FUDMA Journal of Sciences (FJS)
ISSN Online: 2616-1370
ISSN Print: 2645-2944
Vol. 2 No. 3, September, 2018, pp 18 - 23

AQUEOUS WOOD-ASH EXTRACT OF *PARKIA BIGLOBOSA* IMPAIRED SPERMATOGENESIS IN MALE ALBINO MICE

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ABSTRACT

Wood-ash extracts are used as food additives and medicinals by different ethnic groups in Nigeria. Despite these uses, there is paucity of information on the possible adverse effects on the reproductive health in mammals. This study assessed the toxicity of aqueous wood-ash extract of *Parkia biglobosa* on reproductive organ in male albino mice. Four different dose levels of wood-ash extract of *Parkia biglobosa* [0 (control), 5, 50 and 100 mg/kg body weight] were orally administered to 20 mice (5 per group) for seven (7) consecutive days. After 35 days from first day of extract administration, blood samples were collected by retro-orbital phlebotomy and assayed for serum follicle stimulating hormone (FSH), Luteinizing hormone (LH) and testosterone. Mice were sacrificed subsequently by cervical dislocation and testes harvested to examine gonadosomatic index, sperm motility, sperm count, sperm morphology and testicular histopathology using standard methods. ANOVA was used to determine the significant difference among treatments, at $P<0.05$ level of significance. No significant effect was recorded in testicular weight, FSH, LH and testosterone levels; significant decrease in sperm motility, live/dead sperm and sperm count and increase of abnormal sperm cells were recorded. Frequencies of abnormal sperm cells were significantly higher at 100 mg/kg of the extracts than in control. Dose-dependent depletion of spermatogenic cells were recorded in the testes of exposed mice. Aqueous wood ash extract of *Parkia biglobosa* could have damaging effects on sperms and testicular tissues, which can impair reproduction.

Keywords: Wood-ash extract, Reproductive Toxicity, Sperm Quality and Quantity, Seminiferous tubules, Reproductive Hormones

INTRODUCTION

Infertility is one of the common problems of mammals with significant medical, psychological and economic implications (Isidori *et al.*, 2006). Various factors have been indicted in male and female infertility; these include hereditary, environmental, biological, physical and behavioural factors. Among these factors, environmental factors (volatile organic solvents or silicones), biological agents (such as viruses and parasites), physical agents (radiation, exposure to electric shock, excessive heat), chemical dust, pesticides, plant extracts, alcohol, smoking, caffeine consumption, insufficient vitamins intake etc. have been reported to play significant roles in the fertility of dioecious organisms (Mosher and Pratt, 1991; Isidori *et al.*, 2006; Auta and Hassan, 2016).

In recent years, there has been growing concern about the deleterious effects of chemical on developing male reproductive system (Sharma and Garu, 2011). Weight losses of the gonads as well as reduced sperm count and epididymal sperm motility are considered standard criteria for the characterization of toxic agents that may cause fertility problems in males (Ban *et al.*, 1995; Queiroz-Neto, *et al.*, 1997).

Sperms morphology serves as an important and sensitive indicator of chemical toxicity on the reproductive cells. They can be used to evaluate the spermatogenic damage, fertility and heritable genetic changes, which provide a direct measure of the quality of sperm production in chemically treated animals (Bakare *et al.*, 2009; Gautam *et al.*, 2010; Devi *et al.*, 2011).

In Nigeria, the use of aqueous wood-ash extracts as food additives and medicinal by different ethnic groups is raising concern about its deleterious reproductive effects (Auta *et al.*, 2015). At the moment, there is paucity of information on the possible adverse reproductive health effects of wood-ash extracts of *Parkia biglobosa* in mammals which is imperative for safety evaluation. In 2016, Auta and Hassan reported reproductive toxicity of aqueous wood-ash extract of *Azadirachta indica* (neem) on male albino mice. There is scarcity of information on reproductive toxicity of aqueous wood-ash extract of *Parkia biglobosa.* Hence, this study assessed the impairment of spermatogenesis by aqueous wood-ash extract of *Parkia biglobosa* in male albino mice.

MATERIALS AND METHODS

Sample collection

Fresh *Parkia biglobosa* (locust bean) wood was collected from Katsina State, North-Western Nigeria. Stalk of the plant carrying leaves and flowers were collected, and taken to the herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria – Nigeria for authentication and voucher numbers of 90051 was obtained. The woods were processed into ashes by open air burning and extracted using percolation method described by Auta and Hassan (2015).

