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Testiculo-Protective Effect of Stem Bark Extract of *Enantia chlorantha* on Lead Induced Toxicity in Adult Wistar Rat (*Rattus norvergicus*)

Oyewopo AO[1], Saalu LC[4], Dare BJ[1]*, Oyewopo CI[2], Jimoh AA[2], Yama OE[3], Kadir RE[1] and Olaniyan OT[5]

[1]*Department of Anatomy, University of Ilorin Teaching Hospital, University Road, Ilorin, Nigeria*
[2]*Department of Anesthesia, University of Ilorin Teaching Hospital, Ilorin, Nigeria*
[3]*Department of Anatomy, college of Medicine of the University of Lagos, Lagos, Nigeria*
[4]*Department of Anatomy, Lagos State University, Ikeja, Lagos, Nigeria*
[5]*Department of Physiology, Bingham University, Karu, Nasarawa, Nigeria*

Abstract

Background: The study was designed to investigate the effect of stem bark aqueous extract of *Enantia chlorantha* on the sperm parameters and the histo-architecture of the testis of adult Wistar rats treated with lead (IV) oxide.

Methods: A total of 20 adult male rats weighing between 180 g-200 g were randomly selected into 4 groups (n=5). Group 1 was giving only phosphate buffered saline; Group 2 was treated with 150 mg/kg body weight of the extract; Group 3 was treated with 300 mg/kg body weight of the extract and destroying agent [lead (IV) oxide]; Group 4 was treated only with the destroying agent [lead (IV) oxide] orally once daily for 14days of experimental period respectively. Animals were sacrificed by cervical dislocation 24 hours after the last administration of 14 days of experiment. Testis was dissected out following abdominal incision, fixed in 10% formo saline for histological processing using H/E stain. Semen was obtained from caudal part of the epididymis for analysis.

Results: The histological observation revealed that seminiferous epithelium was prominent showing its constituents; the spermatogonia as well as the spermatids and spermatocytes were quite obvious in the control rats and rats treated with the extract (Group 1,2 and 3) as compared with the negative control (Group 4) that received lead IV oxide only. The photomicrograph in group 4 revealed that the interstitial spaces were abnormally widened and the Leydig cells were not observable, hence destroyed. Sperm count, motility, viability and progressivity of the sperm of animals in Group 1,2 and 3 were excellent ($P<0.05$) while those in Group 4 were weak.

Conclusions: The aqueous extract of stem bark of *Enantia chlorantha* maintained the cytological integrity of testes and viability of sperm motility and sperm count of the rats that were treated with the extract when compared with the control rats. This protective effect of *Enantia chlorantha* against the destroying agent used prophylactic might be due to its antioxidant property.

Keywords: *Enantia chlorantha*, Sperm parameters, Testis, Wistar rat

Introduction

Plants of medicinal value are widely used to treat diseases in Nigeria, Africa, Asia, even in parts of Europe and America. These plants are use locally and even in preparation of modern drugs, *Enantia chlorantha* is one of such plants. Since medieval times, the forest has produced food and herbs to maintain good health of humans [1].

Enantia chlorantha is an ornamental tree of up to 30 m high, with dense foliage and spreading crown. The stem is fluted, the outer back is thin, dark brown while, the inner bark is light brown above and pale green beneath. The leaves display up to 20 pairs of lateral veins, and parallel secondary nerves. It is the component of fever preparation by traditional medicine practitioners in forest regions of Nigeria in the management of malaria without recourse to its possible deleterious effect [2]. It is locally called ñDokita Igboò in Nigeria [3]. It is used in treating malaria locally. It has also been reported that cough and wounds are treated through the infusion of the bark of *Enantia chlorantha* [4].

