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Full Length Research Paper

Modulatory effects of kolaviron (*Garcinia kola* extract) on spermogram and reproductive system of adult male wistar rats in lead acetate induced toxiocity

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This study was designed to determine the effects of the concomitant administration of kolaviron (*Garcinia kola* extract) with lead on male fertility by evaluating some spermogram and histopathology of the testis. 20 adult male Wistar rats were randomly divided into 4 groups of 5 rats each. The rats in group O (negative control) were given normal saline, while rats in group A were given 6 mg/kg body weight of lead acetate (positive control) for a period of 2 weeks. Rats in groups B and C (test groups) were given 6 mg/kg body weight of lead and with concomitant administration of 200 mg/kg body weight of the kolaviron extract once daily for a period of 7 and 14 days, respectively. The histopathological analysis revealed distorted morphological alterations of sperm cells, as well as deleterious effects on the seminiferous tubules, with degeneration of interstitial spaces and narrowing of lumen in the lead exposed groups compared to normal architecture in control group. Sperm cell motility was significantly ($P < 0.05$) lowered in animals exposed to lead and kolaviron extract compared to the control group. The kolaviron extract does not prevent further damage of the testes for the period of two weeks. It rather showed worsening effects when compared with Group A which received lead only for 2 weeks. These are indications of interference with maturation stage of spermatogenesis in the seminiferous tubules. We concluded that extract (kolaviron) of *G. kola* do not prevent the toxic effect of Pb on the seminiferous tubular cells but rather worsen the toxic effect after we 2weeks duration of administration. Herbal preparation of *G. kola* should therefore be used cautiously in both man and animal. The possibility of the plant as an anti-fertility drug in man without toxic agents should be carefully explored.

Key words: Kolaviron, lead acetate, wistar rats, reproductive system, toxicity, testis.

INTRODUCTION

Lead has been mined and used as an industrial raw material for many centuries and was considered by chemist as the oldest metal (Neathery, 2007). It is found at low concentrations in the earth's crust predominantly as lead sulphide (galena). Lead is known to be purely toxic as it has no nutritional value or positive biological effect (Sharon, 1999). According to the Wikipedia lead poisoning(also known as plumbism, colica pictonium,

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saturnism, devon colic, or painter's colic) is a medical condition caused by increased levels of the heavy metal lead in the body.

Humans get lead into their body systems through the gastrointestinal tract, respiratory tracts and occasionally through the skin (Neathery, 2007). The oral exposure to lead is said to be more relevant to human environmental exposure (Martina and Krista, 1987). Chronic lead poisoning is commonly seen in young children from sucking lead paint and lead toys or from old house painting. Lead exposure can occur from contact with lead in air, household dust, soil, water, and commercial products (Rossi, 2008). Lead from the atmosphere or soil can end up in groundwater and surface water. It is also potentially present in drinking water, for example from plumbing and fixtures that are either made of lead or have lead solder (Chisolm, 2004; Menkes, 2006).

Concentrations of blood lead > 40 µg/dl seems to be associated with a decrease in sperm count, sperm volume, sperm motility, and morphological alterations (Grant, 2009). A pregnant woman's elevated blood lead level can lead to miscarriage, prematurity, low birth weight, and problems with development during childhood (Cleveland, 2008). The effects of exposure to high doses of lead on reproduction and development have been established, but not so for those caused by low lead doses or the influence that life stage at which contact with the metal takes place might have.

Bitter kola *(Garcinia kola)* is a species of flowering plant in the Clusiaceae or Guttifera family. It is usually found in Benin, Cameroon, Democratic Republic of the Congo, Ivory Coast, Gabon, Ghana, Liberia, Nigeria, and Sierra Leone. It thrives in the subtropical or tropical moist lowland forests. *G. kola* seed, generally known as bitter kola in Nigeria belongs to a family of tropical plants known as Guttifera (Plowden, 1972).

Despite the fact that physiological studies are still lacking to validate the therapeutic ability of *G. kola* (Orie and Ekon, 1993), its use in African traditional medicine is popular. The seeds are rich in flavonoids, which have been shown to have antibiotic property (Hong-xi and Song, 2001) and employed in the treatment of Diabetes (Tita et al., 2001). *G. kola* has however been reported to cause deterioration of the reproductive system by Braide et al. (2003). Nottidge et al. (2008) also reported prolonged administration of bitter kola extract to have destructive effects on the testes of dog.

The researched biological (medicinal) properties of *G. kola* includes: anti-biotic property (Hong-xi and Song, 2001), anti-inflammatory properties (Braide, 1991), anti-microbial properties (Madubunyi, 1995; Okunji et al., 1995; Adefule-Ositelu et al., 2004) and antioxidative properties (Farombi et al., 2004; Terashima et al., 2002).

