorbance at 340 nm was measured at 20s intervals for 5 min using UV-vis spectrophotometer (Hitachi U-2001). Protein content in the enzyme source was estimated by the method of Lowri et al [14] using bovine serum albumin as standard. The enzyme activies were expressed in micromoles of NAD converted to NADH mg/ protein/min for 3ß-HSD or micromoles of NADPH converted to NADP mg/ protein/min for 17ß-HSD.

### 2.5. **Quantitative determination of serum hormone level**

The levels of Serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were determined by established methods after preliminary standardization.

### 2.6. **Serum testosterone**

Serum testosterone was estimated using a sensitive radioimmunoassay (RIA). The antiserum used in the assay was raised in rabbits using a derivative of testosterone (testosterone-3-carboxy methyloxime conjugated to BSA).

### 2.7. **Radioimmunoassay of Testosterone:**

Radioimmunoassay (RIA) of testosterone was done according to the method of Rao *et al*. [15] with minor modifications. One hundred microlitres of standard testosterone (10-1000 pg) diluted in 0.01 M GPBS (pH 7.4) or suitable aliquot (100 – 200 µl) of sample were incubated with 100 µl of tritiated testosterone (10,000-20,000 cpm/ tube) and 100 µl of diluted antiserum (1:5,000) at 4°C for overnight. After incubation, 350 µl chilled dextran coated charcoal was added to each tube in ice bath and incubated at 4°C for 10 minutes. The tubes were centrifuged at 6000 x g for 10 minutes at 4°C. The supernatant was carefully decanted in to vials containing 3.0 ml of toluene-based scintillation fluid and counted in a Liquid Scintillation Counter. After deduction the non-specific binding from all the values, the results were plotted as % of specific binding versus log concentrations of testosterone.

### 2.8. **Serum FSH and LH**

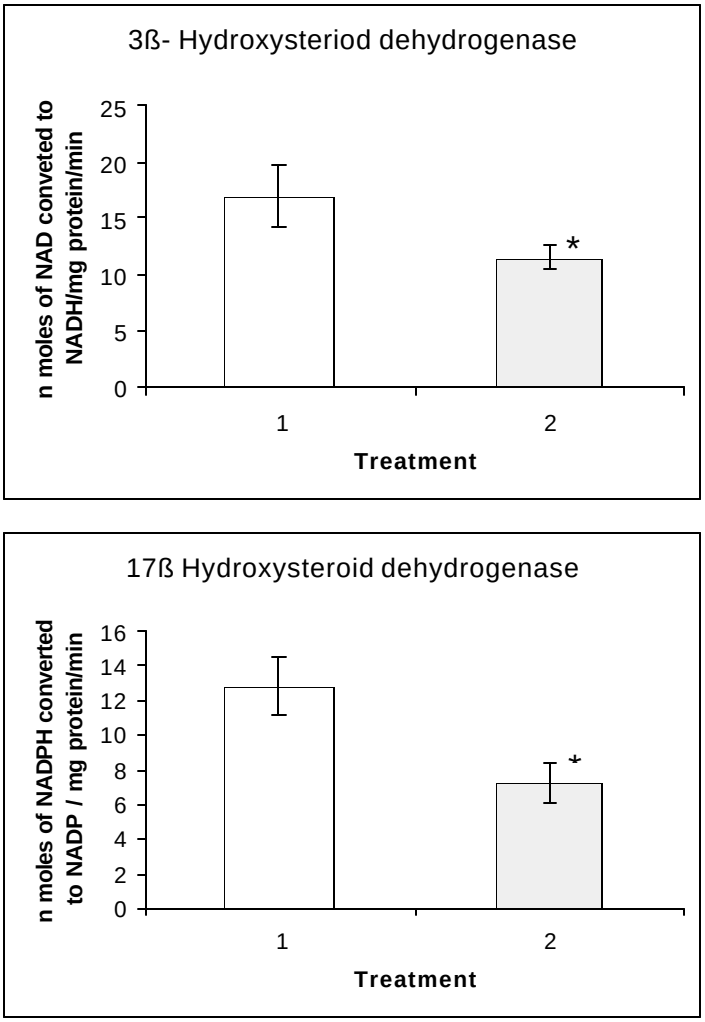
#### **Radio-iodination of FSH and LH**

Highly purified iodination grade follicle stimulating hormone (NIDDK-rFSH) and luteinizing hormone (NIDDK-rLH-RP-3) were radio-iodinated with carrier-free ($^{125}$I) according to the method of Hunter and Greenwood [16] as modified by Greenwood *et al*. (1963) using Chloramine T as an oxidizing agent. The iodination of rat FSH and LH with [$^{125}$I] was performed in eppendorf tubes.