Phytochemical analysis of the aqueous and ethanolic extracts of *Enantia chlorantha* stem bark revealed the presence of phenolics, flavonoids, alkaloids, glycosides and saponins [5]. A work showed that the bark stem extracts of *Enantia chlorantha* inhibited the growth of all the bacteria and fungi which include two Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogens*), six Gram-negative bacteria (*Escherichia coli, Salmonella typhi, Shigella sonnei, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and two fungi (*Aspergillu niger* and *Candida albicans*). These properties suggest that the plant extract is broad spectrum in activity and that its mode of action may not be due to inhibition of cell wall synthesis [6]. Furthermore, it has been found out that administration of aqueous extract of *Enantia chlorantha* lead to a significant reduction of liver alkaline phosphatase activity noticeable after the first dose. For lactate dehydrogenase activity, the reduction in enzyme activity was not significant until after the fifth dose of the extract administration [2]. A more closely related study showed that the extract of *Enantia chlorantha* significantly increased sperm motility and viability dose dependently without a significant increase in the sperm counts [7].

Due to the wide use of *Enantia chlorantha* in treating malaria and some other diseases, it is important that research work be taken on the plant.

This research work is therefore, aimed at finding the possible effect of stem bark aqueous extract of *Enantia chlorantha* on sperm parameters and changes in the histo-architecture of the testes of the adult Wistar rat treated with lead (IV) oxide.

Materials and Methods

Experiment animals

Twenty adult male Wistar rats weighing between 180 g¬¬¬-200 g were obtained from animal house in the Anatomy Department University of Ilorin, Nigeria. The rats were allowed to acclimatize for 2 weeks at photo periodic condition of 12 hours light and 12 hours darkness natural cycles and fed with a standard Pfizer diet from Bendel

***Corresponding author:** Dr. Dare BJ, Department of Anatomy, University of Ilorin Teaching Hospital, University Road, Ilorin, Nigeria, Tel: +234-807-780-5474; E-mail: jdare_tolu@yahoo.com

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Feeds Ilorin and water *ad libitum*, the experiment was carry out in accordance with the Animal Experimentation Committee Regulation of the Institution.

Extract preparation

Fresh stem barks of enantial chlorantia were obtained from Oja Oba market, Ilorin, Nigeria. Authentication was done in the Department of Plant Biology, University of Ilorin, Nigeria. The stem bark was sun-dried, after which it was grinded into a powder form, 490 g of the sample was soaked in 1.6 L of warm distilled water for 24 hours and then filtered. The filtrate was concentrated using a water bath maintained at 30°C, extract was obtained from concentrate which was dissolved in phosphate buffered saline for dosage preparation and media for administration and preserved in refrigeration throughout the experimental period.

Extract administration

The extract was given oro-gastrically with the aid of oral cannula by gavagesô once daily at an interval of about 24 hours at 0700 hour for 14 days of experiment. This was given according to the average body weight of animal in each group. Group 1 received phosphate buffered saline serves as control; Group 2 received 150 mg/kg body weight of the extract and Group 3 received 300 mg/kg body weight of the extract and 1ml of lead IV oxide. Group 4 received 1ml of lead IV oxide only for 14 days of experimental period respectively.

Animal sacrifice

Animals were sacrificed by cervical dislocation 12 hours after the last administration; Testes were collected following abdominal incision from the scrotum.

Seminalysis

The caudal epididymis was dissected out, several incision (1 mm) were made in the caudal epididymis which was suspended in 1mls of Hamôs f-10 solution [8] after 3-5 minutes of incubation at 37°C, the sperm swim up and the sperm concentration and motility were determined by using new improved nebular haemocytometer [9].

Histological analysis

Testes were carefully dissected out following abdominal incision and fixed in 10% formo saline and processed routinely for paraffin embedding. 5 µ sections were obtained with rotary microtome and processed for Haematoxylin and Eosin Stalin (H / E). Sections were observed with light microscope and photomicrograph was taken for further analysis [10].

Statistical analysis

The value are recorded as mean ± SEM at $p < 0.05$ significant difference using student t-test (SPSS).

Results

Semen analysis revealed a significant ($p<0.05$) reduction in the sperm count, sperm motility and viability in the group that was treated with lead only as compared with the control and the group that was treated with the extract only as shown in Table 1. There was an increase in the sperm characteristic of the rats that were treated with the extract only over the rats that were treated with both the extract and lead prophylactically.