This work was designed to study if *G. kola* extract (kolaviron) prevent lead toxicity on the reproductive system of adult wistar rats and to investigate the reported suggestions that G.kola may have adverse effects on the reproductive system (Braide et al., 2003). A lot of humans work in industries that allow frequent exposure to lead; industries like paint companies, battery companies and companies which use fuel additives. This therefore calls for scientific research to find out substances that could prevent lead toxicity as a way to ensure the work environment is safe.

However, lead has been reported to be gonadotoxic and may cause serious damage to the reproductive system. Chowdury (1986) and Guloik (1989) reported testicular histochemical changes and spermatogenic inhibition in wistar rat treated with lead.

Moreover, despite the extensive use of kolaviron as an herbal medicine, much work has not been done to study some of the toxicological implications on other related systems. Motivated by this, it is necessary to study if kolaviron has capability to prevent lead toxicity on the reproductive system of male wistar rats.

METHODOLOGY

Treatments and management of experimental animals

Twenty (20) adult Wistar rats were used for this research. The animals were housed in hutches and fed with rabbit chow purchased from Ladokun Feeds. They had access to feed and water *ad libitum*.

They were acclimatized for three weeks under standard conditions of temperature and illumination (12 h light and 12 h dark). The rats were randomly divided into four groups of 5 animals each (O, A, B, and C). Group O was the control and received 0 mg/kg body weight of lead acetate and kolaviron, respectively, but were given normal saline for the period of the experiment. Group A received 6 mg/kg body weight of lead acetate, with normal daily food ration and clean water. Group B received 6 mg/kg body weight of lead acetate and 200 mg/kg body weight of kolaviron for 7 days, while Group C received 6 mg/kg body weight of lead acetate and 200 mg/kg body weight of kolaviron for 14 days. The animals were sacrificed using the cervical dislocation method, after which a Y-skin incision was made over their perineum and the epididymis and testes were exposed and harvested. The epididymis was crushed and mixed with some drops of normal saline and immediately analyzed for sperm count, sperm motility and morphology while the testes was immediately fixed in bouin's fluid.

Preparation of kolaviron and lead acetate solution

Lead acetate was procured from British Drug (BDH, UK). Five (5 g) of lead acetate was dissolved in 1 L of water. 6 mg/kg body weight of lead acetate was administered to the three groups of animals using an insulin hypodermic injection (40 units to 10 mls). Fresh bitter kola seeds were obtained from the local market in Ogbomosho and certified by the Department of Biology of the Ladoke Akintola University of Technology. The seeds were dried, the outer cover peeled, it was then sliced, air dried and pulverized into powder after which the ethanol extraction was processed to yield kolaviron. Extraction was carried out by using the soxhlet

extractor as described by Tairu et al. (1991). 60 g of the extract was measured out and dissolved in 1 L of distilled water to give 60 mg/ml.

Spermogram

Sperm analysis was done using new improved Neubauer's haemocytometer (Deep 1/10 mm, LABART, Germany) counting chamber and the results recorded (Harrison and Moore, 1980).

Histology

The testes were fixed in Bouin's fluid until processed. Thereafter, they were stained using the haematoxylin and eosin method for routine histological studies. The slides were thereafter viewed on a light microscope and the photo-micrographs were taken.

Statistical analysis

The results were statistically analyzed using the Prism 5 for Windows (version 5.02, © GraphPad Software, Inc). The mean, standard error of mean and standard deviation of the data were calculated. In addition, the student t-test was used to check the significance of the results. The difference of means were considered significant at $P < 0.05$.

RESULTS

Weight

There was an observed increase in the weight of the control Group O which did not receive the treatments (Figure 1). There was an observed decrease in the weight of the rats in Group A which received 6 mg/kg bw of lead for 2 weeks. It was also observed that Groups B and C which received 200 mg/kg bw of kolaviron with concomitant 6 mg/kg bw of lead for 1 and 2 weeks, respectively had observed reduction in weight of the animals. The first week of kolaviron administration however showed little decrease in weight when compared to the two weeks administration. There was however no significant ($P > 0.05$) difference in the weight decrease of Groups A, B and C.

Spermogram

Lead acetate greatly affected the microscopic count of Groups A, B and C as there was an observed significant ($P < 0.05$) reduction in the microscopic count values as presented in Table 1 and Figure 2. It was observed that prolonged administration of lead for two weeks significantly ($P < 0.05$) reduced the microscopic count as observed in Group A. The administration of lead acetate reduced the number of rapid progressive sperm cells significantly ($P < 0.05$) as seen in Group A. Group C which received the lead coupled with kolaviron for two weeks has the lowest number of rapid progressive sperm cells. It is also observed that the number of slow progressive sperm cells in Groups B and C also significantly increased when compared to Group A which received lead only.

The number of non-progressive sperm cells also increased in the Groups A, B, and C with the highest number in Group C which received kolaviron for two weeks. It can be observed that lead administration significantly reduced ($P < 0.05$) the number of normal sperm cells and increased the number of head defects as seen in Groups A, B and C when compared to the control in Group O. However, as observed in Group C, the number of sperms with head defects is reduced when compared to Group A though with no statistical significance. Prolonged kolaviron administration probably improved the incidence of Head defected sperm cells in Group C.

