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REPRODUCTIVE PATHOLOGY OF EXPERIMENTALLY INDUCED LEAD POISONING IN RED SOKOTO BUCK: PROTECTIVE EFFECT OF TIGER NUT *(CYPERUS ESCULENTUS)*

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ABSTRACT

Tiger-nut (*Cyperus esculentus*) is a crop that belongs to family *Cyperaceae* which produces rhizome and tuber that are small spherical in shape. The tubers have aphrodisiac, carminative, diuretics and several health benefits. The aim of the present study was to investigate the influence of methanol extract of tiger nut on the male reproductive pathology of chronic lead poisoning in Red Sokoto buck. The tiger nut was extracted using 95% methanol by cold extraction method. Four adults Red Sokoto bucks (12 to 25 kg) were randomly grouped into four. Group I was administered distilled water (200 mL). Group II was administered lead acetate (200 mg/kg) only. Group III was administered methanol extract of tiger nut (200 mg/kg) and lead acetate (200 mg/kg). Group IV was administered tiger nut (200 mg/kg) only. Evaluation of semen characteristics (semen motility, semen concentration, ejaculate volume and semen pH) were determined by standard method. The serum testosterone changes were determined using commercial kits. The lead acetate (200 mg/kg) group II showed decreased semen characteristics parameters while tiger-nut (LA + TN) group III showed improved values of the semen characteristics. However, the semen characteristics values on the distilled water (DW) group I and sole tiger nut (TN) group IV showed a good semen characteristics values. In conclusion the methanol extract of tiger nut contained antioxidant properties that significantly influence a protective role in ameliorating the pathological effect of lead poisoning in male reproductive pathology in Red Sokoto goat.

Keywords: Tiger-nut, lead-poisoning, testosterone, antioxidant and phytochemical.

INTRODUCTION

Reproduction is central to the continued existence of animals on earth. In sexual animals, reproduction is important for both males and females for life to continue. Reproduction is achieved by an act of sexual interaction which commences the process of fertilization. For this process to be successful, the reproductive system of both the male and female must be in their normal conditions (Rastogi, 2011). Failure or inability to perform this important natural act by either sex will inevitably result in

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reproductive pathology and this will raise many questions bordering infertility. One of the major causes of reproductive disorder with little or no attention is environmental pollution of heavy metals resulting from illegal mining activities (Rastogi, 2011). However, reproductive pathology due to effects of environmental pollutants on sexual function of heavy metals like lead seems to be one of the most abundant and largely distributed metals (water pipes, industrial pollution and illegal mining) in our environment. Its bio-concentration in the seminal plasma has encouraged researchers to investigate its potential effects on the reproductive function (Haouas *et al.,* 2015).

Lead is one of the most abundant toxic heavy metals that have been detected in all parts of the environment and biological system (Xia *et al.,* 2010). It is an abundant, ubiquitous, dangerous and important toxic environmental contaminant of global concern due to its significant role in modern industry. In developing countries, sources of lead are more widespread than in western countries, and they include, in addition to traditional medicines, cosmetic and tooth-cleaning powders, the on-going use of lead-based paints, water contamination from lead pipes and cisterns, use of lead-containing cookware and lead-glazed crockery, and unsafe practices in small factories and mines (Hore *et al.,* 2014; Pfadenhauer, *et al.,* 2014). Lead is used in the manufacture of protective paints for iron and steel, explosives, rodenticide and batteries. Lead causes a number of adverse effects on the reproductive system in both males and females. Common adverse effects induced by lead in males include: reduced libido, abnormal spermatogenesis (reduced motility and number), asthenospermia, hypospermia, teratospermia (Sallman, 2001), chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone. The females, on the other hand, are more susceptible to infertility, miscarriage, premature membrane rupture, pre-clampsia, pregnancy hypertension and premature delivery (Flora *et al.,* 2011). During the gestation period, direct influence of lead on the developmental stages of the foetus has also been reported (Saleh *et al.,* 2009).

Tiger nut (*Cyperusesculentus*) belongs to the *Cyperaceae* family of plant species.The tubers are about the size of peanuts and are commonly found in Nigeria (Adewale, *et al.,* 2015). In Nigeria, the Hausas call it *"Aya"*, Yorubas*"imumu",* the Igbos *"ofio"*. Tiger nuts which are incorrectly called "nuts" or "nutlets," as the origin of their common name, are small, about the size of a peanut growing at the rhizome of the plant (Bamishaiye and Bamishaiye, 2011). Tiger nut is rich in energy, minerals and vitamins. Three varieties of tiger nut were common in Nigeria namely: black, brown and yellow (Oyedepo and Odoje, 2014).

MATERIALS AND METHODS

Experimental animals and Housing: Twelve adults' fertile males Red Sokoto goats within the age range of one year weighing 12 to 25kg were procured from Maiadua goat market in Katsina State, Nigeria. The animals were acclimatized for 2 weeks in the animal house of the Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Kaduna State. They were fed with grass, hay, wheat bran, groundnut haulms at intervals during the early hours of the day and supplemented with 2kg of concentrate feeds later in the day, and gave access to water *ad libitum.* The blood and faecal samples were collected and taken for parasitological analysis and screening for presence of haemoparasites, respectively. Ethical approval for the use of Red Sokoto goats for this study was obtained from the Ahmadu Bello University Committee on Animal Use and Care, Ahmadu Bello University, Zaria Nigeria.

Tiger Nut Extraction: Fresh tiger nuts *(Cyperus esculentus)* were procured from a local market in Zaria, Kaduna State, Nigeria. The seeds (Plate I) were screened for the presence of bad ones, which were discarded

while the rest were washed and air dried for 10 days and, thereafter, pulverised into smooth powder. Crude methanol extracts of the tiger nut was obtained using the cold maceration method with 90% methanol (Ajani *et al.,* 2016). The pulverized sample (2 kg) was suspended in 2.5 L of 90% methanol with regular agitation for 24 hours. The solution obtained was filtered and the resulting filtrate was concentrated using the rotary evaporator coupled to a thermo-circulator. The residue was further air-dried to a constant weight and, thereafter, preserved in a refrigerator at 4°C until required for use (Ajani *et al.,* 2016).

