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 being 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 like 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,
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 °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
Effect of ginger on arsenite-induced reproductive toxicity

was determined by measuring the exponential disappearance of \( \text{H}_2\text{O}_2 \) 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 \( \pm \) 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>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Arsenite</th>
<th>Arsenite + Age</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes (g)</td>
<td>1.06 ( \pm ) 0.03</td>
<td>0.47 ( \pm ) 0.10*</td>
<td>0.87 ( \pm ) 0.08*</td>
<td>1.01 ( \pm ) 0.12*</td>
</tr>
<tr>
<td>Epididymis (g)</td>
<td>0.63 ( \pm ) 0.07</td>
<td>0.18 ( \pm ) 0.02*</td>
<td>0.26 ( \pm ) 0.02*</td>
<td>0.37 ( \pm ) 0.04*</td>
</tr>
<tr>
<td>Prostate (g)</td>
<td>0.17 ( \pm ) 0.04</td>
<td>0.05 ( \pm ) 0.01*</td>
<td>0.14 ( \pm ) 0.02*</td>
<td>0.2 ( \pm ) 0.03*</td>
</tr>
<tr>
<td>Seminal Vesicle (g)</td>
<td>0.38 ( \pm ) 0.05</td>
<td>0.07 ( \pm ) 0.02*</td>
<td>0.21 ( \pm ) 0.07</td>
<td>0.32 ( \pm ) 0.05*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Arsenite</th>
<th>Arsenite + AGE</th>
<th>AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count (10^6/ml)</td>
<td>28.8 ( \pm ) 1.49</td>
<td>18.8 ( \pm ) 0.87</td>
<td>25.3 ( \pm ) 0.88*</td>
<td>35.5 ( \pm ) 1.48*</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>74.2 ( \pm ) 4.17</td>
<td>57.5 ( \pm ) 3.59</td>
<td>75.0 ( \pm ) 1.83*</td>
<td>84.2 ( \pm ) 2.00*</td>
</tr>
<tr>
<td>Morphology (%)</td>
<td>9.7 ( \pm ) 0.67</td>
<td>29.0 ( \pm ) 0.97*</td>
<td>20.7 ( \pm ) 0.49*</td>
<td>12.17 ( \pm ) 1.14*</td>
</tr>
</tbody>
</table>

Table 1: Reproductive organ weights of male rats in control and other experimental rats. Values are expressed as Mean \( \pm \) SEM, \( n = 6 \), *\( p<0.05 \) compared with control group, \( ^* p<0.05 \) compared with arsenite group. “AGE” means aqueous ginger extract.

Table 2: Sperm characteristics of male rats in control and other experimental rats. Values are expressed as Mean \( \pm \) SEM, \( n = 6 \), *\( p<0.05 \) compared with control group, \( ^* p<0.05 \) compared with arsenite group. “AGE” means aqueous ginger extract.
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.

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

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 (Kametchouing 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 (Kametchouing 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 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 meditated 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 organosomatic indices.

REFERENCES


Effect of ginger on arsenite-induced reproductive toxicity


