ORIGINAL ARTICLE

Protective effect of speman on cisplatin-induced testicular and epididymal toxicity in mice

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Testicular cancer is the most common cancer affecting men of reproductive age. Advances in treatment of the disease, which includes the administration of cisplatin, have brought the 5-year survival rate to over 90%. This high cure rate, coupled with young age of patients, makes elucidation of the impact of the treatment on reproduction become increasingly important. The objective of the present study was to investigate the protective effect of speman, a non-hormonal herbal formulation, on cisplatin-induced suppressed male reproductive health in mice. Male mice were treated with cisplatin or speman alone or in combination and assessed for spermatogenesis and steroidogenesis. Significant decrease in the weights of testes and epididymis was observed in cisplatin treated animals. Injection of cisplatin significantly decreased epididymal sperm count, viable sperms, motile sperms and hypo-osmotic swelling (HOS)-tail coiled sperms with a significant reduction in the testicular steroidogenic enzyme activities and serum testosterone levels, whereas co-administration of speman with cisplatin showed a significant improvement in the selected reproductive parameters over cisplatin alone treated mice indicating the beneficial effect of speman to combat cisplatin-induced suppressed reproduction in male mice.

Key words: Cisplatin, male mice, speman, sperm, steroidogenesis

INTRODUCTION

Testicular cancer is the most common cancer disease among 20-35 year olds, with the incidence rates still on the rise.[1] Advances in treatment of the disease, which includes the administration of chemotherapeutic agents, have brought the 5-year survival rate to over 90%. This high cure rate, coupled with young age of patients, makes the post-treatment quality of life of testicular cancer patients a concern. Consideration of the impact of the treatment on reproduction has become increasingly important. Platinum-based chemotherapy is extensively used to treat a range of solid tumours all over the world.[2,3] Cisplatin [*cis*-diamminedichloroplatinum(II)] is a platinum-derived anti-neoplastic, DNA alkylating agent used for cancer treatment. Despite its promising results, cisplatin treatment is coupled with several toxic side effects including short-term or long-lasting suppression of male reproductive health. Earlier, several studies suggested that cisplatin affects Sertoli cells and Leydig cells of testis, thereby causes anti-spermatogenic[4,5] and anti-steroidogenic[6,7] effects, respectively. Further, the toxic effects of cisplatin even extend to accessory sex organs like epididymis and vas deferens, thereby affecting sperm maturation events.[8] Though the molecular mechanism of anti-gonadal effects induced by cisplatin are not clearly understood, it has been claimed that cisplatin-induced gonadal toxicity is either direct, forming DNA adducts due to its strong electrophilic nature, via nucleophilic substitution reactions[9] and/or indirect, by at least in part mediating dysfunction of biosynthesis of testosterone.[6]

Herbal medicine is gaining importance day-by-day in the management of several disorders including male reproductive disorders. Speman is a non-hormonal herbal formulation and is well acknowledged phyto-based medicine to improve male fertility.[10] The protective role of speman in cases of premature ejaculation, spermatorrhoea, oligospermia and enlarged prostate is well recognised.[11] Moreover, speman has also been administered to alleviate suppressed male reproduction during physical[12] and/or chemical[13-15] stress conditions.

Considering the facts that a) cisplatin induces spermatotoxic effects and reduces testosterone biosynthesis and b) speman plays a key role in spermatogenesis and steroidogenesis, the present study was aimed to ascertain the influence of speman on the reproductive toxicity caused by cisplatin in mice.

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MATERIALS AND METHODS

Procurement and Maintenance of Experimental Animals

Male Swiss albino mice (28±3 g) were procured from an authorised vendor (M/s. Raghavendra Enterprises, Bangalore, India). They were housed (four per cage) in polypropylene cages (18″×10″×8″) lined with sterile paddy husk, under standard laboratory conditions (temperature 30±2°C; light and dark 12:12 hours) at the Animal Facility, Department of Biotechnology, S. V. University, Tirupati. The mice were fed on standard pellet chow (purchased from HLL Animal Feed, Bangalore, India) and water *ad libitum*. The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals.[16] Also, this study was carried out according to the guidelines for the care and use of laboratory animals[17] and approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupati, India (vide No. IAEC/No- 438/01/a/CPCSEA).