#### **Radioimmunoassay of serum FSH**

Serum FSH was assayed by the method of Lin *et al*. [17] after suitable modifications. The assay was carried out in duplicate with total, non-specific binding, maximum binding, standard, quality control and unknown serum sample tubes. In brief, 200 µl of standard FSH (0.25 to 64 ng/ml) or serum samples were incubated with 100 µl of iodinated FSH (15,000 - 20,000 cpm/100 µl) and 200 µl of rat FSH antiserum (1:1,25,000) at 4°C for 48 h. At the end of the incubation 200 µl of goat-antirabbit gamma globulin was added to all the tubes (except total count tubes) and mixed. Once again the tubes were incubated for 24 h at 4°C and centrifuged at 1500 x g for 30 minutes. The

**Figure.1.Effect of carboplatin (10 mg/kg body weight) on the activity levels of selected steroidogenic marker enzymes in the testis of rats.**




Control; ▨ Carboplatin

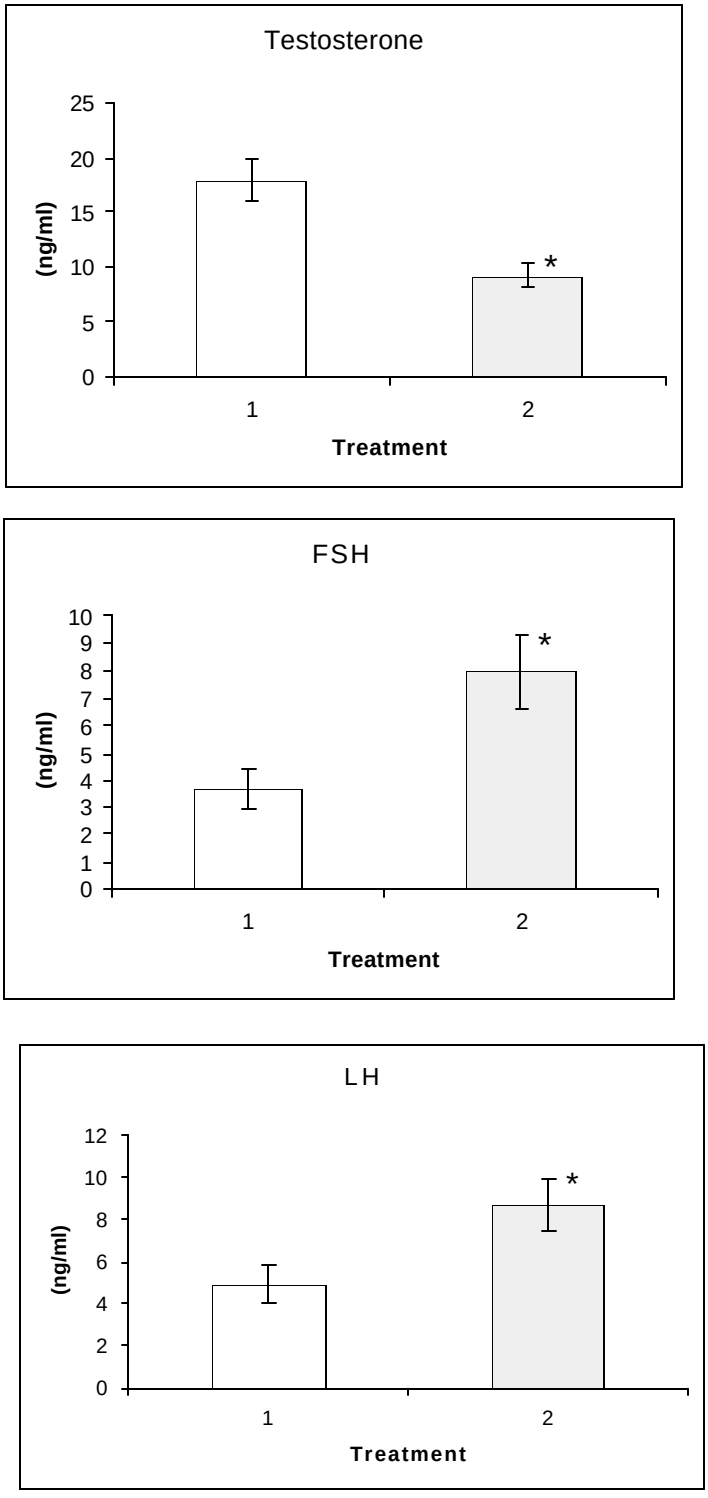
*Values are mean ± S.D of eight individuals. Values are significantly different from control at *p<0.05.*