Histogram

The photomicrographs of the testes taken for the Groups O, A, B, and C at different magnifications (that is, ×40, ×100, ×400) are represented in Figures 5 and 6. Group A shows interepithelial spaces as a result of degenerating epithelial cells, widening of lumen, mild congestion. Group B has a lumen filled with dead germinal cell, severe degeneration of interstitial space. Group C shows vacuolization of testicular epithelium, congestion of spermatozoa cells, and lumen filled with degenerated germinal cells.

DISCUSSION

Since it was first reported by Carlsen et al in 1992, more authors have corroborated and reported a decline in the male reproductive capacity over the last decades (Carlsen et al., 1992; Adami et al., 1994; Carlsen et al., 1995; Swan et al., 2000; Skakkebaek et al., 2001; Zheng et al., 1997; Aitken et al., 2004; Richiardi et al., 2004). A combination of factors including environmental and lifestyle have been reported to contribute to adverse effects on the reproductive health in men (Priya and Reddy, 2012). Metal toxicity in particular (Pizent et al., 2012), including lead have been implicated (Priya and Reddy, 2012). The disruption in spermogram, especially, sperm motility and morphology, coupled with the deleterious effects of lead on the seminiferous tubules, with degeneration of interstitial spaces and narrowing of lumen as observed in our study established the toxicity of lead on male reproductive function. These results have a wide implication for reproductive health in highly polluted

Table 1. Microscopic count values.

Parameter	Group O (control)	Group A	Group B	Group C
Sperm count ×10 ^{6}	78.14±7.84^{abc}	32.28±3.27^{ade}	12.44±3.55^{bd}	11.18±5.07^{ce}
Percentage motility				
Rapid progressive	84±1^{abc}	66±6.7^{ad}	50±4.47^{b}	38±8.6^{cd}
Slow progressive	8±2^{bc}	12±2de	24±2.44^{bd}	24±2.44^{ce}
Non progressive	5±0	11±3.67	14±2.44	18±4
Dead cells	5±0	11±3.67	12±2	19±4
Percentage morphology				
Normal	79±2.9^{abc}	52±3.7^{a}	56±2.4^{b}	54±2.4^{c}
Head defect	10±2.2^{abc}	38±3.7^{a}	34±2.4^{b}	36±2.4^{c}
Middle piece defect	6±1	5±0	5±0	5±0
Tail Defect	6±1	5±0	5±0	5±0

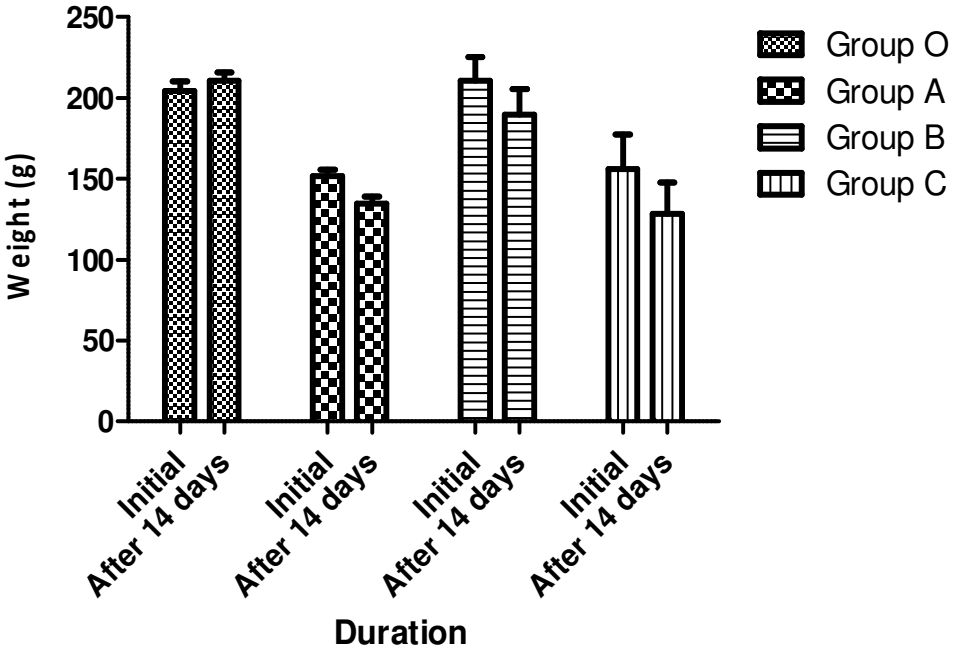

Figure 1. Relationship between initial and final weights of all the groups.