Test Chemicals

Cisplatin [*cis*-diamminedichloroplatinum(II) or CDDP] (Cadila Pharmaceuticals Ltd., Ahmedabad, India) was purchased from a local drug store and used as the test chemical. Speman, a non-hormonal polyherbal drug manufactured by Himalaya Co. (India), was also purchased from a local drug store.

Experimental Design

Healthy male mice were randomly allocated into four groups with each group consisting of eight animals. The mice in first group served as controls and were intraperitoneally injected with physiological saline in a 50 µl volume (0.9% of sodium chloride in distilled water). Mice in groups 2, 3 and 4 served as experimental animals. Animals in group 2 were injected intraperitoneally with cisplatin at a dose of 3 mg/kg body weight in a 50 µl volume for 3 alternate days. Mice in group 3 received 0.5 ml of speman (80 mg/kg body weight) daily for 35 days orally by means of an oral feeding tube, whereas mice in group 4 received the same experimental regimen as that of mice in group 2, but in addition they also received speman (80 mg/kg body weight) through gavage for a period of 35 days. The rationale for choosing 35 days experimental period is based on earlier studies[18] and the dose selection of cisplatin and speman in the present study is based on the studies of Malarvizhi and Mathur[7] and Subbarao *et al*.,[19] respectively.

Necropsy

After completion of the experimental period (35 days), the mice were fasted overnight, weighed and killed by cervical dislocation on the day following the last treatment. Testes, epididymis, seminal vesicles, vas deferens and ventral prostate were weighed to the nearest milligram by using Shimadzu electronic balance (model No: BL-220H) after clearing off the adhering tissues. Reproductive organ indices were determined by using the formula: (weight of the tissue/weight of the mice) × 100.

Sperm Parameters

Sperm count

Epididymal sperm suspension was collected by placing cauda in 10 ml Petri dish containing 2.0 ml of physiological saline at 37°C. The epididymal fluid was subjected to sperm count using Neubauer Chamber as described by Belsey *et al*.[20] Sperm count was expressed as a millions/ml.

Sperm motility

Sperm motility was determined according to the method described by Belsey *et al*.[20] The whole process was performed within 5 min following their isolation from cauda epididymis. First, non-motile sperms were counted, followed by motile sperms. Sperm motility was expressed as a percentage of the total sperm counted.

Sperm viability

The ratio of live and dead sperms was determined using 1% trypan blue by the method of Talbot and Chacon.[21] The viable sperm with its intact cell membrane will not take up the dye (trypan blue) and remains unstained. Sperm viability was expressed as a percentage of the total sperm counted.

Hypo-osmotic swelling test

Hypo-osmotic swelling (HOS) test is an important test to assess sperm membrane integrity. When viable sperms are exposed to hypo-osmotic medium, there will be an influx of fluid causing the tail to coil, which can be seen under phase-contrast microscope and the percent of coiling was estimated by the method of Jeyendran *et al*.[22]

Assay of Testicular 3β-Hydroxysteriod Dehydrogenase (EC: 1.1.1.51) and 17β-Hydroxysteriod Dehydrogenase (EC: 1.1.1.61) Activity Levels

The activity levels of 3β hydroxysteriod dehydrogenase (3β-HSD) and 17β hydroxysteriod dehydrogenase (17β-HSD) in the testes of mice were determined using the procedure described earlier.[23] The testicular tissue was homogenised (5% w/v) in ice-cold Tris HCl buffer (pH 6.8). The microsomal fraction was separated and used as enzyme source. The reaction mixture in a final volume of 2.5 ml contained: 100 mmoles of sodium pyrophosphate buffer (pH 9.0), 0.5 moles of cofactor (NAD for 3β-HSD and NADPH for 17β-HSD) and 0.1 moles of substrate (dihydroepiandrosterone for 3β-HSD and 4-androstene-3,17-dione for 17β-HSD) and 20 mg equivalent of microsomal protein as the enzyme source. The reactions

were carried out in a cuvette of 1 cm path at 23°C. The absorbance at 340 nm was measured at 20 sec intervals for 5 min using a spectrophotometer (Hitachi Model No: U 2001) against blank containing all the components except the enzyme source. The enzyme activities were expressed as nmoles of NAD converted to NADH/mg protein/min (3β-HSD) or nmoles of NADPH converted to NADP/mg protein/min (17β-HSD). The protein content in the enzyme source was determined according to the method of Lowry *et al*.[24] using bovine serum albumin as the standard.

