www.ajbrui.net

Afr. J. Biomed. Res. 13 (January 2010) 39 - 45

Full length Research article

Effect of *Zingiber Officinale* (Ginger) on Sodium Arsenite-Induced Reproductive Toxicity in Male Rats

Morakinyo A.O, Achema P.U And Adegoke O. A

Department of Physiology, College of Medicine. University of Lagos, Lagos, Nigeria.

ABSTRACT: Arsenite is a major environmental chemical and a known reproductive toxicant via the depression of spermatogenesis and androgenesis in males. The possibility of sodium arsenite reproductive toxicity been caused by auto-oxidation was investigated in this study taking advantage of the anti-oxidant properties of ginger and its androgenic activities. The effect of exposure to sodium arsenite (10 mg/kg BW/day) by gavage via oral cannula without or with aqueous ginger extract (500mg/kg BW/day) co-treatments for 30 days was evaluated in adult male rats. The weight of the reproductive organs, sperm count, motility, and morphology were evaluated. Plasma FSH, LH and testosterone levels were assayed. Lipid peroxidation (indexed by MDA) and antioxidants enzymes likes GSH, SOD, CAT were assessed. Sodium arsenite treatment decreased the reproductive organs weight: testis, epididymis, prostate and seminal vesicle; sperm functions: count, motility and normal morphology; plasma hormones level: FSH, LH and testosterone. There was a decrease in the activities of GSH, SOD and CAT as well as an increase in MDA concentration. Co-administration of aqueous ginger extract with arsenite was found to protect against adverse change in the reproductive organ weight, attenuate the decrease in sperm functions, enhance plasma reproductive hormones level along with increased antioxidants activities and reduced peroxidation. This study showed that sodium arsenite apart from being a hormonal disrupter also causes oxidative stress which contributed to the reproductive damage in the male rats. The protective effects of ginger on reproductive toxicity and oxidative stress as evidenced by the clear restoration of sperm functions, testicular steroidogenesis and reproductive organo-somatic indices could be attributed to its antioxidants and androgenic properties.

Key Words: Arsenite, Ginger, Antioxidants and Sperm.

INTRODUCTION

Environmental toxins and radiation are suspected to be responsible in part for the deterioration of semen quality observed worldwide during the recent few decades (Sarkar et al, 2000). Exposure to arsenicals, which is used as herbicides, fungicides and rodenticides may cause soil, air and water pollution (Nickson *et al*, 1993) and might be a factor considering the hormonal disruption that occurs with its use (Sarkar et al, 2003). Arsenical exposure through drinking water is common in many areas of the world (Chartterjee et al, 1993). Its usage in insecticides, acarides and arsenical soap manufacture constitute a major risk factor when one is over exposed to these products. Metabolic disorders, hypertrophy of adrenal glands (Biswas et al, 1994) and anemia (Sarkar et al, 1992), inhibition of the activity of testicular steroidogenic enzymes (Sarkar et al, 2003) and reduction in the weight of the testis and accessory sex organs (Sarkar et al, 1991) are associated with exposure to arsenicals.

Ginger (*Zingiber officinale*) is commonly used as food spice in many Asian and African countries, including Nigeria. It contains a host of compounds which includes acid resins, vitamin C compounds [folic acid, inositol, choline and panthotenic acid] (Arfeen, 2000), gingerol, sesquiterpene, vitamin B3 and B6,

Manuscript received: August 2009; Accepted: December 2009
Author for correspondance :
Department of Physiology, College of Medicine of the University of Lagos, Surulere 23401, Lagos, Nigeria.
Tel. +2348055947623; E-mail: morakinyofemi@yahoo.com

volatile oils and bio-trace elements [Ca, Mg, P and K] (Ernst and Pittler, 2000). The medicinal values of ginger have been intensively reported. Its antioxidant effect on reproductive toxicity induced by Cisplatin in rats was observed as it normalized antioxidant enzyme activity in the testis and associated lipid peroxidative indices (Ahmed et al., 2002). Ginger has been shown to lower lipid peroxidation and increase glutathione content in blood of rats (Amin and Hamza, 2000). Kamtchouing *et. al.* (2002) also reported that ginger extract have androgenic activity in male rats.