Toxicity study: The median lethal dose (LD_{50}) of the lead or the extract was determined using a standard two phase approach as described by Lorke (1983). In the first phase of the trial, nine rats were divided at random into three groups of three rats each. The animals were deprived of feed and water for 12 hours. The groups I, II and III animals were treated with lead or *C. esculentus* extract orally at 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight, respectively. Rats were observed for 48 hours for any sign of toxicity or mortality. In the second phase of the experiment, three animals were assigned into three groups of one animal each (Lorke, 1983). Rats in different groups were administered with lead or extract at three doses, depending on the outcome of the phase one. The LD_{50} was then calculated as the geometric mean of doses that produced 0% and 100% mortality. However, if the lead or extract did not produce mortality at a dose up to 5000 mg/kg, it was considered safe, and the LD_{50} was assumed to be $\geq$ 5000 mg/kg.

Experimental design: Group I (DW): buck served as the control and was administered equivalent volume of distilled water. Group II (LA): buck was administered lead acetate at 200 mg/kg body weight. Group III (TN): buck was administered methanol extract of tiger nut at 200 mg/kg body weight. Group IIV (LA + TN): buck was administered lead acetate at 200 mg/kg + methanol extract of tiger nut at 200 mg/kg body weight. The dose regimens of both lead and extract were administered *per os* from day 1 of lead acetate administration once daily, for a period of 20 weeks. The bucks were monitored for clinical signs of toxicity and death.

Determination of hormone: Serum samples collected were assayed for testosterone levels using the enzyme-linked immunosorbent assay (ELISA) method, based on competitive binding principle. Testosterone present in the sample competed with enzyme-labelled testosterone for binding with anti-testosterone antibody immobilised on the microwell surface (Ali *et al.,* 2016). The amount of conjugate that bound to the microwell surface was expected to decrease in proportion to the level of testosterone in the sample. The unbound sample and conjugate were then removed by washing and the colour development reagents (substrates) were added. Upon exposure to the bound enzyme, a colour change occurred; the intensity of which reflected the amount of bound enzyme-testosterone conjugate and was inversely proportional to the concentration of testosterone in the sample within dynamic range of the assay. After stopping the reaction, the resulting colour was measured using a spectrophotometer (Spectro Lab 23A) at 450nm (Ali *et al.,* 2016).

Semen collection: Semen was collected on days 1 and once every month throughout the course of the study. The rectums of the goats were washed with 6% sodium chloride solution (Matshaba, 2010). The probe was then inserted up to about 30.48 cm and held in a position of rectal floor. Alternate current was passed to increase with gradual increase in voltage from zero to five volts to return again to zero within every 5 to 10 seconds. The subsequent stimulation was progressively higher, so that at about the fifth stimulus a maximum of 10 - 15 volts was reached. Erection and ejaculation occurred at 10 to 15 volts, when 0.5 to 1 ampere current was passed. The source of electric current was AC/220 – 250

volts/single phase/50 cycles. Semen samples collected were kept in a thermo flask at 37 °C, and transported to the laboratory for evaluation within 1 hour after collection (Matshaba, 2010). In the laboratory, semen samples collected were evaluated macroscopically for semen volume, sperm concentration, and microscopically for semen morphology and motility (Malebogoro *et al.*, 2015).

Histopathological slide preparation: The samples of testes of each goat were obtained and fixed in Bouin's solution. They were later dehydrated in graded alcohol, cleared in xylene, infiltrated with molten paraffin wax at 50 °C and blocked in paraffin. Sections were cut at 5-6 µM using a microtome. The sections were then stained with haematoxylin-eosin as described by Luna (1968) and then examined under light microscope at magnification of × 400. Lesions observed were recorded and photomicrographs taken.

RESULTS

Table 1: Phytochemical composition in tiger nut *(Cyperus esculentus)*

Phytochemicals	Composition (mg)	Percentage composition (%)
Alkaloids	0.820	61
Phenols	0.127	9.5
Tannins	0.039	2.8
Flavonoids	0.005	0.3
Saponins	0.213	15.8
Steroids	0.039	2.8
Glycosides	0.100	7.8

Table 2: Effect of methanol extract of tiger nut on semen reaction time, semen ejaculation volume, semen motility, semen concentration, and semen pH in Red Sokoto goats treated with lead acetate at 200 mg/kg for 20 weeks.

Parameters	DW	LA	LA + TN	TN
Semen reaction time (seconds)	30.00 ±2.809^{a}	60.17 ± 2.744^{b}	38.33 ± 2.410^{a}	25.43 ± 2.887^{a}
Semen ejaculation volume (mL)	0.700 ± 0.058^{a}	0.300 ±0.058^{b}	0.600 ± 0.058^{ab}	0.800 ± 0.116^{ab}
Semen motility (%)	70.50 ± 2.179^{a}	25.00 ± 2.887^{b}	50.17 ± 2.887^{ab}	85.00 ± 2.887^{bac}
Semen concentration	220.7 ± 11.53^{a}	80.00 ± 10.89^{a}	120.3 ± 11.55^{a}	226.7 ± 14.53^{a}
Semen pH	7.00 ± 0.289^{a}	5.50 ± 0.289^{a}	6.00 ± 0.2887^{b}	7.167 ± 0.260^{a}

a,b,c=values of mean±SEM in the same row with different super scripts are significantly (P<0.01) different. Disilled water (DW), lead acetate combined with tiger nut (LA+TN), tiger nut extract (TN). (TN).