Radioimmunoassay of Serum Testosterone

Trunk blood was collected from each animal using a heparinised syringe and plasma was separated by centrifugation at 2000 × *g* for 15 min after overnight storage at 4°C and stored at −20°C until all the samples were collected. Radioimmunoassay (RIA) for serum testosterone level determination was performed by the method of Rao *et al*.[25] The sensitivity of the assay was calculated as 0.002 ng and intra-assay variation was found to be 6.5%. All of the samples were run at the same time to avoid inter-assay variation.

Statistical Analysis

Data were statistically analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The values $P<0.05$ were considered significant. The data were presented as mean±SD. All statistical tests were performed using Statistical Package for Social Sciences (SPSS), version 16.0.

RESULTS

General Toxicity

The mice treated with cisplatin and speman alone or cisplatin and speman did not show any clinical signs of toxicity (such as abnormal salivation, fasciculations, tremor, facial movements, and head and forelimb clonuses, convulsions, ataxia, diarrhoea, increased diuresis) or none of the mice were excluded from the experiment. No mortality was observed in both treated and untreated groups of mice during the experimental period.

Body and Organ Weight Gain Profile

No significant changes in the body weight gain were observed in controls and experimental animals [Table 1]. Significant decrease in the weights of testis ($P<0.01$) and epididymis ($P<0.01$) was observed in cisplatin injected mice as compared to the control mice; however, no significant changes were observed in the prostate gland, vas deferens and seminal vesicles after cisplatin treatment [Table 1]. On the other hand, supplementation of speman to cisplatin treated mice significantly increased the weights of testis ($P<0.05$), and epididymis ($P<0.05$) as compared to cisplatin alone injected mice, whereas no significant changes in the body weights and also in the relative weights of reproductive organs were observed in speman alone treated mice [Table 1].

Spermatology

The average sperm count, viable sperm and motile sperm in cauda epdidymal plasma were found to be 41.62±1.76 millions/ml, 82.24±4.20% and 70.84±3.48%, respectively, in the control mice. A significant decrease in epididymal sperm count (-50.84%; $P<0.05$), motility (-33.91%; $P<0.05$) and viability (-34.75%; $P<0.05$) was observed in cisplatin treated mice as compared to controls [Table 2]. Moreover, the number of HOS-tail coiled sperms (HOS test) in cisplatin treated mice was also decreased significantly (−53.72%; $P<0.05$) as compared to controls [Table 2]. Supplementation of speman to cisplatin treated mice significantly ($P<0.05$) increased the sperm count (66.81%), motility (43.55%) and viability (35.32%) and also the number of HOS-tail coiled sperms (55.83%) as compared to cisplatin injected mice [Table 2]. Significant ($P<0.05$) increase in the sperm count (22.63%) and motile sperm (14.69%) was also observed in speman alone treated mice as compared to control mice. However, no significant changes were observed in the viable sperms and HOS-tail coiled sperms in speman alone treated mice as compared to the control mice [Table 2].

Table 1: Changes in body weight gain and reproductive organ indices in cisplatin injected mice with or without speman supplementation

Parameter	Control	Experimental groups		
		Cisplatin	Speman	Cisplatin + speman
Body weights (g)	10.91^{a}±1.21	11.09^{a}±1.65 (1.65)	11.06^{a}±1.87 (1.38)	10.88^{a}±2.04 (-1.89)
Tissue indices (g%)				
Testis	0.92^{a}±0.12	0.61^{b}±0.09 (-33.69)	0.98^{a}±0.06 (6.52)	0.87^{c}±0.07 (42.62)
Epididymis	1.53^{a}±0.11	0.89^{b}±0.11 (-41.83)	1.70^{a}±0.36 (11.11)	1.41^{c}±0.14 (58.43)
Seminal vesicle	0.69^{a}±0.14	0.65^{a}±0.14 (-5.79)	0.72^{a}±0.15 (4.35)	0.73^{a}±0.13 (12.31)
Vas deferens	0.15^{a}±0.05	0.14^{a}±0.03 (-6.67)	0.16^{a}±0.03 (6.67)	0.15^{a}±0.02 (-)
Prostate gland	0.058^{a}±0.003	0.053^{a}±0.012 (-8.62)	0.061^{a}±0.006 (5.17)	0.055^{a}±0.013 (3.77)