The histological integrity of the animals in the control group as shown in plate 1 (Figure 1) and those treated with the extract only as shown in plate 2 (Figure 2) were maintained; spermatic cell at difference stages of development was obvious and the germinal epithelium lining the basement membrane was equally prominent in the rats treated with the extract only (plate 2) and in the control group (plate 1) that received only phosphate buffer saline.

	Sperm count (Million/ml)	Sperm motility (%)	Viability (%)	Progressivity
GROUP 1 (Control)	66.67 ± 0.88	80.96 ± 1.01	87.9 ± 0.54	B
GROUP 2 (Extract only)	53.20 ± 7.85*	71.21 ± 1.98*	76.58 ± 2.019*	C
GROUP 3 (Extract + Lead)	47.60 ± 7.15*	69.59 ± 3.48*	74.25 ± 1.87*	C
GROUP 4 (Lead only)	11.47 ± 5.97*	28.39 ± 3.56*	22.41 ± 4.68*	D

*Significantly different from the control at $P < 0.05$. (n =5)
B- Very Good
C- Good
D- Average

Table 1: Showing results of the sperm parameters of the rats in Mean ± SEM.

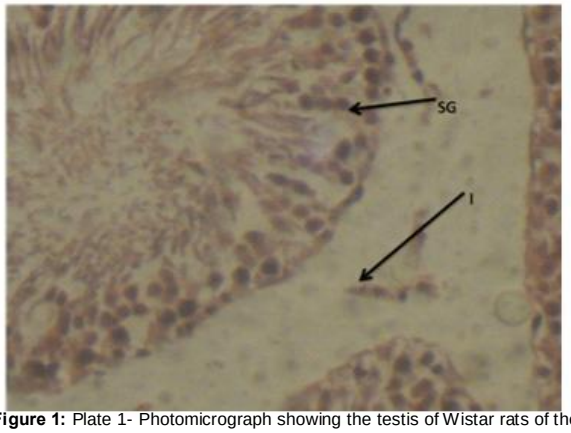

Figure 1: Plate 1- Photomicrograph showing the testis of Wistar rats of the control group using H/E stains Mg × 100. LY- Leydig cells, I- interstitial space, SG- spermatogonia.

The group that was treated with the lead oxide and the extract as shown in plate 3 (Figure 3); shows that the germinal epithelium and spermatic cells were more prominent at different phases of development when compared with the group that received lead oxide only as shown in plate 4 (Figure 4) which shows a distortions in the arrangement of the spermatic epithelium and abnormal widened of the interstitial space.

Discussion

The protective effect of *Enantia chlorantha* was investigated on the testicular cyto-architecture and on the semen analysis in adult Wistar rat. The usual quantity of semen ejaculated at each coitus averages 3.5 millilitreôs in man, when the number of sperm in each millilitre fall below normal, the animal is likely to be infertile even though only a single sperm is necessary for fertilization, the ejaculate usually must contain a tremendous number of sperm for one sperm to fertilize the ovum, whenever the majority of sperm are morphologically abnormal or are found to be non motile, the condition is likely to be infertile, even though the remainder of the sperm appear to be normal [7].

The present study revealed that there was significant ($p<0.05$)

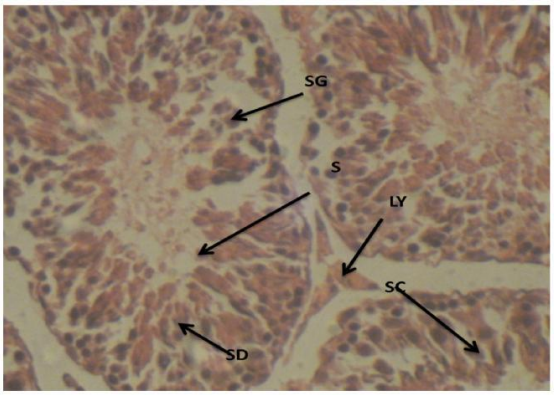

Figure 2: Plate 2- Photomicrograph showing the testis of Wistar rat from Group 2 treated with the extract of *Enantia chlorantha* only using H/E stain Mg × 100. LY- Leydig cells, SG- spermatogonia, SD- spermatid, SC- spermatocytes, S- spermatozoa.