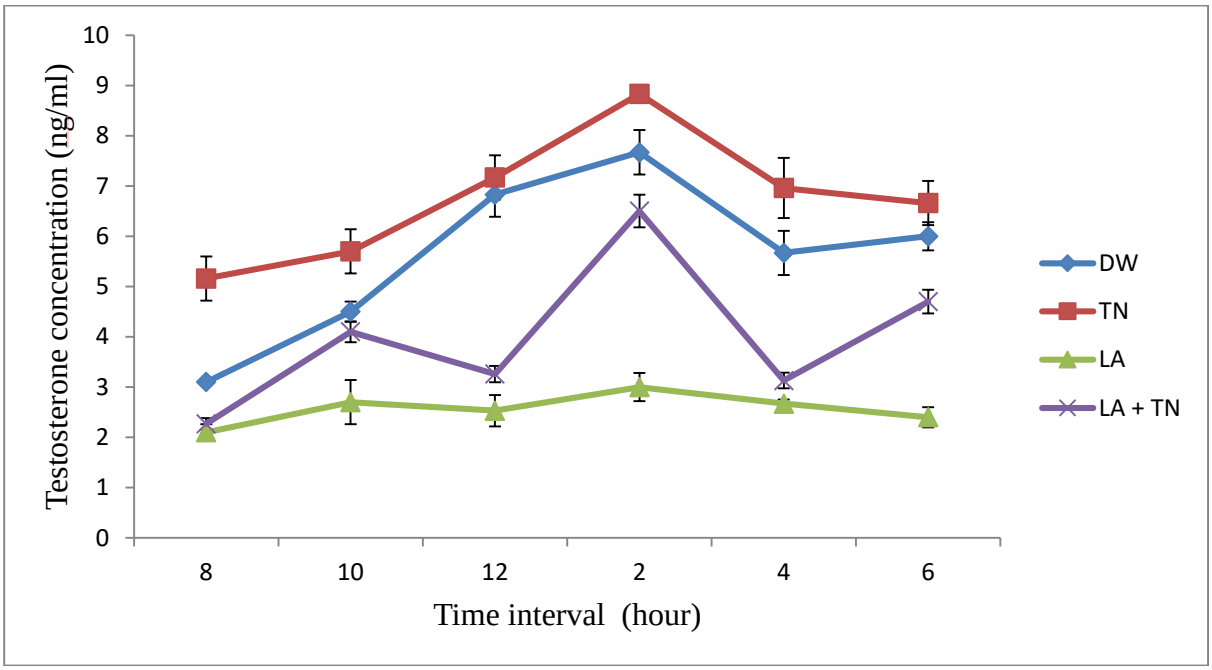

Figure 1: Effect of methanol extract of tiger nut on testosterone concentration in Red Sokoto buck treated with lead acetate at 200 mg/kg for 20 weeks. Distilled water (DW), lead acetate (LA), lead acetate combined with tiger nut (LA+TN), tiger nut (TN).

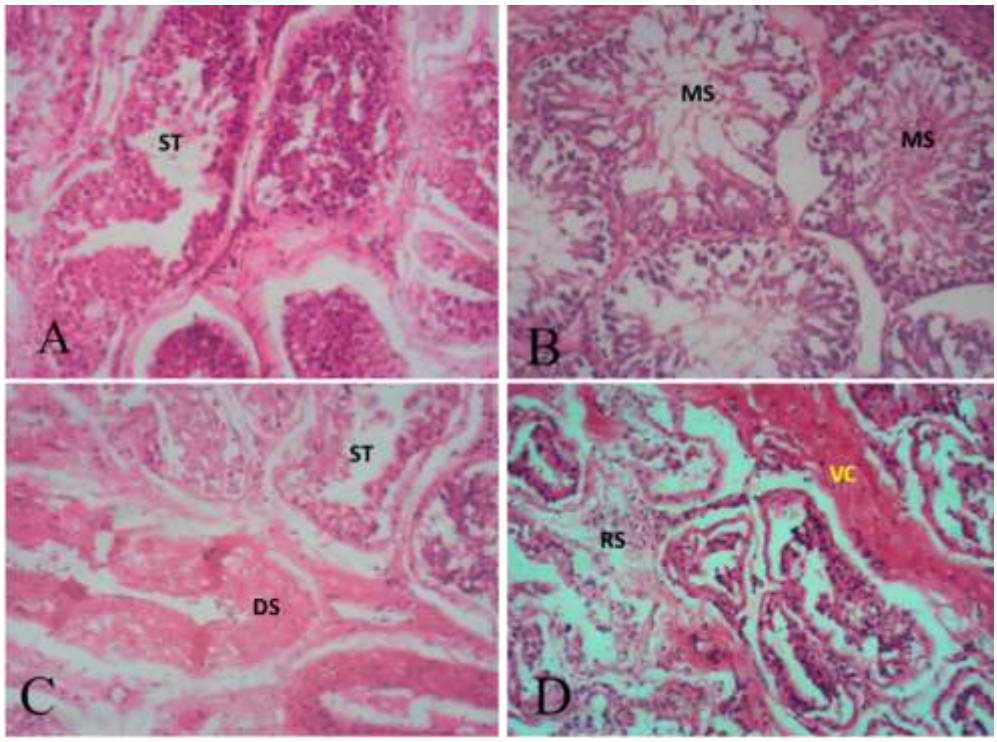

Plate 1: Photomicrograph of section of testis from experimental Red Sokoto goats; **I-A:** administered 200 mL of distilled water (DW) showing no observable microscopic lesions with normal seminiferous tubules (ST). **1-B:** administered 200 mg/kg methanol extract of tiger nut showing normal arrangement of spermatogonia (SG) along the tubular membrane with migration of spermatids (MS) towards the lumen of the seminiferous tubule (ST). **1-C:** administered lead acetate at 200 mg/kg showing areas of necrosis with degenerated spermatogonia (DS) along the tubular membrane of the seminiferous tubule (ST). **1-D:** administered lead acetate combined with methanol extract of tiger nut at 200 mg/kg

showing areas of necrosis with regeneration of spermatogonia (RS) along the tubular membrane of the seminiferous tubule (ST) with vascular congestion (VC). H & E x 400.