Though a lot of studies have been carried out on sodium arsenite effects on male reproductive system, the mechanism underlying these effects have not been extensively studied. The possibilities that it might be due to oxidative stress cannot be ruled out since arsenite is a thio-reactive oxidative stressor (Roybal et al, 2005). The possibility of sodium arsenite reproductive toxicity been caused by auto-oxidation was investigated in this study taking advantage of the anti-oxidant properties of ginger (Ahmed et al, 2000; Sekiwa et al, 2000) and its androgenic activities (Kamtchouing et al, 2002).

MATERIALS AND METHODS

Animals

Twenty four male albino rats (140-170g) of the Sprague-Dawley strain were obtained from the animal house of the College of Medicine University of Lagos. The rats were kept in cages in the quarantine section of the Laboratory Animal Centre, University of Lagos. The rats were allowed to acclimatize for a period of three weeks before the commencement of the experiment. They had access to rats chow and water ad libitum.

Chemical and Drug

Sodium arsenite (Sigma Chemical Co) used was obtained from the Department of Pharmaceutical Chemistry of the University of Lagos while the dried ginger rhizomes were purchased from a local herbal store.

Drug and Extract Preparation

1800mg of Sodium arsenite was dissolved in 300mls of distilled water obtaining a stock concentration of 6mg/ml. Dried ginger rhizomes were pulverised, and soaked in distilled water for 48 hours. A final aqueous ginger extract (AGE) concentration of 100mg/ml was obtained following this maceration method.

Treatment Regime

The rats were randomly divided into four groups comprising 6 rats each and were subjected to the following treatments. Group-1 (control rats) received distilled water as a vehicle. Group-2 (arsenic-treated rats) was given sodium arsenite at the dose of 10 mg per kg body weight per day. Group-3 (AGE-supplemented, arsenic-treated rats) received in addition to exposure of arsenic, dose of AGE at 500mg/kg body weight/day. Group-4 (AGE-treated rats) received 500mg/kg body weight/day AGE. The drug and extract administration were given by gavage via oral cannula and lasted for a period of 30 days.

Sperm Function Analysis

After exposing the reproductive tract, the caudal epididymis was carefully isolated and minced with scissors in 1ml of physiological saline to release the sperm. Each chamber of the haemocytometer was loaded with 10ul of diluted sperm and allowed to stand or settle for 5 minutes. Counting was done under a light microscope at 400X magnification. Sperm morphology was determined using the Eosin and Nigrosin stain. Briefly, 10ul of eosin and nigrosin was mixed with 40ul of sperm suspension. The sperm suspension was incubated at 40 ^{0}C for 5 min and then re-suspended with a micro-pipette. About 100 sperm cells per rats were morphologically examined under the microscope at 400X magnification. Morphological abnormalities were classified as headless sperm, banana head, bent neck and bent tail. Sperm motility was done by placing 10ul of sperm suspension on slide for microscopic evaluation at a magnification of 400X. About 100 sperm cells were examined and classified as either motile or immotile and expressed as percentage.

Biochemical analysis

The reduced glutathione (GSH) content of the testis homogenate was determined using the method described by Van Dooran et al (1978). The GSH determination method is based on the reaction of Ellman's reagent 5,5' dithiobis (2-nitrobenzoic acid) DNTB) with the thiol group of GSH at pH 8.0 to produce 5-thiol-2-nitrobenzoate which is yellow at 412nm. Malondialdehyde (MDA) is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems. And it is used as an indirect index of lipid peroxidation (Draper and Hadley, 1990). We have employed the method of Uchiyama and Mihara (1978) in this study for the determination of MDA which is based on its interaction with thiobarbituric acid (TBA) to form a pink complex with absorption maximum at 535nm. The activity of the SOD enzyme in the testis homogenate was determined according to the method described by Sun and Zigman (1978). The reaction was carried out in 0.05m sodium carbonate buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005N HCl. CAT activities

was determined by measuring the exponential disappearance of H_2O_2 at 240nm and expressed in units/mg of protein as described by Aebi (1984). Absorbance was recorded using Shimadzu recording spectrophotometer (UV 160) in all measurement.

Hormonal assay

An enzyme based immunoassay (EIA) system was employed to determine testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the plasma samples so collected.