countries like Nigeria. Environmental discharge of lead due to the use of petroleum products (especially leaded petrol), construction works, paint removal, demolition, vehicle batteries and car repairs contribute to airborne Pb pollution (Pizent et al., 2012; De Rosa et al., 2003) and possibly introduce high concentrations of this potential reproductive toxicant into the environment. This may be particularly true for Nigeria. Despite the global preference for the use of unleaded petrol and the ban of lead-based paint and lead solder in food cans, these products are still predominant in Nigeria market. Subsequently, the Nigeria general population may be exposed to enormous amount of lead in food and drinks. All these factors put Nigerian population at high risk of Pb toxicity (De Rosa et al.,

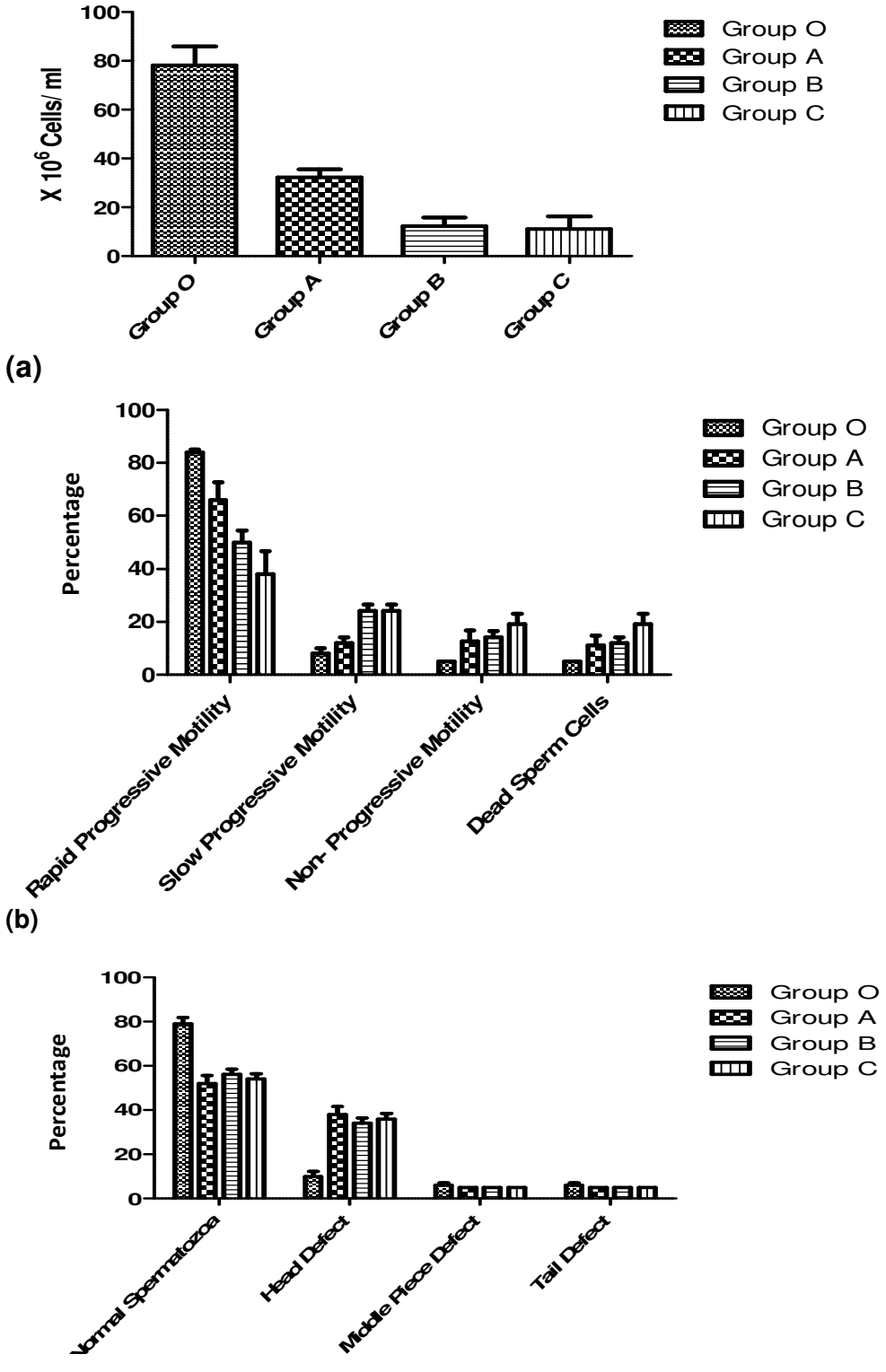

Figure 2. Distribution of spermogram (sperm count, motility and morphology, respectively).