supernatant was carefully decanted and the precipitate was counted for 1 minute in a microprocessor-based gamma counter (Kontron Gammamatic). The concentrations of FSH in the serum samples were calculated from the logit-log representation of the calibration curve expressed as ng/ml.

**Radio immunoassay of serum LH**

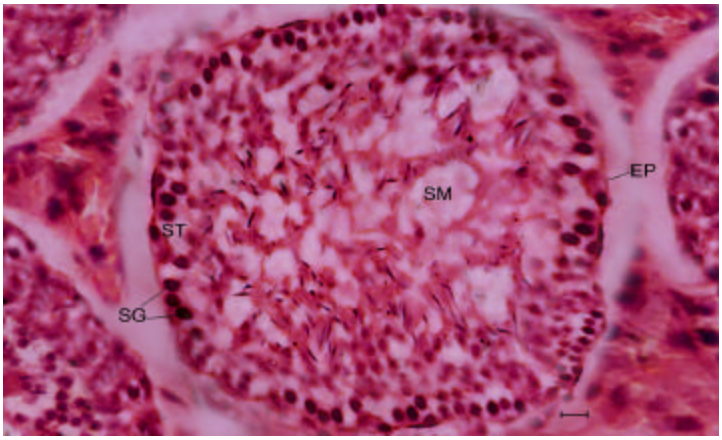
Serum LH was assayed by the method of Lin *et al*. [17] after suitable modifications. The assay was carried out in duplicate with total, non-specific binding, maximum binding, standard, quality control and unknown serum sample tubes. In brief, 200 µl of standard LH (0.25 to 64 ng/ml) or serum samples were incubated with 100 µl of iodinated LH (15,000-20,000 cpm/100 µl) and 200 µl of rat LH antiserum (1:1,80,000) at 4°C for 48 h. At the end of the incubation 200 µl of

**Legend for Figure.2:Effect of carboplatin (10 mg/kg body weight) on serum testosterone, follicle stimulating hormone and lutenizing hormone levels in male rats.**

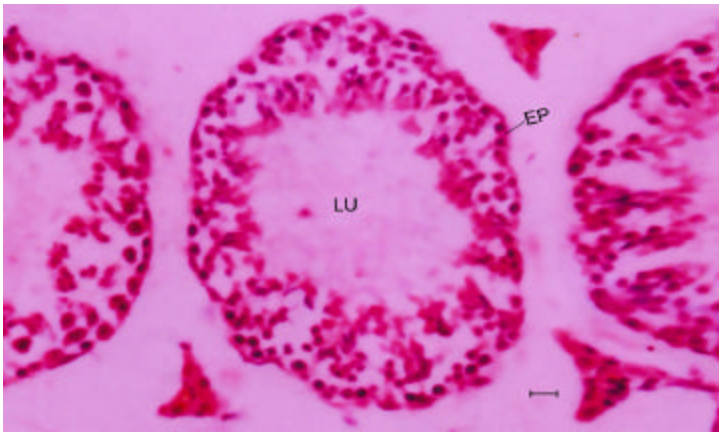



Control; ▨ Carboplatin

*Values are mean ± SD of eight individuals. Values are significantly different from control at * p<0.0001.*