Statistical Analysis

Data obtained were expressed in Mean ± SEM. Statistical analysis was performed by analysis of variance (ANOVA) followed by multiple comparison by two-tailed t-test. The Values for $p< 0.05$ were considered to be statistically significant.

RESULTS

Reproductive Organ Indices

Sodium arsenite significantly decreased the weights of testes ($P < 0.01$), epididymis ($P < 0.001$), prostate gland ($P < 0.001$) and seminal vesicle ($p<0.001$) in rats compared with control. However, there was no significant change in the weights of the testes, epididymis, prostate gland and seminal vesicle of rats administered with sodium arsenite-ginger combination compared with control. Co-treatment with ginger was found to protect against adverse changes in the weights of testes, epididymis, prostate gland and seminal vesicle (Table 1).

Sperm Parameters

The sperm count and motility in the caudal epididymis were significantly reduced ($P < 0.001$) after treatment with sodium arsenite compared with their respective controls (Table 2). However, co-administration of ginger with sodium arsenite attenuates the decrease in sperm count and motility when compared with the arsenite treated rats, with the sperm count and motility reaching the control values. There were significant increases ($p<0.001$) in sperm count and motility with co-administration of ginger and sodium arsenite when compared with sodium arsenite treated group. There was a significant increase ($P < 0.01$) in sperm abnormal morphology in arsenite treated rats, however, co-administration of ginger with sodium arsenite reduces ($p<0.001$) the percentage abnormal morphology in comparison with arsenite treated rats, though the number did not reach the control value.

Plasma FSH, LH and Testosterone

Plasma levels of FSH, LH and testosterone were significantly ($p>0.05$) decreased in the arsenite treated rats when compared with the control (Table 3). The co-administration sodium arsenite and ginger increased the plasma level of FSH and LH above the control rat, whereas the testosterone plasma level was significantly increased compared with arsenite treated but slightly less than control value.

Table 1:

Reproductive organ weights of male rats in control and other experimental rats. Values are expressed as Mean + SEM, n = 6, *P<0.05 compared with control group, #P<0.05 compared with arsenite group. "AGE" means aqueous ginger extract.

Parameter	Control	Arsenite	Arsenite + Age	Age
Testes (g)	1.06 ± 0.03	0.47 ± 0.10*	0.87 ± 0.08#	1.01 ± 0.12*
Epididymis (g)	0.63 ± 0.07	0.18 ± 0.02*	0.26 ± 0.02*	0.37± 0.04*#
Prostate (g)	0.17 ± 0.04	0.05 ± 0.01*	0.14 ± 0.02#	0.2 ± 0.03#
Seminal Vesicle (g)	0.38 ± 0.05	0.07 ± 0.02*	0.21 ± 0.07	0.32 ± 0.05#

Table 2:

Sperm characteristics of male rats in control and other experimental rats. Values are expressed as Mean + SEM, n = 6, *P<0.05 compared with control group, #P<0.05 compared with arsenite group. "AGE" means aqueous ginger extract.

Parameter	**Control**	**Arsenite**	**Arsenite + AGE**	**AGE**
Count (10^6/ml)	28.8 ± 1.49	18.8 ±0.87*	25.3 ±0.88#	35.5 ±1.48*#
Motility (%)	74.2 ± 4.17	57.5 ± 3.59*	75.0 ± 1.83#	84.2 ±2.00#
Morphology (%)	9.7 ± 0.67	29.0 ± 0.97*	20.7 ±0.49*#	12.17±1.14#

Table 3:
LH, FSH and Testosterone levels of male rats in control and other experimental rats. Values are expressed as Mean + SEM, n = 6, *P<0.05 compared with control group, #P<0.05 compared with arsenite group. "AGE" means aqueous ginger extract.