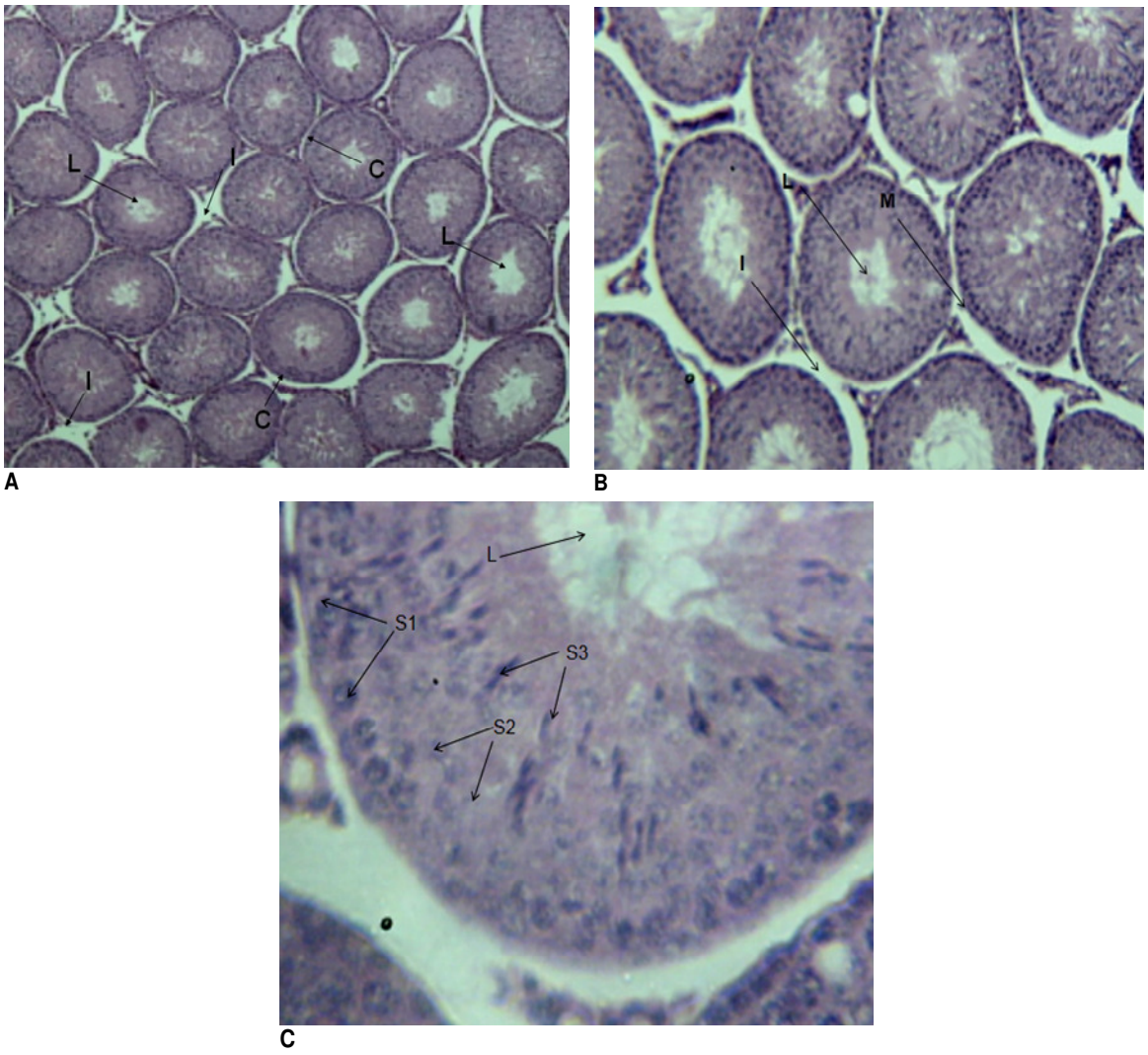

Figure 3. Group O (control, water only). (A) The transverse section of the control group using H&E stain (×40). I = interstitial space, L = lumen, M = basement membrane; (B) transverse section of the transverse section of the control group using H&E stain (×100) I = interstitial space, L = lumen, M = basement membrane; (C) transverse section of the control group using H&E stain (×400). My = myofibroblast, Le = leydig cell, L = lumen Sa = spermatogonia type A, Sb = spermatogonia type B, S1 = primary spermatocytes, S3 = spermatids, S4 = spermatozoa, St = sertoli cell.

2003; Underwood and Smitasiri, 1999). Hence, the gonadotoxic effect of lead as observed in this study may have far more reaching implications in Nigeria population. A more recent study by Pantet al (2003) demonstrated an increase in lead and cadmium levels in the seminal plasma of infertile men. They also reported a significant negative correlation of these toxicants with sperm motility and concentration in oligoasthenozoospermic men. Although, data on reproductive toxicity in men are scanty for most metals (Maines, 1984), we have earlier reported a possible toxicity of cadmium in Nigeria males (Akinloye et al., 2006) and further report lead toxicity in male rats in our current study.