The results of the phytochemical analysis of the methanol extract of tiger nut are as presented in Table 1 Alkaloids (61%) had the highest percentage, followed by saponins (15.8%), phenols (9.5%), glycosides (7.8%), tannins and steroids (2.8%), with flavonoids having the least value, (0.3%).

As shown in table 2, there was a significant ($P < 0.01$) increase in semen reaction in LA (60.17 ± 2.744) group when compared to DW (30.00 ± 2.887) or TN (25.00 ± 2.887) and LA + TN (38.33 ± 2.410) groups. Also, table 2.0 showed a significant ($P < 0.001$) decrease in semen ejaculation volume in LA (0.300 ± 0.058) compared to DW (0.700 ± 0.058), TN (0.800 ± 0.116) or LA +TN (0.600 ± 0.058) group. However, table 2.0, showed a significant ($P < 0.001$) decrease in percentage semen motility in LA (25.00 ± 2.887) compared to DW (70.50 ± 2.179) or LA + TN (50.17 ± 2.887) or TN (85.00 ± 2.887) groups. Similar pattern was observed in the study with semen concentration in the LA (80.00 ± 10.89) groups which showed a significant ($P < 0.01$) decreased in semen concentration compared to LA + TN (120.30 ± 11.59), DW (220.7 ± 14.53) and TN (226.7 ± 14.55) as shown in table 2.0. In this study, semen p^H decreased in LA (5.500 ± 0.289) value although the decrease showed no significant ($P > 0.05$) difference when compared with LA + TN (6.000 ± 0.289), DW (7.000 ± 0.289) and TN (7.167 ± 0.260) (Table2).

As shown in figure 1, at 8.00 am, there was no significant ($P > 0.05$) difference in the serum testosterone concentration in LA (2.000 ± 0.066 ng/ml), LA + TN (2.20 ± 0.013) and DW (3.0 ± 0.289 ng/ml.) groups when compared with TN (4.1 ± 0.557 ng/ml) group. There was also gradual increase in the serum testosterone concentration of LA + TN and DW groups as time progresses from 8.00 am to 10.00 am with peak value of LA + TN (3.80 ± 0.04 ng/ml) and DW (4.20 ± 0.31 ng/ml) respectively. At 12.00 noon, there was a significant ($P < 0.05$) decrease in the serum testosterone concentration in LA + TN (3.1 ± 0.213 ng/ml) when compared with DW (6.40 ± 0.51 ng/ml) and TN (6.80 ± 0.21 ng/ml) groups. However, at 2.00 pm, there was a significant ($P < 0.05$) increase serum testosterone concentration in LA + TN (6.1 ± 0.231 ng/ml), DW (7.2 ± 0.50 ng/ml) and TN (8.5 ± 0.101 ng/ml) groups when compared with LA (2.8 ± 0.030 ng/ml) group. At 4.00 pm there was a significant ($P < 0.05$) decrease in the serum testosterone concentration in DW (5.4 ± 0.02 ng/ml) and TN (6.9 ± 0.04 ng/ml) when compared with LA or LA + TN groups. At 6.00 pm there was a significant ($P < 0.05$) increase in serum testosterone concentration of bucks treated with LA + TN (4.6 ± 0.05 ng/ml) when compared with the LA (2.2 ± 0.03 ng/ml) treated group.

The histopathology of Red Sokoto buck in distilled water (DW) group showed no observable microscopic lesions, with normal seminiferous tubules and well-arranged spermatogonia (Plate I-A). Similarly, the testis of Red Sokoto buck in methanol extract of tiger nut (TN) group showed normal arrangement of spermatogonia along the tubular membrane with migration of spermatids towards the lumen of the seminiferous tubule (Plate II-B). Testicular section from the bucks in lead acetate (LA) group showed spermatogonia degeneration and necrosis along the tubular membrane of seminiferous tubules (Plate III). Necrosis and regeneration of spermatogonia were observed along the tubular membrane with vascular congestion (Plate IV) in group with tiger nut extract combined with lead acetate.

DISCUSSIONS

The recorded high value of alkaloids (61%) in brown tiger nut suggested a wide range of health promoting properties, including enhancement of sexual behaviour. Imam *et al.* (2013) reported 30% of alkaloids in small brown tiger nut. However, Ayo *et al.* (2016)

reported that alkaloids was the lowest phytochemical quantified during the phytochemical composition and functional properties of flour produced from two varieties of tiger nut *(Cyperus esculentus)*. Alkaloid is one of the aphrodisiac bioactive principles (Alok *et al.,* 2013) in tiger nut. It was reported by Kumar *et al.* (2010) that the alkaloid in *Achyranthes aspera linn,* played antifertility role in male albino rat. Alkaloids act peripherally to elevate the testicular cholesterol content in male thereby causing the release of steroids. At the same time it increases the release of nitric oxide from the endothelial and nerve endings resulting in blood vessel dilation in male sexual organ. Again, it relaxes *corpus carvenosum* smooth muscle in male corpulatory organ thereby enhancing sexual behaviour in male (Alok *et al.,* 2013). The result of this study also showed that flavonoid was 0.3%.This was contrary to the result of Imam *et al.* (2013), who reported that flavonoid contained 18% in small brown tiger nut. The difference might be attributed to the type of soil where the tuber was planted or from the methods employed during processing. However, flavonoids are known for their capacity to act as powerful antioxidants which can protect the animal body from free radicals and reactive oxygen species (Manta *et al.,* 2013). The mechanism of action of flavonoids is reduction in reactive oxygen species (ROS) production by either inhibiting enzyme or chelating elements that cause free radical generation. It could also be by scavenging ROS or by shielding the antioxidant defences (Shashank and Abhay, 2013). The result also showed 9.5% phenol. Phenols are essentially natural antioxidants against various degenerative diseases (Wu *et al.,* 2013). Derivative from saponin are used commercially for sex hormone such as progesterone production (Sarker and Nahar, 2007). Therefore, the second highest phytochemical got from tiger nut in this study was saponin (15.8%). This is in contrary to the report of Ayo *et al.* (2016) with the lowest quantifiable saponin. In this study the glycoside (7.8%), steroids (2.8%) and tannins (2.8%) were recorded. On the contrary, Imam *et al.* (2013), reported 32% glycosides and tannins 18% in small brown tiger nut.

