Journal of Medicinal Plants Research Vol. 5(3), pp. 388-397, 4 February, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Long-term treatment with *Kigelia africana* fruit extract ameliorates the testicular toxicity following cisplatin administration in male Sprague-Dawley rats

Azu, O. O.[1]*, Duru, F. I. O.[1], Osinubi, A. A.[1], Oremosu, A. A.[1], Norohna, C. C.[1], Okanlawon, A. O.[1] and Elesha S. O.[2]

[1]Department of Anatomy, College of Medicine of the University of Lagos, P. M. B. 12003, Idiaraba, Lagos, Nigeria.
[2]Department of Morbid Anatomy, College of Medicine of the University of Lagos, P. M. B. 12003, Idiaraba, Lagos, Nigeria.

Accepted 17 November, 2010

Cisplatin is one of the most potent chemotherapeutic antitumor drugs that generates reactive oxygen species and also inhibits the activity of antioxidant enzymes in testicular tissues. In the present study, we investigated the long term effects of treatment with *Kigelia africana* fruit extract (KAFE) and cisplatin in male Sprague-Dawley rats. Our literature survey indicated a lack of any experimental study showing the effects of long term use of KAFE in any experimental chemotherapeutic protocol using cisplatin. 50 Sprague-Dawley rats were used for the study, divided into 10 groups (n = 5 in each group): control group, KAFE alone groups (100 and 500 mg/kg), cisplatin group, KAFE and cisplatin co-treatment group, KAFE prophylactic groups, and KAFE post-treatment groups. Testicular histopathology, MDA, GSH, catalase activities were determined alongside epididymal sperm count and motility. Hormonal assay for testosterone, FSH and LH was determined as well as morphometric parameters. Cisplatin-treated rats suffered 44% attrition rate with significantly reduced weight compared to controls and KAFE treated rats. Similarly, sperm motility was below 50% in cisplatin-treated rats while KAFE treatment resulted in over 70% motility. The cross-sectional area of seminiferous tubules in cisplatin-treated rats was 27.77 ± 0.9 × 10^3 µm^2 as against 35.28 ± 1.6 × 10^3 µm^2 in controls. While in cisplatin treated group the tissue levels of GSH and catalase activities were found to be significantly lower than in control and KAFE treated rats, MDA levels were significantly higher. Administration of KAFE as an adjunct to cisplatin and as post-treatment was not as effective in ameliorating the derangements in histoarchitectural and biochemical parameters caused by cislatin as the case when it is administered as a prophylactic or alone. Long term treatment with KAFE in rats has not shown any serious histoarchitectural alterations in the testis of the animals. While it is possible that KAFE may confer protecting capabilities against cisplatin-induced testicular damage, our studies has proven that pre-treatment offers a better option in reducing the ravages caused by cisplatin in the testis in a mechanism believed to be free-radical mediated.

Key words: *Kigelia africana*, testicular toxicity, cisplatin, long-term treatment.

INTRODUCTION

Ethno-medicinal plants have provided a major focus in global health care and are contributing substantially to the drug development process generally (Graham et al., 2000). While there is a continuous search for novel chemo-protective agents against various diseases in the sub region, efforts at identifying relevant plants with potent pharmaceutical activity remains slow. *Kigelia africana* (Lam Benth.) plant grows commonly in tropical west, east and central African regions especially in low

*Corresponding author. E-mail: amechi2@yahoo.com., amechi2@hotmail.com. Tel: +2348023184606.

Abbreviation: KAFE, *Kigelia africana* fruit extract.

plains along Nigeria. It represents an interesting example of a plant used in traditional medicine for many years, but which is now attracting interest and use far beyond its original geographical range (Kolodziej, 1997). It is commonly called 'Sausage tree' or 'Cucumber tree' due to its long sausage-like fruit and belongs to the *Bignoniaceae* family. Its fruits weigh between 4 - 10 kg and hangs from a long and fibrous stalk.

In folklore, the fruit of *K. africana* represents a symbol of fertility (Burkill, 1985). In South eastern Nigeria, the fruits and flowers are mixed with alcohol or water and used by traditional healers for fertility treatment among women and men of child bearing age (Ogbeche et al., 2002). In South western Nigeria, the bark extract is said to have an aphrodisiac effect as it enhances sexual libido and erection in males (Oliver-Bever, 1986; Abioye et al., 2003).

Despite the successful utilisation of few phytochemicals, such as vincristine and taxol, into mainstream cancer chemotherapy, commercial plant-derived anticancer formations represent only one-fourth of the total repertoire of the available treatment options (Okobia, 2003; Kintzios and Spiridon, 2006). Since the discovery of cisplatin (cis-diaminedichloroplatinum (II)) in 1845 by Barnet Rosenberg and its anticancer properties in 1964, significant success has been recorded in the treatment of cancers of testis, ovary and the lungs even in the face of newer generation of platinum complexes (Weiss and Christian, 1993). However, one of the undesired consequences of ciplatin use is its cytotoxicity to rapidly dividing normal cells especially of the testis with the consequent reproductive implications (Atessahin et al., 2006a) via mechanisms believed to be free radical mediated (Cardinal et al., 2000; Rybak et al., 2000).

We have earlier reported in our laboratory that *K. africana* fruit extract (KAFE) possesses androgenic effects (Azu et al., 2009), antioxidant properties (Azu et al., 2010a) and protects against the ravages of reactive oxygen molecules in renal tissues following cisplatin injury (Azu et al., 2010b) in Sprague-Dawley rats. KAFE also significantly improved the deleterious histo-morphometric changes induced by cisplatin in the short term treatment (Azu et al., 2010c). Therefore, the aim of this study is to further investigate the long term changes following treatment with KAFE in cisplatin-induced testicular toxicity in male Sprague-Dawley rats.

MATERIALS AND METHODS

Plant materials

Matured and ripe fruits of *K. africana* were harvested from the forest in Badagry town, Lagos. The taxonomic identification was done by Mr. T. K. Odewo of the Forestry Research Institute of Nigeria (FRIN), Ibadan (herbarium number FHI/08257) and confirmed by Dr. A. B. Kadiri of the Department of Botany and Microbiology, University of Lagos, Nigeria. The fruits were washed, cut into small pieces, air-dried and ground into a powdery form using a grinding machine. The powdered fruit was weighed and kept for further phyto-chemical screening and extraction. The extraction was done using the Soxhlet apparatus with methanol as the solvent as described by Abioye et al. (2003). A total of 1.15 kg of the powdered fruit was used for the extraction and was packed into the thimble of the Soxhlet apparatus and a total of 2.2 L of methanol was used for the extraction. At the end of the extraction process, the extract was evaporated and further dried in an oven regulated at 38°C and the yield which was 115.33 g was stored in sterile universal containers and kept in the freezer for further use. 100 and 500 mg/kg doses of KAFE were used as stated in Azu et al. (2010c).