Toxicity Study

The reproductive toxicity study in male was carried out using methods described by WHO (1999), Dong *et al.* (2008), Bakare *et al.* (2009) and Alabi and Bakare (2014).

A total of 20 apparently healthy adult male mice were grouped in five (5). Group 1, which served as control was orally, administered distilled water while groups 2, 3 & 4 received 5, 50 and 100 mg/kg bw aqueous wood-ash extract of *P. biglobossa* for five (5) consecutive days. They were kept in plastic cages with five (5) mice per cage in an environment of approximately 12 hour light/dark cycle, a temperature of 24°C (±3°C). The mice were supplied with a standard diet and water *ad-libitum*.

At 5weeks (35days) from the first administration, the mice were sacrificed by cervical dislocation and their caudal epididymis surgically removed, sperm smears were prepared from the epididymis. Gonadosomatic index, sperm motility and sperm count (WHO, 1999), Sperm morphology (Talebi, *et al.,* 2013) FSH, LH and testosterone hormones were measured using microplate immunoenzymometric assay method (ELISA). Histopathology of testes was also carried out using H&E.

Blood Collection and Hormonal Assays

Immediately after sacrificing, the bloods were collected into plain 1.5 ml eppendough tubes. The blood were left to coagulate and then centrifuged at 3000 rpm for 30 minutes to separate the serum. The separated serums were stored at -20°C for subsequent hormonal analyses. The circulating levels of testosterone, FSH and LH were determined using radioimmunoassay kits. TAC was also determined. The body weight of each mouse was determined before sacrificing. After sacrifice and dissection, the testes were removed and weighed to determine the gonadosomatic index:

Semen Analysis

Assessment of sperm motility was carried out according to Robb *et al.* (1978) and WHO (1999). In brief, 10 µL of the sperm suspension was placed on a microscopic slide and covered with slip. Slides were examined under the microscope. A minimum of five microscopic fields were assessed to evaluate sperm motility of at least 200 sperm for each mouse (Robb *et al.*, 1978).

The dissected epididymis of each animal was transferred into 10 mL Ham's F10 medium and cut to small slices, in order for the sperm to swim out into the medium. After 10 min of diffusion, 1 mL of the solution was diluted with 9 mL formaldehyde fixative. The diluted solution was transferred into each chamber of Neubauer haemocytometer and sperm heads was manually counted under a microscope. Sperm count was performed according to methods described by Robb *et al.,* (1978) and WHO (1999) and data were expressed as the number of sperm per mL.

The left epididymis was obtained after sacrifice and dissection of the mice. The epididymis was carefully cut off from the testis, making sure the content was not spilled. It was placed into the prepared eosin stain in a watch glass. The epididymis was teased properly to ensure uniform distribution of the sperms in the stain (Robb *et al.*, 1978).

After teasing, the content was left within the watch glass and covered for 45 mins. This content was later mixed. Using a micropipette, 3 droplets were dropped on a slide, smeared and air dried. Sperm smears were examined by light microscopy at x 40 and 100 respectively. Heads without tails or heads that are in contact or overlaid by other sperm(s) or debris were excluded (Talebi *et al.,* 2012).

Histological Examinations of Testes

The weighed gonads were placed in Bouin's fluid for 72 hrs to enable tissue hardening (Culling, 1974). They were then transferred to 10% phosphate buffered formalin for preservation; dehydrated in a series of ethanol dilutions and embedded in paraffin wax (Barnhoorn *et al*., 2010). Sections of 5µm were cut and stained with Haemoxylin and Eosin (H&E) and observed under the microscope for routine histological examination.

STATISTICAL ANALYSIS

The values are expressed as mean ± Standard error (SE). An analysis of variance (one way ANOVA) was used to determine the significance between different doses of exposure and was followed by Duncan Multiple Range (DMR) test. The significant difference between the groups will be considered significant at $P<0.05$ level.

RESULTS AND DISCUSSION

In the male reproductive system, smaller testes, lower sperm count and poor epididymal sperm motility are considered standard criteria for the characterization of toxic agents that may cause fertility problems in the experimental subject (Ban *et al.,* 1995; Queiroz-Neto *et al.,* 1997).