Values are mean±SD of 10 individuals. Values in the parentheses are percent change from that of control. For calculation of percent change, for cisplatin and speman alone treated mice, control mice served as controls; for cisplatin + speman treated mice, cisplatin injected mice served as controls. Mean values that do not share the same superscript in a row differ significantly from each other at $P<0.05$

Steroidogenic Enzyme Activities and Serum Testosterone Levels

Significant (P<0.05) reduction in the activities of testicular 3β-HSD (–41.81%) and 17β-HSD (–51.24%) was observed in cisplatin injected mice as compared to the control mice [Table 3]. Administration of speman significantly increased the activity levels of 3β-HSD (55.68%) and 17β-HSD (69.71%) in the testis of cisplatin injected mice [Table 3] as compared to cisplatin alone injected mice. Significant increase in the activity of testicular 17β-HSD (17.72%) was also observed in speman alone treated mice as compared to the control mice. The levels of serum testosterone decreased (–57.28%) significantly (P<0.05) in mice treated with cisplatin as compared to the control animals. Supplementation of speman significantly (P<0.05) increased serum testosterone level in control (33.29%) and also in cisplatin treated (95.26%) mice [Table 3].

DISCUSSION

Although patients treated with chemotherapeutic agents for testicular cancer experience significant reproductive problems, no studies to date have confirmed that these symptoms are directly the result of chemotherapy. The use of an animal model allows us to administer cisplatin in a dose regimen that is clinically relevant, without the presence of testicular cancer. This provides an opportunity to elucidate the role of cisplatin on reproduction. The present study also encompasses evaluation of the effect of herbal drug, speman, in protecting the mice from the reproductive toxic effect of cisplatin. In the present study, adult male mice were treated with cisplatin and speman alone or in combination and the changes in reproductive organ weights, sperm density and quality and steroidogenesis were analysed.

In the present study, there was a significant decrease in the weights of testis and epididymis in cisplatin treated mice as compared to the controls. This suggests that testis and epididymis are vulnerable targets to cisplatin (3 mg/kg body weight) in mice. Reduction in the testis weight along with disrupted spermatogenesis is a well-known side effect of cisplatin-based chemotherapy.[26] It is accepted that the weight of the testis largely depends on the mass of differentiated spermatogenic cells which has been used as a crude measure of the damage to spermatogenesis.[27] Moreover, the structural and functional integrity of testis and accessory sex organs requires adequate bioavailability of testosterone. Thus, the decreased weights of testis and epididymis in cisplatin treated mice may be due to reduced spermatogenesis and steroidogenesis. Supplementation of speman to cisplatin treated mice significantly increased the weights of testis and epididymis, which may indicate that speman supplementation sustains the bioavailability of testosterone in cisplatin treated mice. Jayatilak[28] observed improvement in accessory reproductive organ functions in mice after treatment with speman. It was also reported that speman stimulates spermatogenesis in humans[19] and steroidogenesis in mice.[15]

Male fertility depends on spermatogenesis and sperm maturation events. Earlier, analysis of sperm quantity and

Table 2: Changes in sperm parameters in cisplatin injected mice with or without speman supplementation

Parameter	Control	Experimental groups		
		Cisplatin	Speman	Cisplatin + speman
Sperm count (millions/ml)	41.62^{a}±1.76	20.46^{b}±1.39 (-50.84)	51.04^{c}±2.86 (22.63)	34.13^{d}±2.21 (66.81)
Viable sperm (%)	82.24^{a}±4.20	53.66^{b}±3.57 (-34.75)	86.27^{a}±3.70 (4.90)	72.61^{c}±4.93 (35.32)
Motile sperm (%)	70.84^{a}±3.48	46.82^{b}±2.68 (-33.91)	81.25^{c}±3.59 (14.69)	67.21^{d}±4.49 (43.55)
HOS-tail coiled sperms (%)	69.67^{a}±1.94	32.24^{b}±2.86 (-53.72)	74.45^{a}±3.39 (6.86)	50.24^{c}±7.59 (55.83)