Animals

In this investigation, 50 healthy adult male Sprague-Dawley rats (8 weeks old, weighing 180 - 220 g) were used. The animals were obtained from the Animal House of the College of Medicine of the University of Lagos, Nigeria. They were kept under standard laboratory conditions (12 h light: 12 h dark and at 28 ± 2°C). The rats were fed with standard commercial rat chow (Pfizer Feeds Ltd, Ikeja, Lagos, Nigeria). Feed and water were provided *ad libitum*. All experiments in this study were performed in accordance with the guidelines for animal research and approved by the Departmental Ethical Committee on Animal Research.

The rats were randomly divided into ten groups (1 to 10) with each group containing five rats per group. Animals lost prematurely were replaced for completion of the groups' total number; a total of nine (9) additional animals were used (overall 59 animals). The treatment period was for 56 days. All treatments were orally administered except for cisplatin that was intraperitoneally administered. Group 1 received normal saline as the control while groups 2 and 3 received 100 and 500 mg/kg KAFE respectively daily for duration of treatment. Group 4 received 10 mg/kg single i.p injection of cisplatin (Cp) (Korea United Pharm. Inc., Chungnam, Korea) (Azu et al., 2010c) and were sacrificed at end of 56 days. Groups 5 and 6 received 100 and 500 mg/kg KAFE co-treatment with Cp respectively while groups 7 and 8 were the prophylactic groups receiving 100 and 500 mg/kg KAFE respectively for 28 days before Cp administration at end of 28 days. Groups 9 and 10 were the post-treatment groups that received Cp (10 mg/kg) and after 28 days were treated with KAFE at 100 and 500 mg/kg doses respectively for the remaining 28 days.

Sample collection

At the end of the experimental period, rats were sacrificed on the 56th day by i.p injection of ketamine (Rotex Medica, Trittau, Germany) 50 mg/kg (Marcon et al., 2008). Measurement of body and testicular weights and volume, epididymal sperm concentration and motility were recorded. One of the testes was fixed in 10% formalin for further histopathological and morphometric assessments while the other testis was utilized for further biochemical assays. Blood samples were collected and plasma as well as other homogenates stored at -20°C until biochemical analyses.

Epididymal sperm concentration and motility

Spermatozoa in the epididymis were counted as described in the procedure here. Briefly, the epididymis was dissected clear and minced with anatomical scissors in a petri dish in 1 ml of Ham-F-10 solution. Both sperm concentration and motility were determined at room temperature (28°C). Progressive motility was evaluated using a light microscope and classified as either motile or non-motile for

the purposes of this study. The microscopic field was scanned systematically and each spermatozoa encountered was assessed. The procedure was repeated once and the average reading was taken.

Total sperm number (count) was determined using a haemocytometer. A dilution ratio of 1:20 from each well-mixed sample was prepared by diluting 50 µl of epididymal spermatozoa suspended in physiological saline with 950 µl diluent. Both chambers of the haemocytometer were scored and the average count calculated, provided that the difference between the two counts did not exceed 1/20 of their sum that is, less than 10% difference. When the two counts were not within 10%, they were discarded, the sample dilution re-mixed and another haemocytometer prepared and counted. To minimize error the count was conducted three times on each of the samples obtained from each epididymes. The average of all the six counts, 3 from each side, from a single rat was constituted as an observation.

Histopathological and morphometric analysis

Fixed testes in 10% formalin were processed and embedded in paraffin, sectioned serially at 5 µm and were stained with haematoxylin and eosin (H and E). Prior to embedding, it was ensured that the mounted sections to be cut were oriented perpendicular to the long axes of the testes. These sections were designated 'vertical sections'. Light microscopy was used for the evaluations. For each testis, seven vertical sections from the polar and the equitoral regions were sampled (Qin and Lung, 2002) and an unbiased numerical estimation of the following morphometric parameters were determined using systematic random scheme (Gundersen and Jensen, 1987): diameter and cross-sectional area of the seminiferous tubules; number of profiles of seminiferous tubules per unit area of testis and numerical density of the seminiferous tubules. The seven vertical sections were selected by a systematic sampling method that ensured fair distribution between the polar and equatorial regions of each testis. Testicular volume was estimated by water displacement (Inuwa, 2005), while the weight was measured by an electronic balance. The two testes of each rat were measured and the average value obtained for each of the two parameters was regarded as one observation. Values are expressed in cm^3 and g for testicular volume and testicular weight respectively. Diameter (D) of seminiferous tubules with profiles that were round or nearly round were measured for each animal and a mean D was determined by taking the average of two diameters, D1 and D2. D1 and D2 were taken only when D1/ D2 > 0.85. Cross-sectional area (A_C) of the seminiferous tubules were determined from the formula $A_C = \pi D^2/4$, (where π is equivalent to 3.142 and D the mean diameter of the seminiferous tubules). Number of profiles of seminiferous tubules in a unit area of testis (N_A) was determined by using the unbiased counting frame proposed by Gundersen (1977). Using this frame, in addition to counting profiles completely inside the frame we counted all profiles with any part inside the frame provided they do not touch or intersect the forbidden line (full-drawn line) or exclusion edges or their extension. Numerical density (N_V) of seminiferous tubules is the number of profiles per unit volume and was determined by using the modified Floderus equation:

$N_V = N_A/ (D+T)$ (Gilliland et al., 2001)

where N_A is the number of profiles per unit area, D is the diameter and T the average thickness of the section.

For each stereological parameter (D, A_C, N_A, N_V), five randomly selected fields from all the seven sections of a single testis were viewed, and estimation on each carried out. The average from a total of seventy readings from five fields in seven sections of the two testes of one rat was obtained and this was recorded as one observation.

Biochemical measurements

The testicular tissue was homogenized in a Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. The testicular tissue lipid peroxidation level was measured according to modified thiobarbituric acid (TBA) method of Buege and Aust (1978). MDA reacts with thiobarbituric acid to give a red compound absorbing at 532 nm. The stock reagent contains 2 ml 15%w/v trichloroacetic acid, 0.375 %w/v thiobarbituric acid and 0.25 mol/L hydrochloric acid. 0.5 g of testicular tissue sample was homogenized in 5 ml of 0.15 M KCl and the homogenate centrifuged at 1000 g for 10 min in a Uniscope® laboratory centrifuge and the supernatant collected. An aliquot of 2 ml of the stock reagent was added to 1 ml of testicular homogenate supernatant and mixed thoroughly and placed in an Equitron water bath (80 - 90°C) for 15 min. It was then cooled and the flocculent precipitate removed by centrifugation at 1000 g for 10 min and the absorbance of the supernatant determined with a spectronic spectrophotometer at 532 nm against blank containing all the reagents. Concentration of malondialdehyde was calculated using the molar absorptivity coefficient of malondialdehyde which is 1.56×10^5 $M^{-1}cm^{-1}$. The amount of produced malondialdehyde (MDA) was used as an index of lipid peroxidation.