Parameter	Control	Arsenite	Arsenite + AGE	AGE
LH(iu /L)	1.05 ±0.04	0.81 ± 0.06	1.32 ± 0.09*	1.39 ± 0.08*
FSH (iu/L)	0.67 ± 0.04	0.42 ± 0.06	1.06± 0.06*#	0.53 ±0.04#
Testosterone (nmol /L)	3.67 ± 0.04	2.77 ± 0.20*	3.13 ± 0.08*	3.27 ± 0.07*

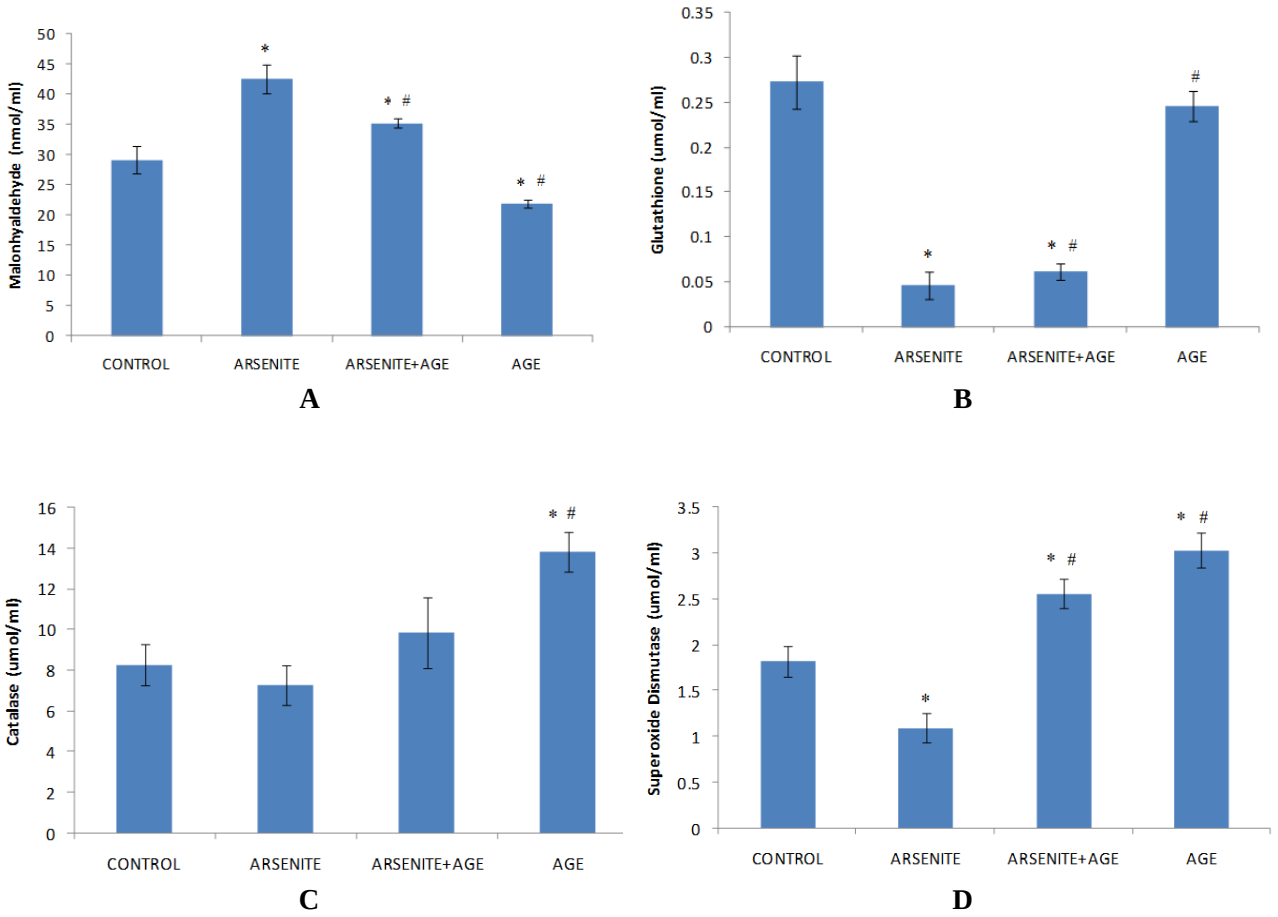


Figure 1a-d:
MDA, GSH, CAT and SOD activities of male rats in control and other experimental rats. Values are expressed as Mean + SEM, n = 6, *P<0.05 compared with control group, #P<0.05 compared with arsenite group. "AGE" means aqueous ginger extract.