A recent study by Adaramoye et al. (2012) reported that kolaviron ameliorate the irradiation induced sperm toxicity by maintaining the sperm quality near normal and further maintain the structure and function of seminiferous tubules of radiationally challenged rat. In contrast, the kolaviron failed to achieve similar goal in our study. Observations from our study show that kolaviron did not protect but rather caused more destructive effects on the spermogram and testicular structure and function of lead treated animals. Surprisingly, kolaviron control animals also show deleterious effect on testis. This result

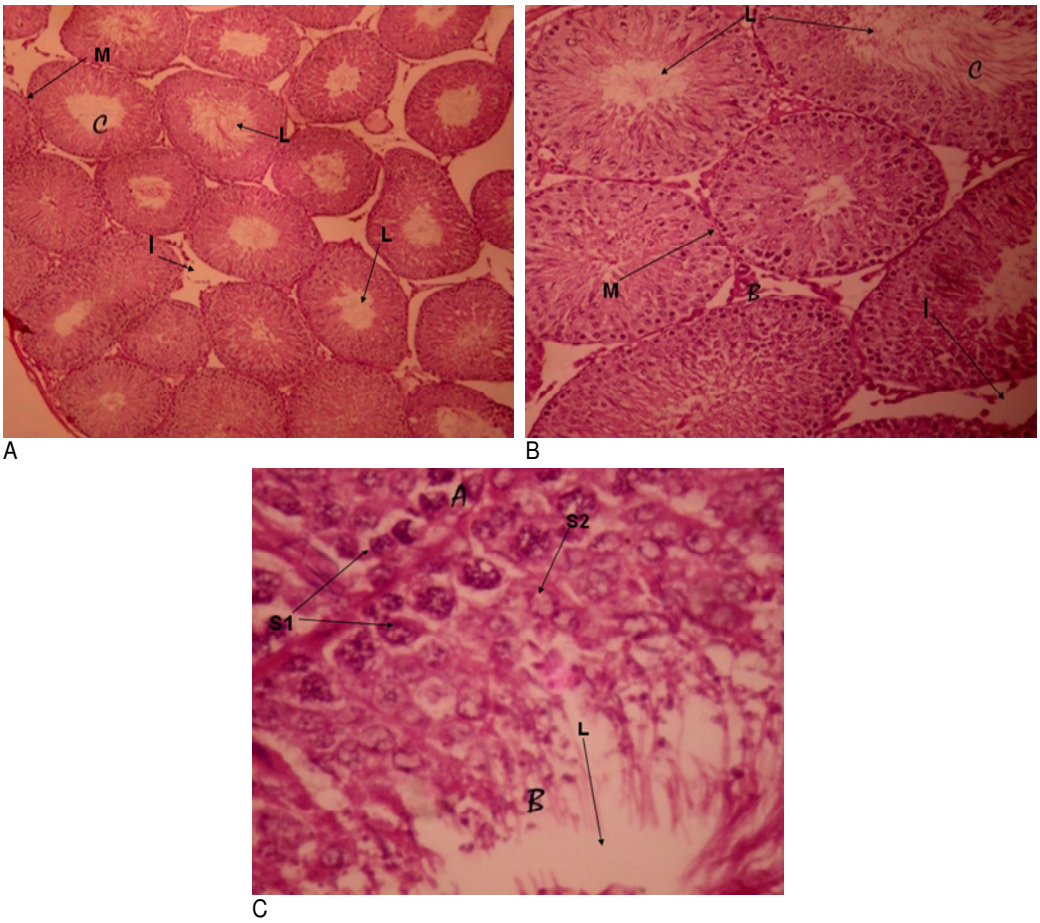

Figure 4. Group A (6 mg/kg body weight Lead, 7 days). (A) Transverse section using H&E stain at Mag (x40) A= tunica albuginea, B= interstitial cells, C=spermatids; (B) Transverse section of the lead group using H&E at Mag (×100) A=tunica albuginea B= interstitial cells C= spermatids; (C) shows a transverse section of the lead group using H&E stain at Mag (x400) A=spermatocytes B= spermatozoas C= sustencular cells.

supports the early report by Braide et al. (2003). Interestingly, this destructive effect of kolaviron seems to be duration dependent.

Braide et al. (2003) reported a marked reduction in serum testosterone concentration of rats administered with methanolic extract of *G. kola.* Though, we did not measure testosterone in our study but the photomicrograph of the treated groups all show some form of mild to moderate testicular atrophy, a generalized degeneration of the interstitial spaces and narrowing of the lumen. The Leydig cells which are the principal cell of the interstitial supporting tissue that synthesize and secrete testosterone in the intact interstitial space suffer most from this degeneration. This probably explains the reduced testosterone described by Braide et al. (2003). Our observation corroborated the findings of Nuttidge et al. (2008) who reported that prolonged administration of *G. kola* for 6 weeks on dogs showed hypoplastic seminiferous tubules, disorganised epithelia cells and absence of viable spermatids and spermatozoa. This probably explains the reduced spermogram of our rats treated with kolaviron which was further worsened when administered with lead. The observations in our study are probably still mild and may worsen on prolonged administration.