The reduced glutathione (GSH) level in testicular tissue was estimated as described by Rukkumani et al. (2004). Briefly, to the homogenate, 10% trichloroacetic acid, TCA was added and centrifuged. 1.0 ml of supernatant was mixed with 0.5 ml of Ellmans reagent, 19.8 mg of 5, 5'-dithiobisnitro benzoic acid-DTNB (Sigma Aldrich Inc., St. Louis, Mo, USA Batch D8130-5G) in 100 ml of 0.1% sodium nitrate and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm on the spectrophotometer. The level of GSH was expressed as µmol/ml.

Catalase was assayed colorimetrically at 620 nm and expressed as µmol of H_2O_2 consumed/min as described in Rukkumani et al. (2004). Briefly, the reaction mixture of 1.5 ml contained 1.0 ml of 0.01M pH 7.0 phosphate buffer, 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent -5% potassium dichromate and glacial acetic acids were mixed in 1:3 ratios.

Hormonal assays

Blood samples were collected into a heparinized bottle and centrifuged at 3000 rpm for 15 min using a bench centrifuge and the plasma stored at 4°C for subsequent assay of testosterone (TT), follicle stimulating hormone (FSH) and luteinizing hormone (LH) using the enzyme-linked immunoabsorbent assay (ELISA) kits from TECO Diagnostics, Anaheim, CA. The samples were collected in the morning to reduce the influence of diurnal variation in hormones.

Statistical analysis

The morphometric and stereological data were subjected to parametric methods of analysis. Results are expressed as means ± standard deviation and subjected to statistical analysis using analysis of variance (ANOVA) followed by Turkey's post-hoc test. The significant level considered was $p < 0.05$.

Table 1. Effects of KAFE treatment following Cp administration on mortality, and body weight of Sprague-Dawley rats.

Groups	No. of rats	No. dead	% mortality	Initial BW	Final BW	% gain
1	5	0	0	184.0 ± 4.18	$216.2\pm13.12^{\beta}$	14.9^{β}
2	5	0	0	186.0± 4.18	$228.4 \pm 4.56^{\gamma}$	18.56^{γ}
3	5	0	0	184.2 ± 4.27	$211.1 \pm 4.38^{\varepsilon}$	12.78^{ε}
4	9	4	44.44	189.2 ± 2.28	$198.0 \pm 3.39^{\gamma}$	4.44^{γ}
5	6	1	16.67	187.0 ± 4.69	$212.8 \pm 8.17^{\gamma}$	11.18^{γ}
6	6	1	16.67	189.0 ± 2.65	$212.8 \pm 8.79^{\beta}$	11.18^{β}
7	5	0	0	187.4 ± 3.72	$212.8 \pm 7.82^{\beta}$	11.94^{β}
8	5	0	0	186.6 ± 4.22	$211.8\pm9.65^{\beta}$	11.89^{β}
9	6	1	16.67	188.0 ± 5.70	$219.6 \pm 8.08^{\gamma}$	14.34^{γ}
10	7	2	28.57	186.0 ± 6.52	$199.4 \pm 7.54^{\beta}$	6.72^{β}

β statistically significant at $P < 0.01$; γ ($P < 0.001$); ε ($P < 0.0001$), BW is body weight.

RESULTS

All animals in control, KAFE alone and pre-treated groups recorded zero percent in mortality after 8 weeks duration. Cisplatin-treated rats recorded 44.44% mortality rate at the end of experiment. Co-administration of KAFE (500 mg/kg) and cisplatin resulted in 16.67% mortality whereas post-treatment with KAFE (500 mg/kg) recorded 28.57% mortality. All animals recorded an increase in body weight that varied according to the groups. The decreasing orders in % increase are as follows: 2 > 1 > 9 > 3 > 5 > 7 > 8 > 6 > 10 > 4 corresponding to 18.56, 14.90, 14.34, 12.78, 12.12, 11.94, 11.89, 11.18, 6.72 and 4.44% respectively. Rats in group 4 gained weight but this weight gain was also significantly less than the control group (Table 1).

After 8 weeks of treatment, there was a statistically significant increase in sperm count in groups 2 and 7 ($p < 0.05$) compared with the control. Group 4 recorded a decrease which was significant at $p < 0.001$ compared with the control. The sperm motility of all groups except group 4 was above 50%. Though groups 2, 7 and 8 were higher than control, the results were not statistically significant (Table 2).

A statistically significant increase ($p < 0.001$) in testicular weight (TW) was observed in groups 2, 7 and 8 while group 4 was significantly decreased after eight weeks compared with the control. Group 3 was high but not significant while group 4 was significantly decreased ($p < 0.001$) when compared to the control. Post-treatment and co-administration of KAFE resulted in significant decline in TW. Testicular volume (TV) after 8 weeks of treatment in groups 2 and 7 were significantly higher than control. Group 4 was significantly lower ($p<0.001$). Co-administration and post-treatment with KAFE resulted in significantly reduced TV (Table 3).

A significant ($p<0.001$) decrease in seminiferous tubular diameter (D) was maximal in cisplatin-alone treated rats after 8 weeks. KAFE (100 and 500 mg/kg) alone and KAFE pre-treatment all resulted in significant ($p<0.001$) elevation in group cross-sectional diameter. Co-treatment with KAFE (100 mg/kg) and post-treatment with KAFE (100 mg/kg) yielded improvement in groups D compared to the controls though not significant. The cross-sectional areas of seminiferous tubules of cisplatin-treated rats was $27.77 \pm 0.9 \times 10^3$ μm^2 which was significantly ($p<0.001$) lower than those of control rats $35.28 \pm 1.6 \times 10^3$ μm^2. KAFE alone and pre-treatment significantly ($p<0.001$) improved the cross-sectional areas of seminiferous tubules at the end of experiment but co-administration and post-treatment did not significantly improve the parameter. Though the numerical density of seminiferous tubules in cisplatin-treated animals were higher than those of control there were not significant. Groups pre-treated with KAFE (100 mg/kg) prophylaxis recorded a significant ($p < 0.05$) increase in number of profiles of seminiferous tubules per unit area (N_A) (Table 3).

Catalase activity was significantly increased in group 2 ($p < 0.05$) and was equally high in group 7 compared to control. There was maximal significant decline in catalase activity in groups 4 ($p < 0.001$). Also, the values obtained in groups 2, 3, 7 and 8 were all higher than those of group 5, 6, 9 and 10. Glutathione levels in groups 2, 3, 7 and 8 were significantly ($p < 0.001$) higher compared to control while it was significantly depressed in group 4 ($p < 0.05$). MDA levels were significantly decreased in groups 2, 3 and 7 ($p < 0.001$). Though MDA values in group 8 was decreased it was not significant but group 4 recorded maximum elevation in MDA levels alongside groups 5, 6, 9 and 10 ($p < 0.001$) (Table 4).