Values are mean±SD of 10 individuals. Values in the parentheses are percent change from that of control. For calculation of percent change, for cisplatin and speman alone treated mice, control mice served as controls; for cisplatin + speman treated mice, cisplatin injected mice served as controls. Mean values that do not share the same superscript in a row differ significantly from each other at P<0.01

Table 3: Changes in testicular steroidogenic enzyme activities and serum testosterone levels in cisplatin injected mice with or without speman supplementation

Enzyme	Control	Experimental groups		
		Cisplatin	Speman	Cisplatin + speman
3β-hydroxysteroid dehydrogenase (nmoles of NAD converted to NADH/mg protein/min)	21.17^{a}±1.72	12.32^{b}±1.41 (-41.81)	22.72^{a}±1.58 (7.32)	19.18^{c}±1.26 (55.68)
17β-hydroxysteroid dehydrogenase (nmoles of NADPH converted to NADP/mg protein/min)	18.62^{a}±1.61	9.08^{b}±1.23 (-51.24)	21.92^{c}±1.44 (17.72)	15.41^{d}±1.35 (69.71)
Testosterone (ng/ml)	7.42^{a}±1.01	3.17^{b}±0.56 (-57.28)	9.89^{c}±0.98 (33.29)	6.19^{d}±0.86 (95.26)

Values are mean±SD of 10 individuals. Values in the parentheses are percent change from that of control. For calculation of percent change, for cisplatin and speman alone treated mice, control mice served as controls; for cisplatin + speman treated mice, cisplatin injected mice served as controls. Mean values that do not share the same superscript in a row differ significantly from each other at P<0.01

quality was used as a good indicator of male fertility.[29] In the present study, significant decrease in the sperm count, motility and viability was observed in cisplatin treated mice. Administration of cisplatin has been reported to affect the numbers of spermatozoa and their motility in rats.[30] Our results are also consistent with earlier reports.[9,26,31] Cisplatin is known to induce apoptotic germ cell loss and also sloughing of seminiferous epithelium,[32-34] which severely affects sperm count. Thus, the reduction in the sperm count, motility and viability of sperm may indicate cisplatin-induced testicular and epididymal toxicity. Significant decrease in the number of HOS-tail coiled sperms in cisplatin injected mice indicates the loss of sperm membrane integrity. Speman supplementation to cisplatin injected mice increased the sperm count and quality. Speman is a well-known polyherbal drug used to improve semen quality of oligospermic men.[35,36]

Testicular 3β-HSD and 17β-HSD play a very important role in the biosynthesis of testosterone from cholesterol. Thus, determining the activity levels of these two enzymes provides valuable information regarding the biosynthesis of testosterone. In the present study, administration of cisplatin to mice decreased the activity levels of 3β-HSD and 17β-HSD, indicating reduced steroidogenesis which in turn indicates dysfunction of Leydig cells. Previous studies also have demonstrated the decreased activity levels of 3β-HSD and 17β-HSD in the Leydig cells cultured with platinum compounds.[37] Accordingly, the circulatory levels of testosterone also decreased in cisplatin treated mice. Significant increase in the activity levels of testicular steroidogenic enzymes and serum testosterone levels was observed in cisplatin treated mice supplemented with speman, indicating stimulation of steroidogenesis. Earlier, it has been reported that speman treatment increased the mean testosterone level from 4.27±0.26 ng/ml to 5.86±0.34 ng/ml in men, without any side effects.[10] Similar increase in the levels of serum testosterone was observed in hexachlorocyclohexane-treated mice after speman supplementation.[11]

Speman is a polyherbal formulation consisting of ingredients from several plants like *Orchis mascula, Asteracantha longifolia, Lactuca scariola, Mucuna pruriens, Suvarnavanga, Argyreia speciosa, Tribulus terrestris, Leptadenia reticulata* and *Parmelia perlata*, most of which are used traditionally as aphrodisiacs.[10] Furthermore, the ingredients from plants like *A. speciosa* and *M. pruriens* in speman result in improvement of sperm density and quality to improve fertility and induce conception.[38] Thus, the mechanism of protective effect of speman against cisplatin-induced spermatotoxic and antisteroidogenic effects in mice could be the stimulation of steroidogenesis, thereby improving spermatogenesis.