**Legend for Figure.3.Transverse section of the testis of the control rat showing the presence of normal tubular structure with spermatogenic cells at different stages of development. Seminiferous tubules are packed closely. The tubular spaces are packed with interstitial tissue, containing clusters of leydig cells. Scale line = 50 µm.**




**Legend for Figure –4.Transverse section of the testis of the rat exposed to carboplatin (10 mg/kg body weight) showing symptoms of arrest of spermatogenesis with ruptured epithelium and very few spematogonia, spermatocytes and spermatids. Scale line = 50 µm.**

goat-antirabbit gamma globulin was added to all the tubes (except total count tubes) and mixed. The tubes were again incubated for 24 h at 4°C and centrifuged at 15000 X g for 30 minutes. The supernatant was carefully decanted and the precipitate was counted for 1 minute in a microprocessor-based gamma counter (Kontron Gammamatic). The concentrations of LH in the serum samples were calculated from the logit-log representation of the calibration curve and expressed as ng/ml.

2.9. **Histological study**

The rats were autopsied and the testes were dissected out. The testicular tissue was fixed in aqueous Bouin's fluid for 24 hours, dehydrated in alcoholic series, cleared in xylol and embedded in paraffin wax. Serial sections of 5 µm thickness were made and stained with Harris haematoxylin-eosin and examined using microscope.

2.10. **Statistical analysis**

The data were presented as mean ± SD. Statistical analysis was performed using analysis of variance (ANOVA) followed by Dunnett's test using SPSS 10.0.

## 3. RESULTS

No mortalities were observed in control or experimental groups. No behavioral abnormalities were observed in experimental animals. Treatment of platinum-based anticancer drugs induced severe reproductive abnormalities in adult male rats. The results represented in (Fig.1) indicate that a significant decrease in activity levels of 3ß-HSD and 17ß-HSD were observed in carboplatin treated rats when compared with control rats.

The levels of serum FSH and LH were increased significantly in rats exposed to carboplatin when compared with respect to the corresponding groups of control rats. Whereas the levels of serum testosterone were significantly decreased in adult rats exposed to carboplatin when compared to the corresponding groups of control rats (Fig .2).

Histological observations of the testes of the control rat consist of seminiferous tubules and inter-tubular elements. The seminiferous tubules show normal spermatogenesis with all cell types and well developed intestinal cells. Each seminiferous tubule consists of the tubular wall the outermost basement membrane. Resting on the basement membrane are the spermatogonia and the sertoli cells. Towards the lumen, the primary spermatocytes, secondary spermatocytes and spermatids adhere to the sertoli cells. Sperms are seen with heads embedded in the sertoli cells and tails lying in the lumen (Fig. 3).

Transverse section of the testis of the rat exposed to carboplatin (10 mg/ kg body weight) shows symptoms of the partial arrest of spermatogenesis. The seminiferous tubules are disorganized. The germinal epithelium, spermatogonia, spermatocytes and spermatids are severely damaged and many are degenerated. Reduction in leydig cells were clearly seen in this group. The degeneration was observed in seminiferous tubules showing necropsied spermatogenic cells and the lumen was empty of active sperms (Fig. 4).

## 4. DISCUSSION

The present study demonstrates the adverse effect of carboplatin on steroidogenesis, pituitary-testicular-axis and testicular architecture in adult male rats. Steroidogensis is the biosynthetic path way that produces steroid hormones. These steroid hormones participate in the control and regulation of the reproductive system. The reproductive steroid hormones are produced in the testes and to a much lesser extent, the adrenal glands. Inside the leydig cell, the steroidogenic pathway begins in the cytoplasm and the final end product hormone i.e., testosterone is produced in the testis [18].

The decreased steroidogenic enzyme activity levels may lead to decreased steroidogenesis in experimental rats which in turn may suppress the reproductive activities in the male rats. It seems carboplatin acts on leydig cells and inhibits the testosterone production which was evident by decrease in the activity levels of 3β-HSD and 17β-HSD enzymes in the testes of experimental rats. Since, the enzyme 3β-HSD is localized exclusively within the leydig cells in the testes [19].