Though kolaviron has been reported as an antioxidant ((Farombi et al., 2013 and 2004), anti-gonadotoxic agent (Farombi et al., 2013, 2012, 2008, Abarikwu 2012, Adedara 2013a and b), exhibited hepatoprotective effect, ameliorate (Farombi et al, 2008 and 2004 Adaramoye et al, 2008). Di-n-butylphthalate- induced testicular toxicity (Farombi et al., 2007) and ameliorate radio-gonatotoxic

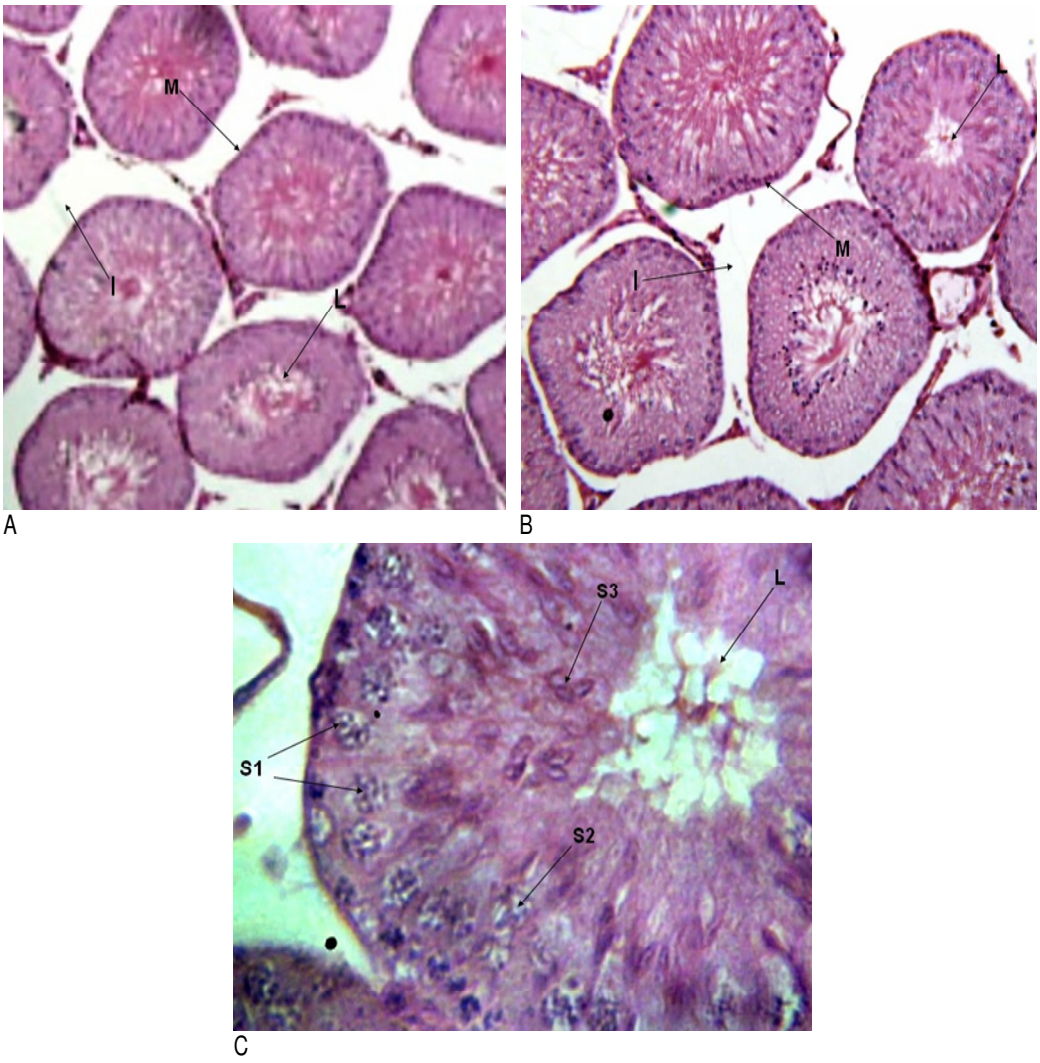

Figure 5. GROUP B (6 mg/kg body weight Lead, 200 mg/kg body weight Kolaviron, 7 days). (A) Transverse section of the control group using H&E stain (×40). I = interstitial space, L = lumen, M = basement membrane, S = Spermatogonia; (B) transverse section of the control group using H&E stain (×100). I = interstitial space, L = lumen, M = Basement membrane, S1 = spermatogonia, S3= spermatids; (C) transverse section of the control group using H&E stain (×400). I = interstitial space, St = sertoli cell, L = lumen, M = basement membrane, S1 = spermatogonia, S3= spermatids, V= vessels.

effect in rats (Adaramoye et al., 2012), our study clearly shows complications in lead induced testicular toxicity. Furthermore, earlier studies have reported a decrease in weight of rats by administration of kolaviron (Uko et al., 2001) and lead (Castellino, 1969). Our study did not only agree with the report but extended the findings that administration of both lead and kolaviron further decrease the animal weight significantly. The implications of this finding on the reproductive performance of this animal remain to be elucidated.