Testosterone (TT) levels in groups 2 and 7 were significantly high ($p < 0.001$) whereas that of groups 3 and 8 were high but not significant. Animals in groups 4, 5, 6, 9 and 10 recorded significantly lower ($p < 0.001$) testosterone levels compared with the control. Generally, the values for TT after 8 weeks were high. FSH levels were significantly higher when compared with the control ($p < 0.001$). Group 4 continued to show significantly high FSH levels. LH values in group 3 was elevated ($p <0.001$)

Table 2. Effects of KAFE treatment following Cp administration on sperm count and motility of Sprague-Dawley rats.

Parameter	Groups (n = 5)									
	1	2	3	4	5	6	7	8	9	10
Sperm count (× 10^6/ml)	122.4 ± 10.97	163.2 ± 6.41$^{\alpha}$	112.8 ± 8.12	73.0 ± 15.02$^{\beta}$	91.4 ± 24.98	86.4 ± 26.25	163.0 ± 26.25$^{\alpha}$	142.0 ±15.15	123.4 ± 6. 23	98.2 ± 7.29
Motility (%)	80.0 ± 7.07	82.0 ± 13.04	70.0 ± 3.54	47.8 ± 9.18$^{\gamma}$	60.6 ± 11.70$^{\alpha}$	52.4 ± 4.34$^{\gamma}$	83.6 ± 4.98	80.4 ± 6.69	69.0 ± 2.65	58.8 ± 8.35$^{\beta}$

α: statistically significant at $P < 0.05$; β ($P < 0.01$); γ ($P < 0.001$); n is number of rats.

Table 3. Effects of KAFE treatment following Cp administration on Testicular weight/volume (TW/TV), diameter (D) and cross sectional area (A_C) of seminiferous tubules, number of profiles of seminiferous tubules per unit area (N_A) and numerical density of seminiferous tubules (N_V) of Sprague-Dawley rats.

Groups	TW (g)	TV (cm^3)	D (um)	A_C (×10^3µm^2)	N_A (×10^{-8}µm^2)	N_V (×10^{-10}µm^{-3}) (n = 5)
1	1.632 ± 0.008	1.61 ± 0.07	211.9 ± 4.7	35.28 ± 1.6	51.25 ± 3.6	23.65 ± 1.9
2	1.840 ± 0.055$^{\gamma}$	1.94 ± 0.06$^{\gamma}$	256.7 ± 5.6$^{\gamma}$	51.78 ± 2.2$^{\gamma}$	53.75 ± 4.1	20.56 ± 1.7
3	1.672 ± 0.023	1.68 ± 0.03	249.3 ± 2.3$^{\gamma}$	48.82 ± 0.9$^{\gamma}$	48.75 ± 3.6	19.17 ± 1.4
4	0.754 ± 0.051$^{\gamma}$	0.76 ± 0.05$^{\gamma}$	1880.0 ± 3.2$^{\gamma}$	27.77 ± 0.9$^{\gamma}$	56.25 ± 8.8	29.14 ± 4.5
5	0.896 ± 0.022$^{\gamma}$	0.90 ± 0.02$^{\gamma}$	219.2 ± 6.2	37.78 ± 2.1	600.0 ± 3.4	26.78 ± 1.7
6	0.884 ± 0.017$^{\gamma}$	0.86 ± 0.03$^{\gamma}$	1950.0 ± 2.2$^{\gamma}$	29.87 ± 0.7$^{\gamma}$	51.88 ± 6.5	25.92 ± 2.9
7	1.838 ± 0.057$^{\gamma}$	1.90 ± 0.01$^{\gamma}$	250.6 ± 3.8$^{\gamma}$	49.34 ± 1.5$^{\gamma}$	64.38 ± 7.5$^{\beta}$	25.20 ± 3.1
8	1.754 ± 0.036$^{\gamma}$	1.75 ± 0.03	232.4 ± 1.6$^{\gamma}$	42.43 ± 0.6$^{\gamma}$	500.00 ± 4.9	210.06 ± 2.1
9	0.790 ± 0.025$^{\gamma}$	0.84 ± 0.06$^{\gamma}$	213.5 ± 1.4	35.81 ± 0.5	44.38 ± 4.1	20.31 ± 1.8
10	0.762 ± 0.037$^{\gamma}$	0.78 ± 0.03$^{\gamma}$	193.4 ± 7.9$^{\gamma}$	29.42 ± 2.4$^{\gamma}$	47.50 ± 9.7	23.84 ± 40.0

β statistically significant at $P < 0.01$; γ ($P < 0.001$).

as was with those of groups 5, 6, 9 and 10. Group 4 continued to record significantly higher LH values ($P < 0.001$). The readings of groups 2, 7 and 8 were similar to those of controls (Table 5).

Histological sections of testes of control rats, KAFE alone as well as KAFE prophylaxis were essentially normal with seminiferous tubules that are uniformly spaced transversely oriented with all the cells of the spermatogenic series present. The seminiferous tubules of cisplatin-treated rats showed marked atrophy, depletion and degeneration of spermatogenic cells. Most of the seminiferous tubular lumen was filled with degenerating/dying spermatogenic cells. The interstitial spaces were also distorted with adjourning vascular congestion. The histological section of testes of rats receiving KAFE (100 and 500 mg/kg) and cisplatin concomitantly (groups 5 and 6) showed some seminiferous tubules with normal spermatogenic components while others show loss of spermatocytes (Figure 1). Post-treatment of rats with KAFE (100 mg/kg) (group 9) showed areas of focal depletion of spermatocytes in the histoarchitectural outline of the seminiferous tubules with areas of vacuolar changes in the epithelium. The rats in group 10 treated with KAFE (500 mg/kg) post-cisplatin injection showed seminiferous tubules with few spermatogonia and areas showing loss of spermatogenic cells in the basement membrane and vacuolar changes as well (Figure 2).

DISCUSSION

Animals treated wit KAFE alone and as prophylaxis h to cisplatin gained weight 3 - 4 times more than cisplatin-alone treated rats despite the marginal increase in animal weight in the latter

Table 4. Effects of KAFE treatment following Cp administration on testicular biochemical parameters of Sprague-Dawley rats.