Although there are no reports on the reproductive toxicity of carboplatin, but many reports are available on such effects of cisplatin. Previous studies also have been demonstrated the decreased activity levels of 3β-HSD and 17β-HSD in the leydig cells cultured with platinum-based anticancer drugs [20] and in the testis of rats treated with diphenyl ethylene diamine platinum complex [21]. Other anticancer drugs such as cyclophosphamide [22], [23] and adriamycin [24] have also been reported to cause decrease in activity levels of 3β-HSD and 17β-HSD enzymes in the testis of rats.

Exposure to toxic substances that are not endogenous to the body can lead to chemical reactions that alter the outcome of several biochemical pathways. Alterations of the hormonal steroidogenic pathway at even one step will change the pattern of production and eventually secretion of steroid hormones. Any chemical interference to steroidogenesis viz., altering enzymatic activity, altering precursor availability, interfering with control mechanisms etc., can cause adverse effects to the reproductive system. Toxic responses to the reproductive system can result in such adverse effects as abnormal sexual and physical development, diminished fertility or sterility [25], [26].

Hormones play a vital role in initiating and maintenance of male reproductive function or testicular function which includes the production of androgens and spermatozoa. The endocrine input is provided by the hypothalamic-pituitary axis. Interactions within the hypothalamic-pituitary-gonadal axis involve a complex array of endocrine, paracrine, and autocrine mechanisms [27], [28].

In the present study, the elevated levels of serum FSH and LH with lowered testosterone levels indicating adverse effect of carboplatin on hypothalamic-pituitary–testicular axis in experimental rats. Decrease in serum testosterone levels in experimental rats indicates impairment of testosterone production by leydig cells. The elevated levels of LH indicate malfunctioning of leydig cells and an impairment of endocrine function. The increased level of FSH was observed in the present study indicating dysfunction of the germinal epithelium [29], [30].

Our results are in agreement with previous studies, which also reported decreased levels of serum testosterone after exposure to cisplatin based chemoetherapy in adult rats [31] and mice [32]. The present observations of elevated serum LH and serum FSH levels are also in agreement with those of previous studies. Increase in both LH and FSH concentrations were observed following procarbazine in rats and humans [33].

Any imbalance in the hormonal status of the body and seminiferous tubules, it leads to disturbances in the process of spermatogenesis. Chemotherapy causes persistent dysfunction of leydig cells. When there is a complete leydig cell failure, the LH concentrations rise and testosterone levels fall down [34]. This likely represents a compensatory mechanism resulting from reduced negative feedback by testosterone at the hypothalamic-pituitary level, thereby reflecting a degree of impairment of testosterone production by the leydig cells [35]. Thus testosterone negatively regulates LH secretion, and in the event of testosterone decline, LH levels increases two-to three-fold. This suggests that carboplatin might have directly affected the hypothalmo-pituitary-testicular axis.

Histological studies on the testis provide the evidence to observe the difference in architecture between control and experimental animals [36]. In the present study the investigation has been aimed to know the effect of exposure to carboplatin on the histological alterations in the testis of adult rats. In the present study histological changes were observed in testes of experimental rats indicating inhibitory effect of carboplatin on spermatogenic compartment with arrest of spermatogenesis.

Within the testes the main target cells for toxicant that disrupt spermatogenesis are the somatic cells, (leydig cells and sertoli cells) and the germ cells themselves. If the toxic exposure is brief or not too severe, the seminiferous epithelium can often recover and rapidly reestablish normal spermatogenesis. However if the injury is severe, the seminiferous epithelium may irreversibly damaged resulting in spermatogenic failure [37].

Spermatogenesis depends on the action of testosterone which is produced by leydig cells in the testis. The sertoli cells play a central role in development of functional testis, and hence in the expression of a male phenotype [38]. Spermatogenic failure has been a recognized consequence of treatment with chemotherapeutic agents [39]. Many drugs particularly alkylating agents have been shown to affect spermatogenesis [40].