The assessment of the weight of the reproductive organs is one of the most sensitive parameters for detection of a substance's influence on the male reproductive system (Mangelsdorf *et al.,* 2003). Among the mice exposed to different doses of aqueous wood ash extract of *P. biglobosa*, significant difference was recorded in gonadosomatic index, LH, FSH and testosterone concentrations in blood serum (Table 1). Alterations in testicular weight can indicate modifications in the seminiferous tubules or interstitial oedema and consequently in sperm production (Sellers *et al.,* 2007). Administration of the extracts could have produced pituitary hypothalamic or sex hormonal effects, as seen in the increased levels of LH and FSH which in turn affected spermatogenesis or exposure, thereby resulting to

abnormalities in seminal fluid leading to functional or structural impairment of sperm similar to what was reported by Odeigah (1997) . Alternations in the male in serum FSH levels could be on the basis of the free radical theory and also could be as result of excessive production of reactive oxygen species (ROS) as reported in work done by Jervis and Robaire (2004).

The results from mice exposed to different dose concentrations of aqueous wood ash extract of *P. biglobosa* also showed significant decrease in percentage sperm motility, live/dead and sperm count (Table 2). The decrease in sperm viability (live/dead) was consistent with reduction in the progressive sperm motility, which may be due to the effect of these extracts on the epididymis by acting as a spermatotoxic agent on maturing or matured spermatozoa (Pacifici *et al.*, 1995).

There is a significant increase in curved tail, curved midpiece, bent midpiece, looped tail and bent tail of sperm cells, with increase in the dose concentration of *Parkia biglobosa* wood-ash extract. Percentage of abnormal cells increased significantly with the dose concentration (Table 3). Abnormal sperm morphology recorded in this study serves as an important and sensitive indicator of aqueous wood-ash extract of *P. biglobosa* toxicity on reproductive cells. They reflect spermatogenic damage, fertility and heritable genetic changes, which provide a direct measure of the quality of sperm production in treated animals (Bakare *et al.*, 2009; Gautam *et al.*, 2010; Devi *et al.*, 2013). This was also established by Wyrobek *et al.* (1983), who reported that several kinds of mutation can lead to abnormal sperm morphology.

Histopathology revealed dose-dependent depletion of spermatogenic cells in the testes of exposed mice, with 100 mg/kg dose group showing very thick (thickened) tunica albuginea, variably sized seminiferous tubules (STs), irregular outlines and severely depleted amounts of spermatogenic cells, indicating testicular atrophy (Figure 1). Histopathological changes in the testis, such as observed in the experiment affect sperm parameters; this was established by Yang *et al.* (2005). Mutation in germ cells prior to or during the reproductive period can be transmitted to later generations resulting in reproductive defects (Taylor, 1980). In general, damage to the sperm cell is said to occur either by physiological, cytotoxic or genetic mechanism (Otitoloju *et al.*, 2010).

Table 1: Gonadosomatic Index and Hormonal Assay of Mice exposed to aqueous wood ash extract of *Parkia biglobosa* at different concentrations

Groups/Dose concentrations	Testicular weight % (g/g BW x 100)	LH (IU/L)	FSH (IU/L)	Testosterone (IU/L)
Control	0.64 ± 0.02^{a}	8.75 ± 0.48^{a}	6.75 ± 0.48^{a}	0.7 ± 0.07^{a}
5 mg/kg	0.74 ± 0.04^{b}	11.25 ± 0.48^{b}	9.25 ± 0.25^{b}	1.0 ± 0.07^{b}
50 mg/kg	0.62 ± 0.03^{ab}	11.75 ± 0.48^{b}	9.5 ± 0.29^{b}	0.9 ± 0.04^{ab}
100 mg/kg	0.69 ± 0.04^{ab}	12.75 ± 1.03^{b}	10.5 ± 1.04^{b}	1.08 ± 0.08^{b}

Values are expressed as Means ± SEM (n = 4 per group). Means in same columns with different superscript letters are significantly different; $p<0.05$.

Table 2: Sperm count and motility assay of mice exposed to aqueous wood ash extract of *Parkia biglobosa*

Groups/Dose concentration	Motility (%)	Live/Dead (%)	Volume(µl)	Count (x106/ml)
Control	91.5 ± 1.19^{a}	98.0 ± 0.0^{a}	5.1 ± 0	137.75 ± 1.84^{a}
5 mg/kg	73.25 ± 2.36^{b}	97.0 ± 0.70^{ab}	5.1 ± 0	116 ± 2.94^{b}
50 mg/kg	66.75 ± 2.36^{b}	96.0 ± 0.71^{ab}	5.1 ± 0	106 ± 3.49^{bc}
100 mg/kg	66.75 ± 2.36^{b}	94.25 ± 1.65^{b}	5.1 ± 0	102.25 ± 5.11^{c}

Values are expressed as Means ± SEM (n = 4 per group). Means in same columns with different superscript letters are significantly different; $p<0.05$.