Lipid Peroxidation and anti-oxidative enzymes

The testicular content of GSH (reduced glutathione) and SOD enzymes were significantly decreased (p< 0.01) after arsenite treatment compared with their respective control. Co-administration of arsenite and ginger significantly (p<0.05) increased the GSH and SOD enzymes levels compared with the arsenite treated rats, although the level was below the controls for GSH but above the control for SOD. The CAT enzyme activities was decreased in the arsenite treated rat which was not significantly (p>0.05) different from the control. However, co-administration with arsenite and ginger increased the CAT enzyme activities above the control level thereby improving its antioxidative capacity. Arsenite treatment significantly increased (p< 0.01) lipid peroxidation index, MDA, in comparison with the control. Lipid peroxidation was attenuated after co-administration of arsenite and ginger as

evidenced by a significant decrease ($p<0.05$) in measured MDA compared with arsenite treated rat.

DISCUSSION

The protective effects of ginger on testicular toxicity of sodium arsenite were investigated in male albino rat. To our knowledge, this is the first study that evaluates the protective effects of plant extract against testicular damage induced by sodium arsenite in experimental animals. The reduction in the absolute weight of the testes, epididymis, prostate and seminal vesicle in the arsenite treated rats indicates the toxic effects of arsenite on these reproductive organs, this effect is in agreement with a previous finding (Sarkar et al, 2003). Testicular mass is a valuable index of reproductive toxicity in male animals (Aman, 1982) and the decrease in testicular mass was consistent with elimination of germ cells (Chapin and Lamb, 1984). Co-administration of arsenite and ginger clearly restored the reproductive organ indices towards normal which may be due to its androgenic activity (Kamtchouing et al, 2002; Jana et al, 2006).

Sperm parameters such as count, motility and morphology are key indices of male fertility, as these are the prime markers in testicular spermatogenesis and epididymal maturation. The diminution of these parameters by sodium arsenite treatment in our study is in agreement with the findings of others, where arsenic treatment was associated with decreased sperm count and motility; and increased abnormal morphology (Sarkar et al, 2003, Jana et al, 2006). Low testosterone concentration may be responsible for the adverse effects of arsenite on sperm parameters, as high level of testosterone in testis is critically required for normal spermatogenesis, development / maintenance of sperm morphology and normal physiology of seminiferous tubules (Sharpe et al, 1988, Sharpe et al, 1992). A decrease in testosterone concentration of arsenic-treated rats may occur due to the reduced levels of LH (Shaw et al, 1979, Kerr et al 2006) as circulating LH is responsible for maintaining normal plasma testosterone concentrations. Co-treatment with arsenite and ginger attenuated spermatogenic / testicular damage induced by arsenite treatment as shown by the return of sperm count, motility and normal morphology toward normal control values. Ginger has been previously shown to stimulate spermatogenesis (Kamtchouing et al, 2002; Jana et al, 2006). The restoration of testosterone, LH, and FSH levels to normal after co-administration with arsenite and ginger might have stimulated the production of quantitatively and structurally normal sperm. Spermatogenesis requires LH and FSH for initiation and maintenance in male rats, LH stimulates Leydig cells to secrete testosterone, normal testicular function is dependent on FSH and testosterone is absolutely require for normal spermatogenesis.

The spermatozoa, in common with all cell types have developed an elaborate antioxidant defence system consisting of enzymes such as catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) that scavenge and suppress the formation of ROS (Record et al, 2001; Wilcox et al, 2004). Estimation of end products of lipid peroxidation such as malondialdehyde (MDA) is an index of the extent of oxidative damage to cellular structures (Sharma and Agarwal, 1996). In this study, arsenite treated rats showed an elevation in MDA level when compared with the control group. An increased MDA concentration might be a consequence of decreased production of antioxidants in the sodium arsenite treated rats' tissues thereby shifting the delicate balance in favour of ROS ultimately in favour of ROS ultimately leading to a plethora of pathologic damage to sperm cells and concomitant loss of function (Sikka, 2004, Morakinyo *et al, 2008*). GSH concentrations and activities of CAT and SOD in the testes were observed to be significantly reduced in arsenite treated rats in this study. Previous studies have shown that sodium arsenite toxicity in the kidney is mediated by the depletion of antioxidants (Khan et al, 2000) and elevation of lipid peroxidation. It has also been suggested that sodium arsenite generates free radicals (Cleric et al, 1996). These free radicals interfere with the antioxidant defence system in the testis and results in the tissue injury, studies have also shown that levels of ROS correlate with motility of spermatozoa (Iwasaki and Gagnon, 1992). ROS appears to play a role in the apoptosis of spermatozoa, thus a decreased sperm count. Therefore, overproduction of free radicals and hence oxidative stress may account at least in part for the testicular toxicity associated with sodium arsenite treatment. There was an inhibition of peroxidative damage evidenced by reduced MDA level, elevation of GSH, catalase and SOD activities in the co-treated arsenite and ginger rats. This finding is consistent with previous findings that ginger significantly lowered lipid peroxidation (Morakinyo et al, 2008) by maintaining the activities of the antioxidant enzymes; SOD, CAT and GSH in the rat testes (Ahmed et al, 2002).