These results clearly show that cisplatin treatment has a deleterious impact on spermatogenesis and steroidogenesis in male mice. Speman supplementation restores the sperm quantity and quality along with enhanced steroidogenesis in cisplatin treated mice. If the same is true for humans, speman could be supplemented to patients who are undergoing cisplatin-based chemotherapy to protect their reproductive health. Further studies to clarify the exact mechanism of speman-induced improvement of suppressed reproduction are warranted.

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Authors' Contributions

PSR conceived the idea, supervised the work, provided the grants for the study, evaluated the data and coordinated the study. SBS and TS carried out the treatment of animals and performed the enzyme assays and sperm analysis. PM, KPR and BPG were involved in testosterone determination. PSR and SBS drafted the manuscript for publication. All the authors have read and approved the final version of manuscript.

REFERENCES

1. Bergström R, Adami HO, Möhner M, Zatonski W, Storm H, Ekbom A, *et al*. Increase in testicular cancer incidence in six European countries: A birth cohort phenomenon. J Natl Cancer Inst 1996;88:727-33
2. Lebwohl D, Canetta R. Clinical development of platinum complexes in cancer therapy: An historical perspective and an update. Eur J Cancer 1998;34:1522-34.
3. Taguchi T, Nazneen A, Abid MR, Razzaque MS. Cisplatin-associated nephrotoxicity and pathological events. Contrib Nephrol 2005;148:107-21.
4. Stephenson WT, Poirier SM, Rubin L, Einhorn LH. Evaluation of reproductive capacity in germ cell tumor patients following treatment with cisplatin, etoposide, and bleomycin. J Clin Oncol 1995;13:2278-80.
5. Zhang X, Yamamoto N, Soramoto S, Takenaka I. Cisplatin-induced germ cell apoptosis in mouse testes. Arch Androl 2001;46:43-9.
6. Vawda AI. Effect of testosterone on cisplatin-induced testicular damage. Arch Androl 1994;32:53-7.
7. Malarvizhi D, Mathur PP. Effect of cisplatin on testicular function in rats. Indian J Exp Biol 1996;34:995-8.
8. Narayana K. Cisplatin induces duplex 3′ overhangs and 5′ blunt ends in epididymal epithelium in a Bax-dependent manner without any protection from L-ascorbic acid. Eur J Pharmacol 2010;641:238-45.
9. Ali BH, Al Moundhri MS. Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: A review