Evidence from the result showed that exposure to lead is detrimental to semen reaction time. Therefore, increase or delay in semen reaction time in the present study establishes decrease in libido of the Red Sokoto buck on administration of lead acetate. Libido is typically calculated by means of the reaction time, defined as the elapsed time between exposure to stimuli and first service (Rehman *et al.,* 2016). Significant decrease in sperm concentration from lead acetate administered group established hypospermia. This may be linked to lead-induced ROS damage on the polyunsaturated fatty acids of cell membranes of germ cells, and subsequently the spermatozoa and mature sperms (Gandhi *et al.,* 2017).

In this study also, decrease sperm motility in the lead acetate (LA) and lead acetate and tiger nut groups implies inability of sperm fertilization capacity in red Sokoto buck. Sperm motility is an important functional measurement to predict sperm fertilizing capacity. Any negative impact on motility would seriously affect fertilizing ability (ElMazoudy *et al*., 2011). This result is in conformity with the report of Gandhi *et al.* (2017) who reported that lead-induced ROS may reduce phosphorylation of sperm axonemes which further weakens sperm motility. Reduction in sperm motility may also be ascribed to ATP deficiency from the lack of axoneme protein phosphorylation, as glyceraldehyde-3-phosphate dehydrogenase in the fibrous sheath become limited to produce sufficient energy for motility through the glycolytic pathway (Gomes *et al.,* 2015; Gandhi *et al.,* 2017). In terms of direct effects on motility, studies showed that lead impairs axonemal microtubule sliding and displaces the role of calcium in binding to calmodulin for tail protein tyrosine phosphorylation (Oliveira *et al.,* 2009). Also, an *in-vitro* study found an increase in flagellum abnormalities, with an

increased presence of coiled tails (Gomes *et al.*, 2015). It was also reported that lead may interact with the sulfhydryl groups on the proteins of the outer dense fibres and fibrous sheath, which are cytoskeletal components of the flagellum, causing its detachment from the plasma membrane (Gomes *et al.*, 2015). Also, sperm apoptosis is the most fatal consequence of oxidative stress induced by lead (Aitken *et al.*, 2015).

Lead-induced ROS generation leading to impaired capacitation of sperm may also compromise penetrative ability (Gandhi *et al.*, 2017). High levels of ROS produce a state of oxidative stress leading to a lower percentage of chemotactic spermatozoa (Sanchez *et al.*, 2010; Du Plessis *et al.*, 2015). The deprivation of ROS scavenging may also initiate spontaneous sperm hyper-activation, impairing sperm transport in the lower female reproductive tract or leading to premature capacitation (Gandhi *et al.*, 2017). Furthermore, lead exposure studies in animals showed a dose-dependent decrease of sperm adherence to the ova along with compromised DNA, RNA, and protein synthesis under the sperm *zona pellucida* binding conditions (Gandhi *et al.*, 2017).

For the lead acetate plus tiger nut (LA + TN) group the resultant effect on serum testosterone level at different time intervals was small compared to the values obtained in the lead acetate group (LA) group. This might be due to the toxic effect of lead alongside the toxic effect of alkaloid from the supplemented tiger nut which produced a synergetic effect resulting to tissue peroxidation and testosterone depletion. This result agrees with the reports of (Taiwo *et al.*, 2010; Fatima *et al.*, 2011). However, the negative effect of lead on testosterone involved a decrease in cytochrome P450 Scc, P-450 c17 and 17-beta hydroxysteroid dehydrogenase 3 in the biosynthesis of steroid hormones (Mokhtar and Maryam, 2011). The reduction in serum testosterone level in lead acetate (LA) group may also be attributed to effect of lead acetate on the endocrine control of reproduction and direct toxicity on reproductive organs. It could also be as a result of a direct toxic action of lead acetate on the hypothalamic-pituitary axis (El-Sayed and El- Neweshy, 2010) which causes decrease in the weight of reproductive organs (Elias *et al.*, 2014) and impairment in testicular morphology and histological alterations in the various components of the testis (Garu *et al.*, 2011; Elias *et al.*, 2014). The current study showed a significant increase in the serum testosterone levels at different times in the tiger nut groups (Figure 1.0). Highly significant values were obtained between (12 noon and 4.00 pm) with peak or highest values at afternoon (12.00 noon and 2.00 pm). These findings corroborate previous studies (Allouh *et al.*, 2015) who observed a significant increase serum testosterone level of tiger nut treated Red Sokoto bucks. The increase serum testosterone level in the tiger nut group may be linked to the direct action of tiger nut on testicular cells and not through the hypothalamus-pituitary axis (Allouh *et al.*, 2015).

The morphology of sperm in this study was totally abnormal and not visible in lead acetate treated acute and chronic groups. This may be as a result of toxic effect of lead acetate which might cause increased generation of free radicals leading to oxidative stress and impairment of testicular-architecture (Sabeti *et al.*, 2016). The visible number of normal sperm morphology recorded in groups administered tiger nut only might be as a result of pro-oxidant effect of tiger nut which prevent the oxidative challenge in the testis and seminiferous tubules in the red Sokoto goat bucks. Groups treated with tiger nut plus lead acetate showed no ameliorative effect, as there were increasing numbers of morphologically abnormal spermatozoa. The abnormal sperm morphology in this study was shown to be high in the lead acetate and LA + TN treated in acute and chronic groups. This might be attributed to oxidative injury to the testis and the seminiferous tubules which affected the process of spermatogenesis. During the period of sperm

chromatin condensation, coupled with polyunsaturated fatty acid, nature of sperm made it highly susceptible to oxidative injury (Sabeti *et al.,* 2016).