Table 3: Sperm morphology of mice exposed to aqueous wood ash extract of *P. biglobosa at different concentrations*

Parameters	Experimental Groups			
	0 (Control)	5 mg/kg	50 mg/kg	100 mg/kg
Tailless head	4.25 ± 0.63	4.75 ± 0.48	4.25 ± 0.63	5.0 ± 0.41
Headless tail	4.25 ± 0.25	4.25 ± 0.48	4.75 ± 0.63	4.5 ± 0.65
Rudimentary tail	1.75 ± 0.48	2.75 ± 0.25	2.0 ± 0.41	2.5 ± 0.29
Curved tail	7.0 ± 0.41 ^{a}	8.75 ± 0.25 ^{bc}	8.0 ± 0.41 ^{ab}	9.25 ± 0.25 ^{c}
Curved midpiece	8.0 ± 0.41 ^{a}	8.75 ± 0.63 ^{ab}	9.75 ± 0.63 ^{b}	10.0 ± 0.41 ^{b}
Bent midpiece	7.75 ± 0.63 ^{a}	9.0 ± 0 ^{b}	9.25 ± 0.25 ^{b}	10.0 ± 0.41 ^{b}
Looped-tail	1.25 ± 0.25 ^{a}	2.0 ± 0.41 ^{ab}	2.25 ± 0.25 ^{b}	1.75 ± 0.25 ^{ab}
Bent tail	7.25 ± 0.25 ^{a}	9.0 ± 0.41 ^{b}	9.25 ± 0.25 ^{b}	10.0 ± 1.41 ^{b}
Total no of ABS	41.5 ± 0.5	49.25 ± 2.17	49.5 ± 1.04	53.0 ± 1.08
Total no of NMS	367.25 ± 3.61	355.75± 4.15	350.5 ± 1.04	348.25 ± 1.65
Total NS	408.75 ± 3.15	405.0 ± 2.04	400.0 ± 0	401.25 ± 1.25
% of Abnormal cells	10.16 ± 0.19 ^{a}	12.17 ± 0.59 ^{b}	12.38 ± 0.26 ^{b}	13.21 ± 0.27 ^{b}

Values are expressed as Means ± SEM (n = 4 per group). Means in same column with different superscript letters are significantly different; $p<0.05$.