Histological observations of the testes of the control rat consist of seminiferous tubules and inter-tubular elements. The seminiferous tubules show normal spermatogenesis with all cell types and well developed interstitial cells. Each seminiferous tubule consists of the tubular wall with the outermost basement membrane. Resting on the basement membrane are the spermatogonia and the sertoli cells. Towards the lumen, the primary spermatocytes, secondary spermatocytes and spermatids adhere to the sertoli cells. Sperms are seen with heads embedded in the sertoli cells and tails lying in the lumen (Fig.3).

Transverse section of the testis of the rat exposed to carboplatin (10 mg/ kg body weight) shows symptoms of the partial arrest of spermatogenesis. The seminiferous tubules are disorganized. The germinal epithelium, spermatogonia, spermatocytes and sperma-

tids are severely damaged and many are degenerated. Reduction in leydig cells were clearly seen in these two groups. The degeneration was observed in seminiferous tubules showing necropsied spermatogenic cells and the lumen was empty of active sperms (Fig. 4).

The present observations of symptoms of the partial arrest of spermatogenesis in the testis of rat exposure to carboplatin are also in agreement with those of previous studies. Chemotherapy with cisplatin can have profound and long-lasting effect on spermatogenesis. Studies in mice have demonstrated an acute damage to spermatogenesis following cisplatin treatment. Detailed investigations in animal models indicate that cisplatin has broad toxic activity, targeting leydig cells, sertoli cells and germ cells [36], [37]. These degenerative changes of seminiferous tubules could be due to the decreased intra-testicular synthesis of testosterone. However testosterone concentrations in the testis of the treated animals would be otherwise sufficient to support complete spermatogenesis [41].

The result indicate that exposure of rats to carboplatin affects the spermatogenesis which may be due to an imbalance in the androgens which are essential for normal spermatogenesis. These changes could be due to the decreased intratesticular concentrations of testosterone. The decreased activities of 3$\beta$-HSD and 17$\beta$-HSD might be responsible for decreased testosterone levels in the testis of rats exposed to carboplatin.

## ACKNOWLEDGEMENTS

We are grateful to Head, Department of Biotechnology, S.V. University for providing necessary laboratory facilities. The research was conducted in accordance with the regulations in the country and approved by the Ethical Committee of Department of Biotechnology, S.V.University, Tirupati.