Changes such as aspermia, sperm head, tail and mid-piece abnormalities with complete necrospermia were observed in lead acetate and LA + TN treated in acute and chronic groups in this study, but in tiger nut treated groups, no noticeable difference in the sperm tail, head or mid-piece abnormality when compared with the distilled water group. Therefore, oxidative injury to sperm mid-piece as a result of lead acetate toxicity could have subjected the sperm morphological abnormality and dysfunction, since the mid-piece of spermatozoa contains a lot of mitochondria making it the center for production of ATP required for sperm motility. Thus, the protection of tiger nut was attributed to its antioxidant effect, protecting the membranes of the cell organelle. Abnormality in sperm tail that was observed common in all the groups treated with lead acetate which could also have been due to oxidative injury to the sperm cells and this agreed with the report of Sabeti *et al.,* (2016).
Aspermia observed in lead acetate treated groups might be attributed to oxidative damage to the testis and seminiferous tubules which hinders the process of spermatogenesis. This was reported that energy in the form of ATP, through oxidative phosphorylation was required by sperm to carry out motility function (Bedford, 1983). But toxic effect of lead had been shown to impair the process of oxidative phosphorylation, which energy is essential for normal spermatozoa movement. Hence any deprivation of ATP may lead to reduction in sperm motility which caused sperm mortality leading to infertility. In support of this, was a reported correlation between testosterone, motility and fertilizing capacity of the spermatozoa (Eliasson, 1985).

In this present study, the histopathological changes of testis of red Sokoto buck administered lead acetate revealed congestion of interstitial blood vessels, atrophy of seminiferous tubules, degeneration of spermatozoa cells, disorganization of seminiferous tubule architecture and sloughing of seminiferous tubular basement membrane with complete loss of interstitial connective tissue and necrosis of seminiferous tubules. This implies loss or necrosis of Leydig cell responsible for production of testosterone hormone. The result of this study is in agreement with the report of Haousa *et al.* (2015) on reproductive pathology of lead acetate in adult male rats. These structural changes could be attributed to a protein interaction mechanism as reported by El Shafai *et al.* (2011). In the present study, the testis of red Sokoto buck administered methanolic extract of tiger nut and lead acetate showed normal seminiferous tubules with spermatozoa in the lumen, partial loss of interstitial connective tissue with necrosis of seminiferous tubular, partial disorganization of seminiferous tubule architecture and sloughing of seminiferous tubules membrane. This result is suggestive of mild ameliorative effect of tiger nut compared to the result in LA treated group.

Congested blood vessels in interstitium, mild interstitial oedema and thickened basement membrane of seminiferous tubules, in focal areas, disruption of basement membrane and germinal cells of basement membrane were observed. In focal areas, separation of epithelium on basement membrane of seminiferous tubules was observed by the end of 2nd week.

CONCLUSION

In conclusion the reproductive hormones and sperm characteristics evaluated in Red Sokoto bucks in this study were altered in a way that was harmful to normal reproductive performance. However, the Pathological changes observed and recorded in testes were suggestive of toxic injury but were

ameliorated to some degrees with methanol extract of tiger nut (*Cyperus esculentus*).

REFERENCES

Adewale, A.; Chiagoziem, A.; Otuechere, Z.O.; Oteglolade, O.B. and Emmanuel, I.U. (2015): Evaluation of the safety profile and antioxidant activity of fatty hydroxamic acid from underutilised seed oil of *Cyperus esculentus*. J. Acute Dis., V. 4(3), P. 230–235.

Aitken, R.J.; Baker, M.A. and Nixon, B. (2015): Are sperm capacitation and apoptosis the opposite ends of a continuum driven by oxidative stress? Asian J Androl., V. 17, P. 633–639.

Ajani, E.O.; Sabiu, S. and Bamisaye, F.A. (2016): Four weeks daily dose oral administration assessment of *Cyperus esculentus*, aqueous extract on key metabolic markers of Wistar rats. Pharmacologia, V. 7, P. 125-133.

Alghazal, M.A.; Lenartova, V.; Holovska, K.; Sobekova, A.; Falis, M. and Legath, J. (2008): Activities of Antioxidant and detoxifying enzymes in rats after lead exposure. Acta Vet Brno., V. 77, P. 347-354.

Ali, M.M.; Ali, M.L.; Islam, M.S. and Rahman, M.Z. (2016): Preliminary assessment of heavy metals in water and sediment of Karnapuli River, Bangladesh. Environ Monit Mgt, V. 5, P. 27 – 35.

Allouh, M.Z.; Daradka, H.M. and Abu Ghaida, J.H. (2015): Influence of *Cyperus esculentus* tubers (tiger nut) on male rat corpulatory behavior. BMC Complement Altern Med., V. 15(331), P. 2 – 7.

Almansour, M.I. (2009): Histological Alterations Induced by Lead in the Testes of the Quail *Coturnix coturnix*. Res J Environ Toxicol., V. 3, P. 24-30.

Alok, S.; Ratendra, K. and Ramandeep, S. (2013): Nature's aphrodisiacs - A review of current scientific literature. Int. J Recent Advan Pharm Res., V. 3(2), P. 1-20.

Ayo, J.A.; Adedeji, O.E. and Ishaya, G. (2018): Phytochemical composition and functional properties of flour produced from two varities of tiger nut *(Cyperus esculentus).* FUW Trends Sci Tech J., V. 1(1), P. 261-266.

Bamishaiye, E.I. and Bamishaiye, O.M. (2011): Tiger nut: As a plant, its derivatives and benefits. Afr J Food Nutr Dev., V. 11, P. 5157 – 5170.

Bedford, J.M. (1983): Biology of Reproduction. 3rd edition; Oxford University Press, Oxford, P. 28-108.

Du Plessis, S.S.; Agarwal, A.; Halabi, J. and Tvrda, E. (2015): Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. J Assist Reprod Gene., V. 32, P. 509 –20

Elias, A.; Oputiri, D.; Oru-Bo P.G. and Akuegbe, E.D. (2014): Lead organ and tissue toxicity: roles of mitigating agents (Part 2). Brit J Pharmacol Toxicol, V. 5 (1), P. 1-15.