Groups	MDA (µmol/mg protein)	CAT (µmol/min)	GSH (µmol/ml) (n = 5)
1	0.812 ± 0.02	20.493 ± 0.145	0.0368 ± 0.0017
2	0.574 ± 0.34$^{\gamma}$	20.741 ± 0.104$^{\alpha}$	0.0836 ± 0.0049$^{\gamma}$
3	0.482 ± 0.33$^{\gamma}$	10.678 ± 0.183$^{\gamma}$	0.0605 ± 0.0039$^{\gamma}$
4	10.846 ± 0.55$^{\gamma}$	00.696 ± 0.078$^{\gamma}$	0.0282 ± 0.0020$^{\alpha}$
5	10.136 ± 0.06$^{\gamma}$	10.073 ± 0.103$^{\gamma}$	0.0325 ± 0.0009
6	10.192 ± 0.02$^{\gamma}$	00.826 ± 0.073$^{\gamma}$	0.0313 ± 0.0004
7	0.670 ± 0.05$^{\gamma}$	20.685 ± 0.086	0.0933 ± 0.0085$^{\gamma}$
8	0.738 ± 0.04	20.000 ± 0.118$^{\gamma}$	0.0969 ± 0.0043$^{\gamma}$
9	10.206 ± 0.04$^{\gamma}$	10.060 ± 0.133$^{\gamma}$	0.0347 ± 0.0003
10	10.376 ± 0.03$^{\gamma}$	00.879 ± 0.048$^{\gamma}$	0.0321 ± 0.0006

MDA; malondialdehyde: CAT; catalase activity: GSH; reduced glutathione; α statistically significant at $P < 0.05$; β ($P < 0.01$); γ ($P < 0.001$).

Table 5. Effects of KAFE treatment following Cp administration on hormonal parameters of Sprague-Dawley rats.

Groups	TT (ng/ml)	FSH (mIU/ml)	LH (mIU/ml) (n = 5)
1	0.82 ± 0.414	90.44 ± 0.407	80.25 ± 0.265
2	10.26 ± 0.043$^{\gamma}$	120.42 ± 0.645$^{\gamma}$	80.66 ± 0.287
3	0.91 ± 0.035	140.03 ± 0.253$^{\gamma}$	90.36 ± 0.290$^{\gamma}$
4	0.47 ± 0.015$^{\gamma}$	360.52 ± 10.325$^{\gamma}$	150.99 ± 0.379$^{\gamma}$
5	0.61 ± 0.016$^{\gamma}$	180.33 ± 0.356$^{\gamma}$	110.02 ± 0.166$^{\gamma}$
6	0.60 ± 0.007$^{\gamma}$	220.65 ± 0.423$^{\gamma}$	110.74 ± 0.281$^{\gamma}$
7	10.06 ± 0.109$^{\gamma}$	130.25 ± 0.295$^{\gamma}$	80.36 ± 0.169
8	0.96 ± 0.149	140.55 ± 0.460$^{\gamma}$	80.61 ± 0.399
9	0.64 ± 0.067$^{\beta}$	180.02 ± 0.924$^{\gamma}$	90.27 ± 0.267$^{\gamma}$
10	0.31 ± 0.069$^{\gamma}$	210.51 ± 0.639$^{\gamma}$	90.35 ± 0.233$^{\gamma}$

TT; testosterone: FSH; follicle stimulating hormone: LH; luteinizing hormone; β statistically significant at $P < 0.01$; γ ($P < 0.001$).

group (4.44%) as against controls (14.9%) and these positively correlated with reduced testicular weight and volume of same groups in the experiment. Co-administration as well as post-treatment with KAFE was not as effective as the prophylactic groups but was definitely higher than the cisplatin-alone group. This attenuation in loss of body weight could possibly be a protective effect on the animals due to the constituents of KAFE as earlier reported in our previous study (Azu et al., 2010c). It is therefore reasonable to believe that KAFE contents could probably be responsible for this weight gain in the animals.

Derangement in ionic metabolism (Yao et al., 2007) is a common feature following cisplatin administration causing profuse loss of ions with fluids. This polyuria occurs in two distinct phases but it is the phase two that is responsible for ion losses. Whether the concomitant loss in ions and fluids contributes to the weight loss in cisplatin-treated rats remains speculative. Also, attrition rate in cisplatin-treated rats was over 40% as against nil mortality recorded in KAFE alone or prophylactic groups.

Administration of KAFE for 56 days brought about an increase in testicular weight in animals treated with KAFE alone and prophylaxis to cisplatin. Similarly, decline was observed in animals treated with cisplatin alone, post-treatment with KAFE as well as those that had KAFE co-administration with cisplatin. This is an indication that the circulating level of androgen was enough to maintain testicular weight which is in agreement with the fact that the structural and functional integrity of reproductive tissues depends on the circulating androgens hence variations may result in reductions in weight of reproductive organs as seen in cisplatin-treated animals (Venkatesh et al., 2002). This significant increase in absolute testicular weight in KAFE alone and pre-treatment groups that could be attributed to increased androgen biosynthesis is evidenced by a corresponding increase in serum testosterone levels. It is also likely that the increased TW and TV could be as a result of the increased sperm content in the testes of the animals.

Long term treatment with KAFE alone and as prophylaxis to cisplatin in this experiment resulted in

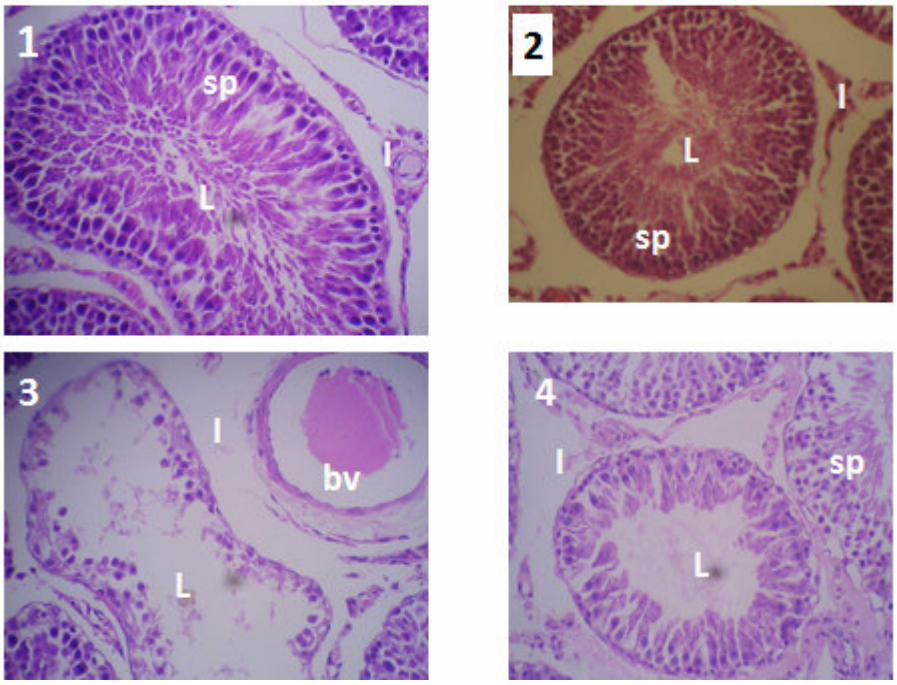

Figure 1. Histopathological slides of control (1), KAFE 100 mg/kg (2),Cp (3) and KAFE 100 mg/kg + Cp (4) treated Sprague-Dawley rats. H and E × 400.Sp is spermatogenic cells, L is seminiferous tubular lumen, I is interstitium, bv is blood vessel. Note marked atrophy of seminiferous tubule in 3.