## REFERENCES

1. Olivi, A., Gilbert, M., Duncan, K.L., Corden, B., Lenartz, D. and Brem, H. (1993**).** Direct delivery of platinum-based antineoplastics to the central nervous system: a toxicity and ultrastruactural study. Cancer Chemother. Pharmacol., 31 (6): 449-454.
2. Boulikas T Vougiouka M. (2003). Cisplatin and platinum drugs at the molecular level. Oncol. Rep; 10: 1663-1682.
3. Taylor RC, Ward SG. (1991). The antiviral activity of some selected inorganic and organometallic complexes: possible new chemotherapeutic strategies, in Lectures in Bioinorganic Chemistry (Nicolini M and Sindellari L, eds) Raven Press,NewYork, pp. 63- 90.
4. Rixe O, Ortuzar W, Alvarez M, Parker R, Reed E, Paull K, and Fojo T (1996). Oxaliplatin, tetraplatin, cisplatin, and carboplatin: Spectrum of activity in drug resistant cell lines and in the cell lines of the National Cancer Institute's Anticancer Drug Screen panel. Biochem. Pharmacol; 52: 1855-1865.
5. Van Hoff, D.D., Schilsky, R. and Reichert, C.M. (1979).Toxic effects of cis dichloro diammine platinum (II) in man. Cancer Treat. Rep., 63: 1527-1531.
6. Evans, B.D., Raju, K.S., Calvery, A.H. and Harland, S.J. (1983). Phase II study of JM8, a new platinum analog in advanced ovarian carcinoma. Cancer Treat. Rep., 67: 997-1000.
7. Martindale Y. (2002). The Extra Pharmacopoeia. 32nd Edition James E, Reynolds F (Eds.) London: Pharmaceutical Press. Gaynon, P.S., Ettinger, L.J.,Moel, D., Siegel, S.E., Baum, E.S., Krivit, W. and Hammond, G.D. (1987). Pediatric phase trial of carboplatin: a Children Cancer Study Group report. Cancer Treat. Rep., 71 (11): 1039-1042.
8. Farrell N (1989). Transition metal complexes as drugs and chemotherapeutic Agents; Kluwer Academic Publishers; pp. 1-276.
9. Micetich KC, Barnes D, Erickson LC (1985). A comparative study of the cytotoxicity and DNA-damaging effects of cis (diammino) (1,1cyclobutanedicarboxylato) platinum (II) and cis-diammine dichloroplatinum (II) on L1210 cells. Cancer Res; 45: 4043-4047.
10. Muggia, F.M. (1989). Overview of carboplatin: Replacing, complementing and extending the therapeutic horisons of cisplatin. Seminars in Oncology, 16 (2): suppl. 5: 7-13.
11. Kai Suichi, Kohmura H, Ishikawa K, Takeuchi Y, Ohta S, Kuroyanagi K, Kadota T, Kawano S, Chikazawa H, Kondo H, Sakai A. (1988). Reproduction studies of carboplatin (II) intravenous administration to rats during the prenatal and lactational periods. J. toxicol. Sci; 13: 63-81.
12. Chung, M.K., Kim, J.C. and Roh, J.K. (1998). Embryotoxic effects of SKI2053R, a new potential anticancer agent, in rats. Reprod. Toxicol., 12 (3):375-381.
13. Bergmeyer HU: $\beta$-hydroxy steroid dehydrogenase. In: Methods of Enzymatic analysis. Edited by H.U. Bergmeyer, Academic Press, New York, 1974, 1, 447-489.
14. Lowry OH, Rosebrough MJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem, 1951, 193, 265-275.
15. Rao AJ, Charaborti R, Kotagi SG, Ravindranath N. (1990). Effect of constant infusion of gonadotropins releasing hormone (GnRH) agonist buserelin and anatagonist CDB 2085 A using osmotic minipumps on testicular function in adult male bonnet monkey (Macac radiata). Andrologia; 22: 567-573.
16. Hunter WM, Greenwood FC.(1962). Preparation of iodine-$^{131}$labeled human growth hormone of high specific activity. Nature; 194: 495-496.
17. Lin KC, Kawamura N, Okamura H, Mori T. (1988). Inhibition of ovulation, steroidogenesis and collagenolytic activity in rabbits by sulphide induced hyperprolactinemia. J. Reprod. Fertil; 83: 611-618.
18. Chen H, Luo L, Zirkin BR. (1996). Leydig cell structure and function during aging. In: Payne AH, Hardy MP, Russell LD (eds.), the Leydig Cell. Illinois: Cache River Press; pp. 222-230.
19. Dupont V, Labrie F, Lvuthe V, Pelletier G: Ontogeny of 3$\beta$-hydroxy steroid dehydrogenase / $\Delta^5$- $\Delta^4$ isomerase (3$\beta$-HSD) in rat testis as studies by immunocytochemistry. Anatomy and Embryology, 1993, 187, 583-589.
20. Malarvizhi D Mathur PP: Effects of cisplatin on rat leydig and sertolicells in vitro. Bio Med Letters, 1996, 53, 121-128.
21. Sergejew TF, Hartmann RW. (1996). Effect of a diphenyl ethylenediamine platinum complex on steroidogenesis in rats. J. Steroid Biochem. Molec. Biol; 58: 243-248.
22. Das UB, Mallick M, Debnath JM, Ghosh D: Protective effect of ascorbic acid on cyclophosphamide – induced testicular gametogenic and androgenic disorders in male rats. Asian J Androl, 2002, 4, 201-207.
23. Ghosh D, Das UB, Misro M: Protective role of $\alpha$-tocopherol-succinate (provitaminE) in cyclophosphamide induced testicular gametogenic and steroidogenic disorders. A correlative approach to oxidative stress. Free radical research, 2002, 36, 1209-1218.
24. Chidambaram N, Selvakumar E, Varalakshmi P. (2005). Protective effect of lipoic acid on adriamycin-induced testicular toxicity. Clin. Chim. Acta; 360: 160-166.
25. Kelce WR, Wilson EM. (1997). Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications. J. Mol. Med; 75: 198-207.
26. Gray LE, Wolf C, Lambright C, Mann P, Price M, Cooper RL, Ostby

J. (1999). Administration of potentially antiandrogenic pesticides (procymidone, linuron,iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicol. Ind. Health; 15: 94-118.