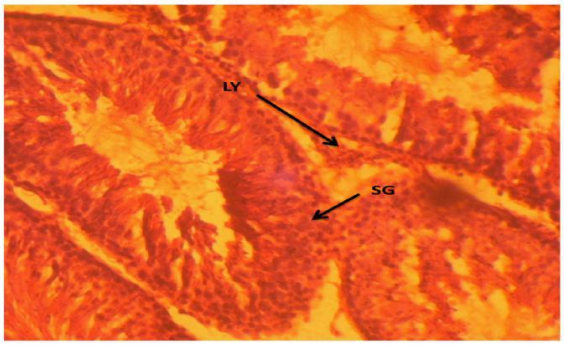

Figure 3: Plate 3- Photomicrograph showing the testis of Wistar rats of Group 3 treated with both *Enantia chlorantha* and lead (IV) oxide Using H//E Stain Mg × 100. LY- Leydig cells, I- interstitial space, SG- spermatogonia.

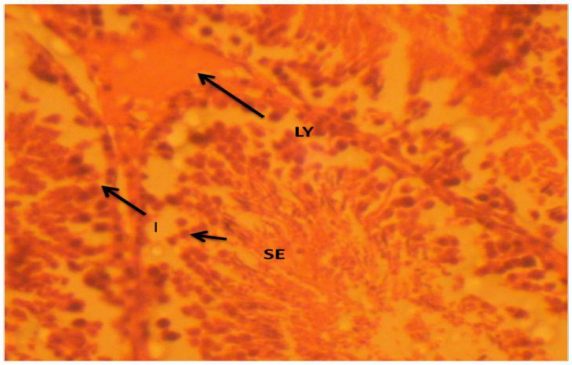

Figure 4: Plate 4- Photomicrograph showing the testis of Wistar rats of Group 4 treated with the destroying agent, lead (IV) Oxide Using H/E Stain Mg × 100. LY- Leydig cells, I- interstitial space, SE- seminiferous epithelium.

decreased in the sperm count and reduction in motility of sperm in the group that was treated with the lead oxide only, but the group that was treated with both the extract and the lead oxide revealed that there is an improvement in the sperm count and motility over the group that received lead oxide only. The control and the group that received extract only showed a marked improvement in sperm integrity and characteristic. The improvement in the sperm quality and quantity of the rats treated with the extract might be due to the antioxidant property of the *Enantia chlorantha* according to Salman and Adesokan [7]. This will consequently enhance fertility in animals treated with the extract. The photomicrographs revealed the histology of the testis of the animal in the control group. It portrays the normal histology of the testis showing clearly the testicular lobules with their seminiferous epithelium and its constituent cells. The testicular histology of the group that received extract only has close resemblance with the control group; the seminiferous epithelium is prominent showing its constituents; the spermatogonia as well as the spermatids and spermatocytes are quite obvious. The testicular integrity of the rats treated with the extract only was maintained in close proximity with the control rats an indication of fertility enhancing ability of the aqueous extract of *Enantia chlorantha* [7].

The photomicrograph of the testis of the animal in group 4 shows that lead (IV) oxide disrupts spermatogenesis by either destroying the spermatids, spermatocytes and the spermatozoa or by interrupting spermatogenesis. Also the interstitial spaces were abnormally widened and the Leydig cells were not observable, hence destroyed; this revealed the antifertility action of lead oxide [11]. Photomicrograph of the testis of animal in group treated with both extract of *Enantia chlorantha* and lead (IV) oxide, shows degenerating of the seminiferous epithelium. However, the spermatogonia are still most prominent; other cells are fairly observable. The Leydig cells are also observable. This shows the regenerative effect of the extract on the seminiferous epithelium as well as the Leydig cells in this group of animals [7].

In summary, the extract produce a regenerative effect on the testis of the animal treated with both the extract of *Enantia chlorantha* and lead (IV) oxide, particularly in relation to the Leydig cells and the germinating epithelium.

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