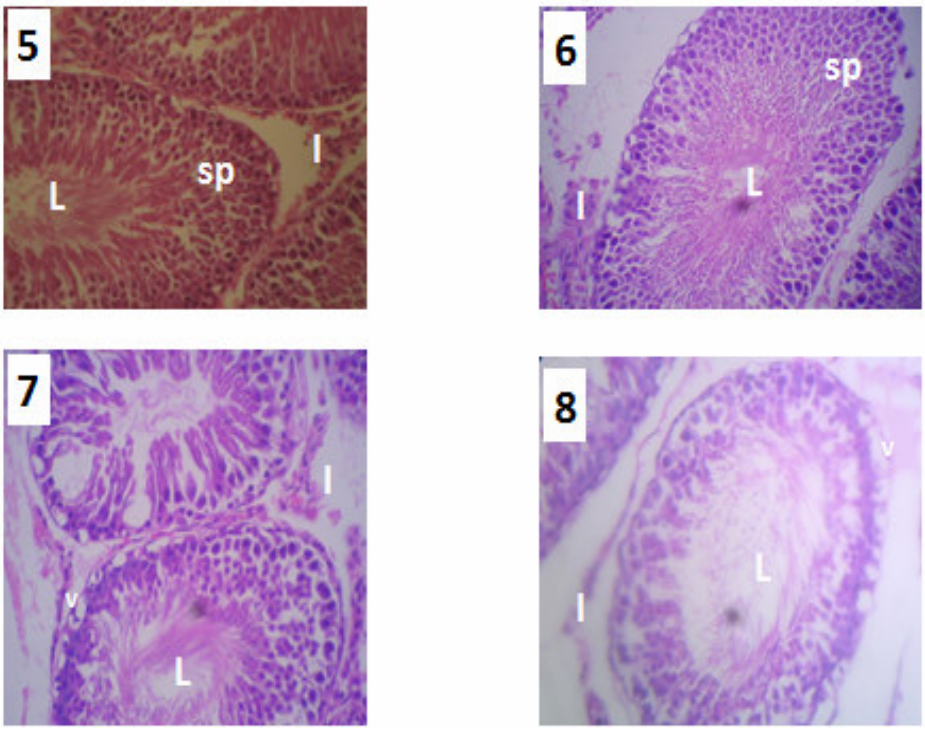

Figure 2. Histopathological slides of KAFE 100 mg/kg pretreatment to Cp (5), KAFE 500 mg/kg pretreatment to Cp(6), KAFE 100 mg/kg post treatment to Cp (7) and KAFE 500 mg/kg post treatment to Cp (8) treated Sprague-Dawley rats. H and E ×400.Sp is spermatogenic cells, L is seminiferous tubular lumen, I is interstitium, v is vacuolar changes in seminiferous tubules in 7 and 8.

significant increase in epididymal sperm count and motility of the rats by 25% with a corresponding decline in cisplatin-treated rats by over 50% of these parameters. This is in agreement with our previous study following short term treatment with KAFE in cisplatin-induced testicular injuries. However, the percentage increase was lower (41 as against 25% observed in this study). Of note however is the fact that the relative values of these parameters where significantly higher than those recorded in the short term treatment experiment which possibly suggests that KAFE offers less deleterious effects on long term usage.

Testicular exocrine function is even more affected by chemotherapy and the differentiating spermatogonia appear to be most vulnerable to this cytotoxicity (Spermon et al., 2006). Cisplatin is known to induce apoptotic germ cell loss which would decrease the sperm count (Seaman et al., 2003; Sawhney et al., 2005). Also, the loss of germ cells and sloughing of epithelium further supports the significant decline in sperm count also observed in studies by Schrader et al. (2002) where azospermia develops after more than 8 weeks of initiation of chemotherapy accompanied by raised serum levels of FSH. Histopathological and morphometric results from this experiment indicate a significant reduction in the tubular and epithelial areas in the testis of cisplatin-treated rats in comparison to controls and KAFE-treated rats. These results also mirror other testicular parameters that were measured and further alludes that cisplatin-induced damage may be via Sertoli cell destruction since the nutritional and structural support of germ cells are maintained by Sertoli cells (Richburg and Boekelheide, 1996). Seminiferous epithelial damage was observed in testicular tissues treated with cisplatin with epithelial sloughing, cell death and retention of spermatids as well as tubular atrophy indicating damage to Sertoli cells consequent upon microtubular disruption (Nakai et al., 1995; Sawhney et al., 2005). The consequent decline in seminiferous tubular diameter was due to sloughing and cell death (Narayana et al., 2006). In most of the seminiferous tubules of cisplatin-alone treated rats, only few spermatogonia were left. This significant deterioration in the histology of cisplatin-alone treated rats corroborates with the decline in other morphometric as well as seminal parameters.

The induction of cisplatin toxicity in renal tissues is said to be a rapid process (Montine and Borch, 1990). Since the kidneys and testes are very sensitive and active sites noted for cisplatin selective toxicity, it is therefore important that any protective agent be present in the tissue before cisplatin administration if it is to confer this protection. This might partly explain why complete protection did not result when KAFE was administered post-cisplatin injection further suggesting that it might be better as a prophylactic treatment than as an adjunct or post-treatment recovery to cisplatin. Higher dose of KAFE (500 mg/kg) administered together with cisplatin and post-cisplatin resulted in mild histological alterations to the seminiferous epithelium with few focal vacuolar changes. The precise component of KAFE responsible for this and its mechanism of damage are not known but it may be due to a direct toxic effect of one or more of the constituents in KAFE. This observation is at variance with the histological observations where KAFE (100 mg/kg) was administered alone and as prophylaxis to cisplatin injection. KAFE is expected to protect testicular tissues to the ravages of cisplatin-induced injuries due to its antioxidant and anti-inflammatory properties of the flavonoids and saponin contents (Picerno et al., 2005; Owolabi and Omogbai, 2007). A high dose administration of KAFE may impair the antioxidant defense system and give way to oxidative damage in a mechanism not yet understood.