Eliasson, R. (1985): Semen analysis as a foot in the search for a male contraceptive. In: *Male contraception*: Advances and future prospects (Eds: Zatuchi, G.I.; Goldsmith, A. S.; Pieler, J. M. and Sciarra, J. J. Happer and Row) Philadelphia, P.114 – 123.

ElMazoudy, R.H.; Attia, A.A. and El-Shenawy, N.S. (2011): Protective role of propolis against reproductive toxicity of chlorpyrifos in male rats. Pestic Biochem Physiol, V. 101, P. 175-181.

El-Sayed, Y.S. and El-Neweshy, M.S. (2010): 'Impact of lead toxicity on male rat reproduction at "hormonal and histopathological levels". Toxicol Environ Chem., V. 92 (4), P. 765 — 774.

El-Shafai, A.; Zohdy, N.; El-Mulla, K.; Hassan, M. and Morad, N. (2011): Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible

protective effect of ascorbic acid. Food Chem Toxicol., V. 49, P. 734-743.

Farmand, F.; Ashkan, E.; Roberts, C.K. and Sindhu, R.K. (2005): Lead induced dysregulation of superoxide dismutase, catalase, glutathione peroxidase and guanylate cyclase. Env Res., V. 98, P. 33-39.

Fatima, R.; Umar, A.K.; Muhammad, A. and Saffia, S. (2011): Protective role of ginger on lead induced derangement in plasma testosterone and luteinizing hormone levels of male sprague dawley rats. J Ayub Med Coll Abbottabad., V. 23(4), P. 24 – 27.

Flora, S.J.S., Pachauri, V. and Saxena, G. (2011): Academic Press; Arsenic, cadmium and lead. Reprod Dev Toxicol., V. 1, P. 415 – 438.

Gandhi, J.; Hernandez, R.J.; Chen, A.; Smith, N.L.; Sheynkin, Y.R.; Joshi, G. and Khan, S.A. (2017): Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning. Zygote., V. 25, P. 103–110.

Gani, M.U.; Siddiqui, M.S.I.; Islam, K.; Ahmed, S.; Rashid, M.H.; Moonmoon, S. and Mostofa, M. (2017): Study on hematological alterations in experimental lead toxicosis in long evans rats. Malays J Vet Res., V. 8(1), P. 11-18.

Garu, U.; Sharma, R. and Barber, I. (2011): Effect of lead toxicity on developing testis of mice. Int J Pharm Sci Res, V. 2, P. 2403-2407.

Gomes, M.; Goncalves, A.; Rocha, E.; Sa, R.; Alves, A.; Silva, J.; Barros, A.; Pereira, M. L. and Sousa, M. (2015): Effect of *in vitro* exposure to lead chloride on semen quality and sperm DNA fragmentation. Zygote., V. 23, P. 384–393.

Gross, R.A.; Macdonald, R.L. and Ryan-Jastrow, T. (1987): Calmodulin-mediated adenylatecyclase from mammaliansperm. J Biol Chem., V. 262, P. 8672-8676.

Haouas, Z.; Zidi, I.; Sallem, A.; Bhouri, R.; Ajina, T.; Zaouali, M. and Mehdi, M. (2015): Reproductive Toxicity of Lead Acetate in Adult male Rats: Histopathological and Cytotoxic Studies. J Cytol Histol., V. 6, P. 293.

Ho, H.C. and Suarez, S.S. (2001a): An inositol 1,4,5-trisphosphatereceptor-gated intracellular Ca(2+) store is involved inregulating sperm hyperactivated motility. Biol Reprod, V. 65, P. 1606-1615.

Hore, P.; Ahmed, M.; Nagin, D. and Clark, N. (2014): Intervention model for contaminated consumer products: a multifaceted tool for protecting public health. Am J Public Health, V. 104, P. 1377 - 83.

Ignotz, G.G. and Suarez, S.S. (2005): Calcium/Calmodulin andCalmodulin Kinase II Stimulate Hyperactivation inDemembranated Bovine Sperm. Biol Reprod., V. 73, P. 519-526.

Imam, T.S.; Aliyu, F.G. and Umar, H.F. (2013): Preliminary phytochemical screening, elemental and proximate composition of two varieties of *Cyperus esculentus* (Tiger Nut). Nig J Basic Appl Sci., V. 21(4), P. 247-251.

Imran, A.; Muhammad, S. and Khalid, F.Y. (2003): Study of the effects of lead poisoning on the testis in albino rats. Pak J Med Res., V. 42 (3), P. 1-9.

Jaiswal, B.S. and Conti, M. (2003): Calcium regulation of thesoluble adenylyl cyclase expressed in mammalianspermatozoa. Proc Natl Acad Sci, U S A, V. 100, P. 10676-10681.

Kumar, S.B.; Sathyanarayana, J.; Estari, M.; Reddy, K.M. and Prasad, M.S.K. (2010): Effect of alkaloids of *Achyranthes aspera Linn.* on fertility in male albino rats *(Rattus norvegicus*). Asian J Anim Sci, V. 5(2), P. 126 - 130.

Lindemann, C.B.; Gardner, T.K.; Westbrook, E. and Kanous, K.S. (1991): The calcium-induced curvature reversal of ratsperm is

potentiated by cAMP and inhibited by anticalmodulin. Cell Motil Cytoskel, V. 20, P. 316-324.

Lorke, D. (1983): A new approach to tropical acute toxicity testing. Arch Toxicol, V. 53, P. 275 - 287.

Luna, G.H. (1968): Manual of Histologic Staining Method of Armed Forces Institute of Pathology. 35th Edition, McGraw-Hill Book Company, NewYork, pp. 250 - 258.

Malebogoro, A.B.; Khoboso, C.L.; TIou, C.C. and Tshimangadzo, L.N. (2015): Comparison of electro ejaculator and artificial vagina on semen collection from South African indigenous goat following assessment by computer aided sperm analysis. Open J Anim Sci, V. 5, P. 210-218.