Conclusion

The results of this study established the gonadotoxic effect of Pb on male rats as well as extended and supported previous findings implicating Pb-related male infertility. Infertility is currently a serious global phenomenon which may be modulated by nutritional status. In Nigeria, this problem may be more deleterious because of the well-recognized deficiency of protective micronutrients in addition to heavily environmental challenges

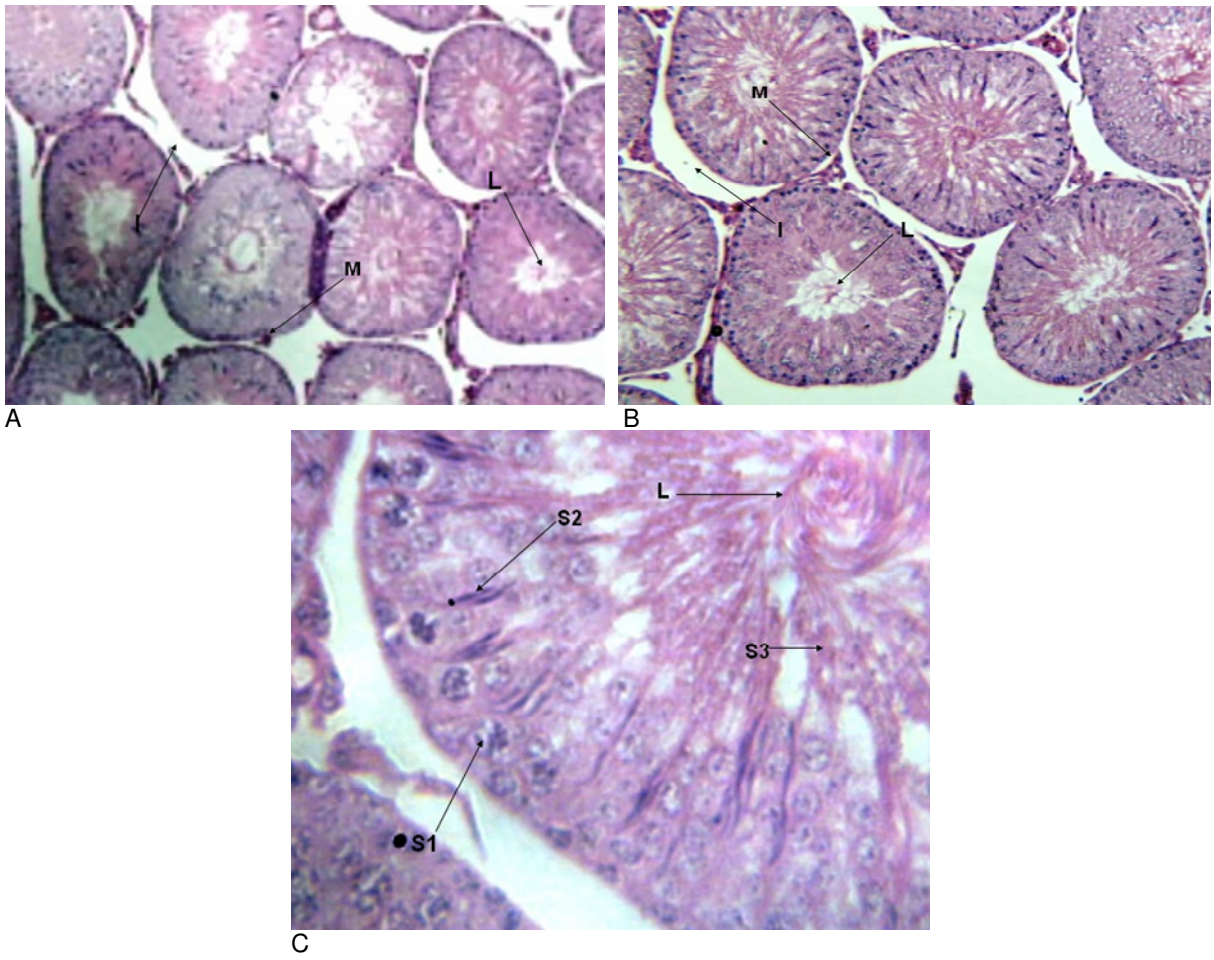

Figure 6. Group C (6 mg/kg body weight lead, 200 mg/kg body weight kolaviron, 2 weeks). (A) Transverse section of the control group using H&E stain (×40). I = interstitial space, L = lumen, S1 = spermatogonia; (B) transverse section of the group using H&E stain (×100). I = interstitial space, L = lumen, M = basement membrane, S1 = spermatogonia;(C) Transverse section of the control group using H&E stain (×400). St = sertoli cell, L = lumen, M = basement membrane, S1 = spermatogonia, S2= spermatocytes, S3= spermatids.

in this sub-region (Underwood and Smitasiri, 1999). Furthermore, we clearly showed that kolivaron, anflavonoid extract of *G. kola* fails to ameliorate but complicated the gonadotoxic effect of lead.

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