27. Halvorson LM, Kaiser UB, Chin WW. (1996). Stimulation of LH beta gene promoter activity by the orphan nuclear receptor, SF-l. J. Biol. Chem; 271: 6645-6650.
28. Meeker JD, Godfrey-Bailey L, Hauser R. (2007). Relationships between serum hormone levels and semen quality among men from an infertility. clin. J. Androl; 28: 397-406.
29. Hansen SW, Berthelsen JG, Von der maase H. (1990). Long-term fertility and leydig cell function in patients treated for germ cell cancer with cisplatin, vinblastine, and bleomycin versus surveillance. J. Clin. Oncol; 8: 1695-1698.
30. Howell SJ, Radford JA, Ryder WD, Shalet SM. (1999) Testicular function after cytotoxic chemotherapy: evidence of Leydig cell insufficiency. J. Clin. Oncol; 17: 1493-1498.
31. Bieber AM, Marcon L, Hales BF, Robaire B: Effects of chemotheropeutic agents for testicular cancer on the male rat reproductive system, spermatozoa, and fertility. J Androl, 2006, 27, 189-200. Meistrich ML, Wilson G, Huhtaniemi I: Hormonal treatment after cytotoxic therapy stimulates recovery of spermatogenesis. Cancer Res, 1999, 59, 3557-3560.
32. Seethalakshmi L, Flores C, Kinkead T, Carboni AA, Malhotra RK, Menon M (1992). Effects of subchronic treatment with cis-platinum on testicular function, fertility, pregnancy outcome, and progeny. J. Androl; 13: 65-74.
33. Gerl A, Muhlbayer D, Hansmann G, Mraz W, Hiddemann W. (2001). The impact of chemotherapy on Leydig cell function in long term survivors of germ cell tumors. Cancer; 91: 297-1303.
34. Pectasides D, Pectasides M, Farmakis D, Nikotaou M, Koumpou M, Kostopouliou et al: Testicular function in patients with testicular cancer treated with bleomycin-etoposide-caraboplatin (BEC 90) combination chemotherapy. Eur Urol, 2004, 45,187-193.
35. Chatterjee R, Mills W, Katz M. (1994). Germ cell failure and leydig cell insufficiency in post-pubertal males after autologus bone marrow transplantation with BEAM for lymphoma. Bone Marr. Transp; 13: 519-522.
36. Petersen PM, Hansen SW, Giwercman A, et al (1994). Dose-dependent impairment of testicular function in patients treated with cisplatin- based chemotherapy for germ cell cancer. Ann. Oncol; 5: 355-358.
37. Boekelheide K, Schoenfeld HA, Hall SJ, Weng CC, Shetty G, Leith J. (2005). Gonadotropin-releasing hormone antagonist (Cetrorelix) therapy fails to protect nonhuman primates (Macaca arctoides) from radiation-induced spermatogenic failure. J. Androl; 26: 222- 234.
38. Mackay S. (2000). Gonadal development in mammals at the cellular and molecular levels. Int. Rev. Cytol; 200: 47-99
39. Atessahin A, Karahan I, Turk G, Gur S, Yilmaz S, Ceribasi AO: Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Rep Tox, 2006, 21, 42-47.
40. Howell SJ, Shalet SM. (2005). Spermatogenesis after Cancer Treatment: Damage and Recovery. J. Nat Cancer Inst Monographs; 34: 12-17.
41. Meistrich ML, Finch M, da Cunha MF, Hacker U, Au WW. (1982). Damaging effects of fourteen chemotherapeutic drugs on mouse tes tis cells. Cancer Res; 42:122-131.

**Source of support: Nil, Conflict of interest: None Declared**