of some recent research. Food Chem Toxicol 2006;44:1173-83.
10. Mukherjee K, Tripathi A, Kulkarni KS. Evaluation of the efficacy of 'Speman' in the management of male subfertility. Indian J Clin Pract 2003;13:29-31.
11. Sahu KC. Clinical observations on the use of speman in the treatment of spermatorrhoea. Med Digest 1954;9:485.
12. Mukherjee M, Rai DK, Dubey GP. Testicular changes in electroshock-induced stress and effect of Speman. Probe 1990;29:300-6.
13. Mathur R, Kulshrestha R. Restitution of ceric sulphate-induced sterility in rats by zinc and Speman. J Sci Res 1984;6:11-4.
14. Rathore HS. Protection of mouse testes, epididymis and adrenals with Speman against cadmimum intoxication. Med Surg 1986:26:33-5.
15. Bhan A, Rathore H. Prevention of Hexachlorocyclohexane (HCH)-induced changes in selected reproductive organs of mice with a herbal drug speman in acute exposure. Indian J Occup Health 1996;39:1-7.
16. CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol 2003;35:257-74.
17. NRC (National Research Council). Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources (ILAR), Commission on Life Sciences. Washington, DC: National Academy Press; 1996. p. 125.
18. Mclaren A. Germ cells and germ cell transplantation. Int J Dev Biol 1998;42:855-60.
19. Subbarao VV, Gupta ML, Arora HL. Effect of Speman on the testes. Probe 1973:12:197-200.
20. Belsey MA, Moghissi KS, Eliasson R, Paulsen CA, Callegos AJ, Prasad MR. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. Singapore: Press concern. 1980. p. 10.
21. Talbot P, Chacon RS. A triple stain technique for evaluating normal acrosome reaction of human sperm. J Exp Zool 1981;215: 201-8.
22. Jeyendran RS, Van der Ven HH, Zaneveld LJ. The hypoosmotic swelling test: An update. Arch Androl 1992;29:105-16.
23. Bergmayer HU. Methods of enzymatic analysis. In: Bergmayer HU, editor. Verlag Chemie Weinheim, New York: Academic Press; 1974. p. 447-89.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
25. Rao AJ, Chakraborti R, Kotagi SG, Ravindranath N. Effect of constant infusion of gonadotropin releasing hormone (GnRH) agonist buserelin and antagonist CDB 2085 A using osmotic minipumps on testicular function in adult male bonnet monkey (*Macaca radiata*). Andrologia 1990;22:567-73.
26. Bieber AM, Marcon L, Hales BF, Robaire B. Effects of chemotherapeutic agents for testicular cancer on the male reproductive system, spermatozoa, and fertility. J Androl 2006;27:189-200
27. Schlappack OK, Kratzik C, Schmidt W, Spona J, Schuster E. Response of seminiferous epithelium to scattered radiation in seminoma patients. Cancer 1988;62:1487-91.
28. Jayatilak PG, Pardanani DS, Dattatreya B, Sheth AR. Effect of an indigenous drug (Speman) on accessory reproductive functions of mice. Ind J Exp Biol 1976;14:170-3.
29. Ohl DA, Menge AC, Sonksen J. Penile vibratory stimulation in spinal cord injured men: Optimized vibration parameters and prognostic factors. Arch Phys Med Rehabil 1996;77:903-5.
30. Seethalakshmi L, Flores C, Kinkead T, Carboni AA, Malhotra RK, Menon M. Effects of subchronic treatment with cis-platinum on testicular function, fertility, progeny outcome, and progeny. J Androl 1992;13:65-74.
31. Azu OO, Duru FI, Osinubi AA, Oremosu AA, Norohna CC, Okanlawon AO. Long-term treatment with *Kigelia africana* fruit extract ameliorates the testicular toxicity following cisplatin administration in male Sprague-Dawley rats. Eur J Pharmacol 2010;641:238-45.
32. Schrader M, Muller M, Straub B, Miller K. Testicular sperm extraction in azoospermic patients with gonadal germ cell tumours prior to chemotherapy-a new therapy option. Asian J Androl 2002;4:9-15.
33. Seaman F, Sawhney E, Giammona CJ, Richburg JH. Cisplatin-induced pulse of germ cell apoptosis precedes long-term elevated apoptotic rates in C57/BL/6 mouse testis. Apoptosis 2003;8:101-8.
34. Sawhney P, Giammona CJ, Meistrich ML, Richburg JH. Cisplatin-induced long-term failure of spermatogenesis in adult C57/B1/6J mice. J Androl 2005;26:136-45.
35. Khaleeluddin K, Santpur SR, Rajaram P, Durgamba KK. Clinical trials in cases of oligozoospermia with Speman. Probe 1973;4:203.
36. Pardanani DS, Delima RJ, Rao RV, Vaze AY, Jayatilak PG, Sheth AR. Study of the effects of Speman on semen quality in oligospermic men. Ind J Surg 1976;38:34.
37. Dupont E, Labrie F, Luu-The V, Pelletier G. Ontogeny of 3β-hydroxy steroid dehydrogenase/Δ^5-Δ^4isomerase (3β-HSD) in rat testis as studied by immunocytochemistry. Anat Embryol (Berl) 1993;187:583-9.
38. Nalini B, Shenoy SK, Geetha B, Anuradha, Vijay KV. The role of herbal drugs in infertile couples. Obstet Gynaecol Today 2003;8:281-7.

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