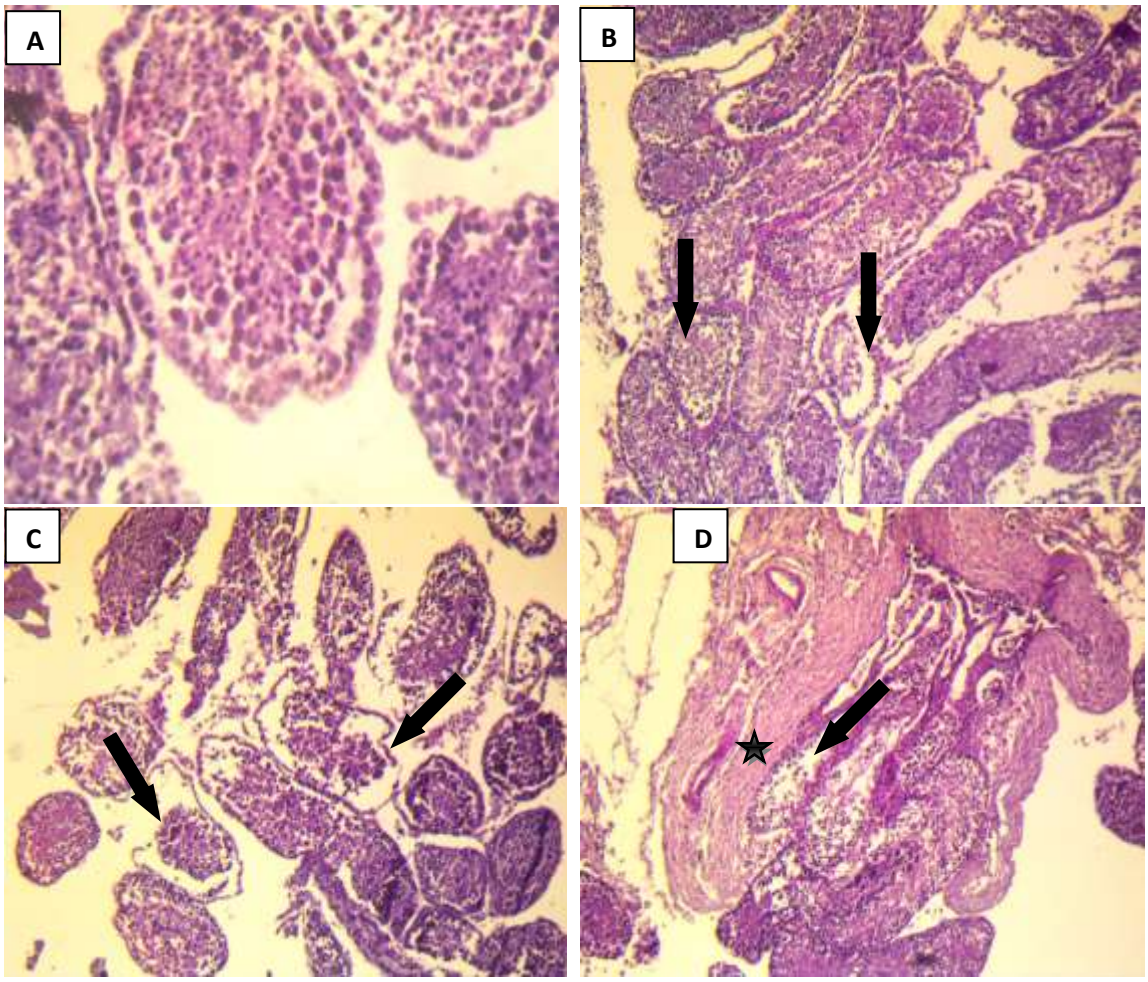

Figure 1: Photomicrograph of mice testes exposed to aqueous wood ash extract of *P. biglobosa*. Control (A) with numerous regular variably sized ST packed full with normal spermatogenic cells. B (5 mg/kg) shows closely packed, numerous and large STs with moderate amounts of spermatogenic cells. There are also a few STs (arrows) which are depleted of spermatogenic cells. C (50 mg/kg) showing variably-sized STs (arrows) with irregular outline and contain moderately depleted amounts of spermatogenic cells. The usual gradation from basal to apical/lamina compartment is generally absent. D (100 mg/kg) shows tunica albuginea (star) is thickened, variably-sized STs with irregular outlines and severely depleted amounts of spermatogenic cells (arrows) suggestive of testicular atrophy. H&E. 100X (B-D) 400X (A). *ST:* seminiferous tubules

CONCLUSION

Aqueous wood-ash extract of *Parkia biglobosa* caused decrease in sperm motility, live/dead sperms and an increase in the number of abnormal sperm cells, which is an indication of infertility. Histopathological changes in the testis such as vacuolation and swelling of the round spermatids, necrosis of the late elongated spermatids, numerous apoptotic cells and formation of multinucleated giant cells in the seminiferous tubules imply that the extracts have reprotoxic effects on spermatogenesis and mice testes.

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CONFLICT OF INTEREST

Authors declare there is no known conflict of interest in regards to the article. The research finding was presented in 2016 at the 2nd World Bio Summit and Expo, Dubai, UAE and power point presented during the conference is available online.

ACKNOWLEDGEMENT

The authors wish to acknowledge financial support received from Federal University Dutsin-Ma, Katsina State – Nigeria. Also, The Head, Department of Zoology is well appreciated for providing the laboratory for this research.

REFERENCES

Alabi, O. A. and Bakare, A. A. (2014). Cytogenotoxic Effects and Reproductive Abnormalities Induced by e-Waste Contaminated Underground Water in Mice. *Cytologia* 79(3):331-340.

Auta, T., Hassan A. T. (2016). Reproductive toxicity of aqueous wood-ash extract of *Azadirachta indica* (neem) on male albino mice. Asian Pacific Journal of Reproduction. 5(2): 111–115.

Auta, T., Otalu E. J., Hassan, A. T. (2015). Evaluation of chemical constituents in aqueous wood ash extracts of *Azadirachta indica* (neem) and *Parkia biglobosa* (locust bean). J. Environ. Tox. and Pub. Health 1(1): 36-40.