Manta, S.; Jyoti, S.; Rajeev, N.; Dharmendra, S. and Abhishek, G. (2013): Phytochemistry of Medicinal Plants. J Pharmacogn Phytochem, V. 1(6), P. 168 – 182.

Marin-Briggiler, C.I.; Jha, K.N.; Chertihin, O.; Buffone, M.G.; Herr, J.C.; Vazquez-Levin, M.H. and Visconti, P.E. (2005): Evidence of the presence of calcium/calmodulin-dependentprotein kinase IV in human sperm and its involvement inmotility regulation. J Cell Sci, V. 118, P. 2013-2022.

Matshaba, B. (2010): Characterisation and cryopreservation of South African unimproved indigenous buck semen. *Master's Dissertation,* University of Free State, Bloemfontein. Johannesburg, South Africa, pp 45 – 51.

Milan, C. and Gyan, C.J. (2014): Toxic effects of transition metals on male reproductive system: A review. J Environ Occup Sci, V. 3(4), P. 204 – 213.

Mokhtar, M. and Maryam, Z. (2011): The behaviour and sexual effects of lead. The rats male adult in testosterone of level. Int J fertil steril, V. 15(1), P. 13 – 20.

Oliveira, H.; Spano, M.; Santos, C. and Pereira Mde, L. (2009): Lead chloride affects sperm motility and acrosome reaction in mice: lead affects mice sperm motility and acrosome reaction. Cell Biol Toxicol, V. 25, P. 341–53.

Oyedepo, T.A. and Odoje, O.F. (2014): Hepato-protective Activities of Tiger Nut (*Cyperusesculentus*) Against Hepatotoxicity Induced by Carbon Tetrachloride in Rats. J Pharm Toxicol Stud, V. 2(4), P. 37 – 41.

Pfadenhauer, L.M.; Burns, J.; Rohwer, A. and Rehfuess, E.S. (2014): Protocol for a systemic review of the effectiveness of interventions to reduce exposure to lead through consumer product and drinking water. Syst Rev, V. 3, P. 36.

Rastogi, S.C. (2011): Essentials of Animal Physiology. 4th Edition, New Age International Limited Publishers, New Delhi. pp. 12 – 24.

Rehman, H.; Alhidary, I.A.; Khan, R.U.; Qureshi Sadique, M.S.U.; Khan, H. and S.H. Yaqoob, S.H. (2016): Relationship of age, breed and libido with semen traits of cattle bulls. Pak J Zool, V. 8(6), P. 1793-1798.

Reid, T.M.; Daniel, F. and Lawrence, A.L. (1994): Mutagenesis by metal – induced oxygen radicals. Environmental Health Protection, V. 102, P. 57-61.

Rusnak, F. and Mertz, P. (2000): Calcineurin: form and function. Physiol, V. 80, P. 1483-1521.

Sabeti, P.; Pourmasumi, S.; Rohiminia, T.; Akyash, F. and Talebi, A.R. (2016): Aetiologies of sperm oxidative stress. Int J Reprod Biomed, V. 14 (4), P. 231 – 240.

Saleh, H.A.; El-, G.A.; El-Fark, M.M and El-Gohary, M. (2009): Effect of maternal lead exposure on craniofacial ossification in rat fetuses and the role of antioxidant therapy. Anat,Histol Embryol, V. 38, P. 392–399.

Sallman, M. (2001): Exposure to lead and male fertility. Int J Occup Med Environ Health, V. 14(3), P. 219-222.

Sanchez, R.; Sepulveda, C.; Risopatron, J.; Villegas, J. and Giojalas, L.C. (2010): Human sperm chemotaxis depends on critical levels of reactive oxygen species. Fertil Steril, V. 93, P. 150–153.

Sarker, S.D. and Nahar, L. (2007): Chemistry for pharmacy students general, organic and natural product chemistry. 2nd edition; John Wiley and Sons, England, PP. 283-359.

Sasikala, N.; Dakshinamoorthy, J.; Mahalakshmi, S. and Archana, M. (2014): Correlation of semen pH with other semen parameters in a sub fertile male population attending a tertiary art center in south India. IJSRP, V. 4(8), P. 1 – 5.

Sharpe, R.M. and Skakkebaek, N.E. (1993): Are oestrogens in falling sperm counts and disorders of the male reproductive tract? Lancet, V. 341, P. 1392 – 1395.

Shashank, K. and Abhay, K.P. (2013): Chemistry and biological activities of flavonoids: an overview. Sci. World J, V. 1(1), P. 1 – 17.

Snyder, L.D. and Walensky, S.H. (1995): Inositol 1,4,5-trisphosphate receptors selectively localized to theacrosomes of mammalian sperm. J Cell Biol, V. 130, P. 857-869.

Soares, E.R.; Sheridan, W.; Haseman, W. and Segall, J.K. (1979): Increased frequency of aberrant sperm as indicators of mutagenic damage in mice. Mutat Res, V. 64, P. 27-35.

Taiwo, A.M.; Ige, S.O. and Babalola, O.O. (2010): Assessments of possible gonadotoxic effect of lead on experimental male rabbits. Glob Vet, V. 5(5), P. 282-286.

Vigeh, M.; Smith, D.R. and Hsu, P.C. (2011): Review article: How does lead induced male infertility?. Iran J. Reprod Med, V. 9(1), P. 1- 8.

Wasco, W.M. and Orr, G.A. (1984): Function of calmodulin inmammalian sperm: presence of a calmodulin-dependentcyclic nucleotide phosphodiesterase associated withdemembranated rat caudal epididymal sperm. Biochem Biophys Res Commun, V. 118, P. 636-642.

Wu, J.Q.; Kosten, T.R. and Zhang, X.Y. (2013): Free radicals, antioxidant defense systems, and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry, V. 46, P. 200-206.

Xia, D.; Yu, X.; Liao, S.; Shao, Q.; Mou, H. and Ma, W. (2010): Protective effect of Smilax glabra extract against lead-induced oxidative stress in rats. J. Ethnopharmacol, V. 130(2), P. 414-20.