Testicular MDA levels, which have been shown to correlate positively with tissue damage and therefore widely used as a marker for lipid peroxidation (Duru et al., 2008) were significantly reduced in animals treated with KAFE alone and as prophylaxis to cisplatin. Conversely, all cisplatin-treated animals suffered significantly elevated MDA levels. The increased MDA level in groups receiving KAFE and cisplatin concomitantly and KAFE post-cisplatin suggest that testicular oxidative stress induced by cisplatin may not be alleviated completely by co-administration of KAFE and further agrees with that the generation of free radicals and or a disturbance in the antioxidant status of the testis is one mechanism of testicular damage by toxicants. It would be preferable to administer KAFE as a prophylaxis before cisplatin injection. Also in agreement with this is the fact that glutathione, an important substrate for glutathione-related enzymes and acts as a free radical scavenger (Rudin et al., 2003), was significantly increased following KAFE treatment. This is in agreement with the results obtained from the histological studies and seminal parameters. It is known that the lipid composition of the sperm membrane exert a significant effect upon the functional quality of spermatozoa (Zalata et al., 1998). Also, the improved sperm/testicular function in this study could be linked to the reduction in MDA levels as the latter is an indirect indicator of TOS hence influences sperm quality and function (Morakinyo et al., 2008). Sperm plasma membranes have a high content of polyunsaturated fatty acids (PUFA) thereby making them sensitive to damage by free radicals resulting in loss of membrane integrity (Suzuki and Sofikitis, 1999). Low catalase activities closely relate to low motility of ejaculated spermatozoa (Kawakami et al., 2007). This can be gleaned from the results on sperm count and motility in the experiment. Cisplatin-treated animals displayed consistently low catalase enzyme activities while KAFE alone and pre-treatment ameliorated the oxidative effects induced by cisplatin.

The normal physiological response to reduction in primary spermatogenesis is a relative elevation in the

blood levels of the gonadotropins. The mechanisms responsible for the rapid increase in gonadotropins, FSH and LH, upon administration of cisplatin to rats are not clearly understood (Aydiner et al., 1997). Upon a single administration of a therapeutic dose of cisplatin 10 mg/kg, the levels of testosterone in the peripheral blood decreased while luteinising hormone increased. An explanation can be deduced from the fact that damage to Leydig cells and withdrawal of the controlling effects of the germinal epithelium on the LH-Leydig cell axis could explain the changes in serum hormone levels as corroborated by other studies (Aydiner et al., 1997). As a result of low testosterone level, prostatic and epididymal secretory function declines and abnormal seminal pH and osmotic pressure develop. This culminates into compromise in spermatogenic integrity and consequent low epididymal spermatogenic parameters observed in the cisplatin treated rats (Kawakami et al., 2007).

Taken together, these findings suggest that cisplatin contributes to the perturbation of steroid and peptide hormone levels in patients undergoing cisplatin-based chemotherapy (LeBlanc et al., 1990). Also, it is established that the effects of cisplatin on hepatic P-450 enzyme expression result from its interruption of the hypothalamic-pituitary stimulation of testicular androgen production and this in turn leads to depletion of circulating normal P-450 enzymes hence feminization (LeBlanc and Waxman, 1988). The mechanism by which KAFE raises testosterone levels is not quite understood but could be linked to the abundance of flavonoids, which is an effective aromatase inhibitor in KAFE (Jeong et al., 1999). The cytochrome P-450 aromatase is required for the conversion of androgens to estrogens and hence aromatase inhibitors would decrease the concentration of estrogens and maintain a higher level of testosterone.

Conclusion

The use of antioxidants as adjuncts for the treatment and/or amelioration of toxicities of chemotherapeutic regimes have been attracting focus in recent times. From our results, cisplatin treatment induces a decrease in plasma antioxidant levels which may be caused from the consumption of antioxidants due to the induced oxidative stress and is ameliorated by KAFE administration. KAFE has also been seen not to be deleterious on testicular epithelium on long term use.

REFERENCES

Abioye AIR, Duru FIO, Noronha CC, Okanlawon AO (2003). Aqueous extract of the bark of *Kigelia africana* reverses early testicular damage induced by methanol extract of Carica papaya. Nig. J. Health Biomed. Sci., 2: 87-89.

Atessahin A, Karahan I, Turk G, Gur S, Yilmaz S, Ceribasi AO (2006a). Protective role of lycopene on cisplatin-induced changes in sperm characteriastics, testicular damage and oxidative stress in rats. Reprod. Toxicol., 21: 42-47.

Aydiner A, Aytekin Y, Topuz E (1997). Effects of cisplatin on testicular tissue and Leydig cell-pituitary axis. Oncol., 54: 74-78.

Azu OO, Duru FIO, Osinubi AA, Norohna CC, Elesha SO, Okanlawon AO (2009). Preliminary study on the androgenic effects of *Kigelia africana* fruit extract in male Sprague-Dawley rats. Mid. East Fert. Soc. J., (MEFSJ) 14(3): 1-7.

Azu OO, Duru FIO, Osinubi AA, Noronha CC, Elesha SO, Okanlawon AO (2010a). Preliminary studies on the antioxidant effect of *Kigelia africana* fruit extract (Bignoniaceae) in male Sprague-Dawley rats. Afr. J. Biotechnol., 9(9): 1374-1381.

Azu OO, Duru FIO, Osinubi AA, Noronha CC, Elesha SO, Okanlawon AO (2010b). Protective agent, *Kigelia africana*, against cisplatin-induced renal oxidative injury in Sprague-Dawley rats. Asian J. Pharm.l Clin. Res., 3: 84-88.

Azu OO, Duru FIO, Osinubi AA, Oremosu AA, Noronha CC, Elesha SO, Okanlawon AO (2010c). Histomorphometric effects of *Kigelia africana* (Bignoniaceae) fruit extract following short-term treatment with cisplatin in male Sprague-Dawley rats. Mid. East Fert. Soc. J., (MEFSJ) doi:10.1016/j.mefs.2010.07.001.

Buege JA, Aust SD (1978). Microsomal lipid peroxidation. Methods Enzymol., 52: 302-10.

Burkill HM (1985). The useful plants of west tropical Africa. Planta WT Afr., 1: 254-257.

Cardinal R, De Groot J, Huizing E, Veldman J, Smoorenburg G (2000). Cisplatin-induced ototoxicty: morphological evidence of spontaneous outer hair cell recovery in albino guinea pigs? Hear Res., 144: 147-56.

Duru FIO, Olabiyi O, Noronha CC, Akinwande AI, Okanlawon AO (2008). Brief ischaemia reduces testicular lipid peroxidation following subsequent ischaemia: an evidence for ischaemic preconditioning. Nig. Q. J. Hosp. Med., 18: 149-152.

Gilliland KO, Freel CD, Lane CW, Fowler WC, Costello MJ (2001). Multilamellar bodies as potential scattering particles in human age-related nuclear cataracts. Molecular Vision., 7: 120-130.

Graham JG, Quinn ML, Fabricant DS, Farnsworth NR (2000). Plants used against cancer-an extension of the work of Jonathan Hartwell. J. Ethnopharmacol., 73: 347-377.

Gundersen HJG (1977). Notes on the edge of the numerical density of arbitrary profiles: the edge effect. J. Microsc., 111: 219-223.