Bakare, A. A., Okunola, A. A., Adetunji, A. O. and Jenmi, B. H. (2009). Genotoxicity assessment of a pharmaceutical effluent using four bioassays. Genetic and Molecular Biology 32(2):373-381.

Ban, Y., Komatu, K. M., Inagaki, S. and Nakatsuka, M. H. (1995). Testicular spermatid and epididymalvsperm head count as an indicator for reproductive toxicity in rats. Experimental Animals 44:315-22.

Devi, S. A., Umasankar, M. E. and Babu, S. (2011). A Comparative Study of Antioxidant Properties in Common Indian Spices. International Research Journal of Pharmaceuticals 3(5): 465 – 468.

Dong, L., Zhang, H., Duan, L., Cheng, X. and Cui, L. (2008). Genotoxicity of testicle cell of mice induced by microcystin-LR. Life Science Journal 5(1): 43-45.

Gautam, D., Sharma, G. and Goyal, R. P. (2010). Evaluation of toxic impact of Tartrazine on male swiss albino mice. Pharmacologyonline 1:133-140.

Isidori, A. M., Pozza, C. D. and Gianfrilli, I. A. (2006). Medical treatment to improve sperm quality. Journal of Reproduction Biomedicine 12:704-714.

Jervis, K. M. and Robaire, B. (2004). The effects of long-term vitamin E treatment on gene expression and oxidative stress damage in the aging Brown Norway rat epididymis. Biology of Reproduction71:1088-1095.

Mangelsdorf, I., Buschmann, J. and Orthen, B. (2003). Some aspects relating to the evaluation of the effects of chemicals on male fertility. Regulatory Toxicology and Pharmacology 37: 356-369.

Mosher, W. D. and Pratt, W. F. (1991). Fecundity and infertility in the United States: incidence and trends. Journal of Fertility and Sterility 56: 192-193.

Odeigah, P.G.C. (1997). Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutation Research 389: 141-148.

Otitoloju, A. A., Obe, I. A., Adewale, O. A., Otubanjo, O. A. and Osunkalu, V. O. (2010). Preliminary Study on the Induction of Sperm Head Abnormalities in Mice, Mus musculus, Exposed to Radiofrequency Radiations from Global System for Mobile Communication Base Stations. Bulletin of Environmental Contamination and Toxicology 84:51–54.

Pacifici, R., Altieri, I., Gandini, L., Lenzi, A., Passa, A.R. and Pichini, S. (1995). Environmental tobacco smoke: nicotine and cotinine concentration in semen. Environ. Res. 68.1:69-72

Queiroz-Neto, A., Mataqueriro, M. I., Santana, A. E. and Alessi, A. C. (1997). Toxic effects of *Annona squamosa* seed extract in rats and swine. Revista Brasileira de Toxicologia 10:11-15.

Sellers, R. S., Morton, D., Michael, B., Roore, N., Johnson, J. K., Yano, B. L., Perry, R. and Schaffer, K. (2007). Society of Toxicology Pathology Position paper: Organ Weight Recommendation for Toxicology Studies. Pathology 35: 751-755.

Sharma, R. and Garu, U. (2011). Effects of Lead Toxicity on Developing Testes in Swiss Mice. Universal Journal of Environmental Research and Technology 1(4): 390-398.

Talebi, A. R., Khorsandi, L. and Moridian, M. (2013). The effect of zinc oxide nanoparticles on mouse spermatogenesis. Journal of Assisted Reproduction and Genetics 30(9):1203-1209.

Taylor, R. J. (1980). Food Additives. John Wiley and Sons, New York. In: Aduloju R. K., Otubanju, O. A. and Odeigah, P. G. C. (2008). An *In Vivo* Assay of the Mutagenic Potential of Praziquantel (PZQ) using sperm head abnormality test. J. Hum. Ecol., 23.1:59-63.

World Health Organization (1999). WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd edition. New York , Cambridge University Press.

Wyrobek, A. J., Gordon, L. A., Burkhart, J. G., Francis, M. W., Kapp, R. W.Jr., Litz, G., Malling, H. V., Topham, J. C. and Whorton, M. D. (1983). An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic. Mut. Res. 115: 73-148.

Yang, H. J., Lee, S. H., Jin, Y., Choi, J. H., Han, C. H. and Lee, M. H. (2005). Genotoxicity and toxicological effects of acrylamide on reproductive system in male rats. Journal of Veterinary Science 6:103–9.