Conclusion

Recently, much attention has been focused on the protective effects of antioxidants and the possibility of using antioxidants in the treatment of male infertility. Accumulating evidences suggest that the effects of ginger against oxidative damage may be attributed to its antioxidant properties (Vimala et al, 1999; Katiyar et al, 1996). The prevention of sodium arsenite induced

oxidative stress damage in rats by ginger as shown by the result obtained from this study, suggests that part of the mechanism of the reproductive damage is attributed to the over production of free radicals. The protective effect of ginger against sodium arsenite reproductive damage might also be mediated through its androgenic activities. In conclusion, this study showed that sodium arsenite apart from being a hormonal disrupter (Sarkar et al, 2003) also causes oxidative stress which contributed to reproductive toxicity in the male rats. The protective effects of ginger on reproductive toxicity and oxidative stress have also been shown as evidenced by a clear attenuation of arsenite-induced damage sperm functions, testicular steroidogenesis and reproductive organo-somatic indices.

REFERENCES

Aebi H. (1984). Catalase in vitro. *In meth enzymol*.121-126

Ahmed RS, Seth V, Banerjee BD. (2000). Influence of dietary ginger on antioxidant defence system in rat: comparison with ascorbic acid. *Ind J Exp Biol.* 38:604-606.

Aman RP. (1982). A critical review of methods for evaluation of spermatogenesis from seminal characteristics. *J Androl*. 2:37-38.

Amin A, Hamza AA. (2006). Effects of Roselle and Ginger on Cisplatin-induced reproductive toxicity in rats. *The Seventh Annual U.A.E. University Research Conference*. SCI – 117-124.

Arfeen Z (2000) Ginger, the review of natural products facts and comparisms.

Biswas NM, Chowdhury RG, Sarkan M. (1994). Effects of Sodium arsenite on adrenocortical activities in male rats. Dose-duration dependent responses. *Med Sci Res.* 23:153-154.

Chapin RE, Lamb JC. (1984). Effect of ethylene glycol monoethyl ether on various parameters of testicular function in the F344 rats. *Environ Health Perspectives*. 57:219-224.

Chatterjee A, Das D, Chatter D. (1993). The study of underground water contamination by arsenic in the residential area of Behela and Calculta, due to industrial pollution. *Environmental Pollution.* 80: 57-65.

Cleric WJ, Hensley KD, Martino DL, Allan Butterfield D. (1996). Direct detection of ototoxicants induced reactive oxygen species generation in cochlear explants. *Hearing Res*. 98:116-124

Draper H, Hadley A. (1990). Malondialdehyde determination as index of lipid peroxidation I. *In method in enzymology: (Packer L, Glazer A.Eds): Academic press.*

Ernst E, Pittler MH. (2000). Efficacy of ginger for nausea and vomiting: a systematic review of randomised clinical trials. *Br J Anaesth.* 84: 367-71

Iwasaki A, Gagnon C. (1992). Formation of reactive oxygen species in spermatozoa of infertile patients. *Fertil Steril.* 57; 409-416.

Jana K, Jana S, Samanta PK. (2006). Effect of chronic exposure to sodium arsenite on hypothalamo-pituitary- testicular activities in adult rats: possible an estrogenic mode of action. *Reprod Biol Endocrinol.* 4: 91-96.

Kamtchouing P, Fandio GYM, Dimo T, Jastra HB. (2002). Evaluation of androgenic activities of *zingiber Officinal* and *Pentadiplandra brazzeano* in male rats. *Asian J Androl*. 4: 299-301.