Gunderson HJG, Jenson EB (1987). The efficiency of systematic sampling in stereology and its prediction. J. Microsc., 147: 229-263.

Inuwa IM (2005). First order stereology in diabetes and endocrine research-number and volume estimation. Int. J. Diabetes Metabol., 13: 10-17.

Jeong UJ, Shin YG, Kim IH, Pezzuto JM (1999). Inhibition of aromatase activity by flavonoids. Arch. Pharm. Res., 22: 309-312.

Kawakami E, Takemura A, Sakuma M, takano M, Hirano T, Hori T, Tsutsui T (2007). Superoxide dismutase and catalase activities in the seminal plasma of normospermic and asthenozoospermic beagles. J. Vet. Med. Sci., 69: 133-136.

Kintzios SE (2006). Terrestrial plant-derived anticancer agents and plant species used in anticancer research. Crit. Rev. Plant Sci., 25: 79-113.

Kolodziej H (1997). Protective role of *Kigelia africana* fruits against benzo(a)pyrene-induced fore-stomach tumourigenesis in mice and against albumen-induced inflammation in rats. Pharm. Pharmacol. Lett., 7: 67 – 70.

Leblanc GA, Waxman DJ (1988). Feminisation of rat hepatic P-450 expression by cisplatin: Evidence for perturbations in the hormonal regulation of steroid-metabolising enzymes. J. Biol. Chem., 263: 15732-15739.

LeBlanc GA, Kantoff PW, Sze-Fong N, Frei E, Waxman DJ (1990). Hormonal perturbations in patients with testicular cancer treated with cisplatin. Cancer Cytopathol., 69: 2306-2310.

Marcon L, Hale BF, Robaire B (2008). Reversibility of subchronic exposure to cancer chemotherapeutics, bleomycin, etoposide and cisplatin (BEP) on spermatogenesis, fertility and progeny outcome in the male rat. J. Androl., Epub ahead of print.

Montine TJ, Borch RF (1990). Role of endogenous sulfur containing nucleophiles in an in vitro model of cis-diamminedichloroplatinium-induced nephrotoxicity. Biochem. Pharmacol., 39: 1751–7.

Morakinyo AO, Adeniyi OS, Arikawe AP (2008). Effects of *Zingiber*

officinale on reproductive functions in male rat. Afr. J. Biomed. Res., 11: 329-333.
Nakai M, Hess RA, Netsu J, Nasu T (1995). Deformation of the rat Sertoli cell by oral administration of carbendazim (methyl 2-benzimidazole carbamate). J. Androl., 16: 410-6.
Narayana K, Prashanthi N, Nayanatara A, Biary LK, D'Souza UJ (2006). An organophosphate insecticide methyl parathion (O, O-dimethyl O-4-nitrophenyl phosphorothioate) induces cytotoxic
damage and tubular atrophy in the testis despite elevated testosterone level in the rat. J. Toxicol. Sci., 31: 177-89.
Ogbeche KA, Ogunbiyi YO, Duru FIO (2002). Effect of methanol extract of *Kigelia africana* on sperm motility and fertility in rats. Nig. J. Health Biomed. Sci., 2: 113-116.
Okobia MN (2003). Cancer care in sub-saharan Africa-urgent need for population-based Cancer Registries. Ethiop. J. Health Dev., 17: 89-98.
Oliver-Bever B (1986). Medicinal plants in Tropical West Africa, Cambridge University Presss: Cambridge, London, New York, New Rochelle, Melbourne., p. 240.
Owolabi OJ, Omogbai EKI (2007). Analgesic and anti-inflammatory activities of the ethanolic stem bark extract of *Kigelia africana* (Bignoniaceae). Afr. J. Biotechnol., 6: 582-585.
Picerno P, Autore G, Marzocco S, Meloni M, Sanogo R, Aquino RP (2005). Anti-inflammatory activity of verminoside from *Kigelia africana* and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. J. Nat. Prod., 68: 1610-1614.
Qin D, Lung MA (2002). Morphometric study on Leydig cells in capsuloptomised testis. Asian J. Androl., 4: 49-53.
Richburg JH, Boekelheide K (1996). Mono-(2-ethylhexyl) phthalate rapidly alters both Sertoli cell vimentin filaments and germ cells apoptosis in young rat testes. Toxicol. Appl Pharmacol., 137: 42-50.
Rudin CM, Yang Z, Schumaker LM, VanderWeele DJ, Newkirk K, Egorin MJ (2003). Inhibition of glutathione synthesis reverses Bcl-2-mediated cisplatin resistance. Cancer Res., 63: 312-318.
Rukkumani R, Aruna K, Varma PS, Rajasekaran KN, Menon VP (2004). Comparative effects of curcumin and an analogue of curcumin on alcohol and PUFA induced oxidative stress. J. Pharm. Pharm. Sci., 7: 274-283.
Rybak L, Hussain K, Morris C, Whitworth C, Somani S (2000). Effect of protective agents against cisplatin oto-toxicity. Am. J. Otol., 21: 513-520.
Sawhney P, Giammona CJ, Meiatrich ML, Richburg JH (2005). Cisplatin-induced long-term failure of spermatogenesis in adult C57/B1/6J mice. J. Androl. 26: 136-145.
Schrader M, Muller M, Straub B, Miller K (2002). Testicular sperm extraction in azoospermic patients with gonadal germ cell tumours prior to chemotherapy-a new therapy option. Asian J. Androl., 4: 9-15.
Seaman F, Sawhney E, Giammona CJ, Richburg JH (2003). Cisplatin-induced pulse of germ cell apoptosis precedes long-term elevated apoptotic rates in C57/BL/6 mouse testis. Apoptosis., 8: 101-108.
Spermon JR, Ramos L, Wetzels AMM, Sweep CGJ, Braat DDM (2006). Sperm integrity pre-and post-chemotherapy in men with testicular germ cell cancer. Hum. Reprod., 21: 1781-1786.
Suzuki N , Sofikitis N (1999). Protective effects of antioxidants on testicular functions of varicocelized rats. Yonago Acta Medica., 42: 87-94.
Venkatesh V, Sharma JD, Kamal R (2002). A comparative study of effect of alcoholic extracts of *Sapindus emarginatus*, *Terminalia belerica*, *Cuminum cyminum* and *Allium cepa* on reproductive organs of male albino rats. Asian J. Exp. Sci., 1 and 2: 51-63.
Weiss RB, Christian MC (1993). New cisplatin analogues in development. A review. Drugs, 46: 360-377.
Yao X, Panichpisal K, Kurtzman N and Nugent K (2007). Cisplatin nephrotoxicity: A review. Am. J. Med Sci., 334: 115-124.
Zalata AA, Christophe AB, Depuydt CE, Schoonjans F, Comhaire FH (1998). White blood cells cause oxidative damage to the fatty acid composition of phospholipids of human spermatozoa. Int. J. Androl., 21: 154-162.