Katiyar S.K, Agarwal R, Mukhtar H (1996). Inhibition of tumor promotion in SENCAR mouse skin by ethanol extract of Zingiber Officinal Rhizomes. *Cancer Res*. 56 (5) : 1023-1030.

Kerr JB, Sharpe RM. (2006). Effects and interaction of LH and LHRH agonist on testicular morphology and function in hypophysectomised rats. *J Reprod Fertil*. 76:175-192.

Morakinyo AO, Adeniyi OS, Arikawe AP. (2008). Effects of *Zingiber officinale* on reproductive functions in male rats. *Afr J Biomed Res.* 11(3):329-333.

Nickson R, McArthur J, Burges W, Ahmed KM, Rarensero FP, Rahman M. (1998). Arsenic poisoning of Bangladesh ground water. *Nature*. 395:338-345.

Record IR, Dreosti IE, McInerney JK. (2001). Changes in plasma antioxidant status following consumption of diets high or low in fruits and vegetables or following dietary supplementation with an antioxidant mixture. *Br J Nutr*. 85: 4459-4464.

Roybal CN, Hunsaker LA, Barbash O, VanderJagt DL, Abcouwer SF. (2005). The oxidant stressor arsenite activates endothelial growth factor mRNA transcription by an ATF4-dependent mechanism. *The Journal of Biological Chemistry*. 280 (21): 20331-9.

Sarkar M, Biswas NM, Ghosh D. (1991). Effect of sodium arsenite on testicular and 3beta and 17beta hydroxysteroid dehydrogenase activation in albino rats: dose and duration dependent response. *Med Sci Res*. 19: 789-793.

Sarkar M, Chaudhuri GR, Chattopadhyay A, Biswas NM. (2003). Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. *Asian J Androl.* 1:27-31.

Sarkar M, Ghosh D, Biswas H.M. (1992). Effect of Sodium arsenite on haematology in albino rats. *Ind J Physiol Allied Sci.* 46: 116-120.

Sarkar R, Mohanakumar KP, Chowdhury M. (2000). Effects of an organophosphate pesticide, quinalphos, on the hypothalamogonadal axis in adult male rats. *J Reprod Fertil*. 118:29-38.

Sekiwa Y, Kubota K, Kobayashi A. (2000) Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *J Agric Food Chem*. 48 : 373-377.

Sharma RK, Agarwal A. (1996). Role of reactive oxygen species in male infertility. *Urology*. 48: 835-50.

Sharpe RM, Donachie K, Cooper I. (1988). Re-evaluation of the intratesticular level of testosterone required for quantitative maintenance of spermatogenesis in the rat. *J Endocrinol*. 117: 19-26.

Sharpe RM, Maddocks S, Millar M, Saunders PTK, Kerr JB, Mckinnell C. (1992). Testosterone and spermatogenesis: identification of stage dependent, androgen- regulated proteins secreted by adult rat seminiferous tubules. *J Androl*. 13: 172-184.

Shaw MJ, Georgapouls LE, Payne AH. (1979). Synergistic effect of FSH and LH and testicular Δ5, 3β-hydroxysteroid dehydrogenase isomerase. Application of a new method for the separation of testicular compartments. *Endocrinology*. 104: 912-918.

Sikka SC. (2004). Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *J Androl*.25: 5-18.

Sun M, Zigman S. (1978). An improved spectropholometric assay for Superoxide dismutase based on epinephrine antioxidation. *Anal Biochem*. 247(10): 82-89.

Tappel AL. (1978). Glutathione peroxidate and hydro peroxides. *Meth Enzyme*. 52: 506-513.

Uchiyama M, Mihara M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituris acid test. *Anal Biochem.* 86: 271-278.

Van Dooran R, Liejdekker C.M, Handerson PT. (1978). Synergistic effects of phorone on the hepatotoxicity of bromobenzene and paracetamol in mice. *Toxicol.* 11: 225-233.

Vimala S, Norhanom AW, Yadav M. (1990). Antitumor promoter activity of Malaysian ginger rhizobia used in traditional medicine. *Br J Cancer.* 80 (1-2): 110-116.

Willcox Jk, Ash SL Catignani GL. (2004). Antioxidants and prevention of chronic disease. *Crit Rev Food Sci.* 44: 275-295