Aqueous Extract of *Juglans Nigra* Prevents Lead Induced Testicular Toxicity in Rats

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**Abstract**

**Background.** Lead is a heavy metal that has been known for its adverse effects on many body organs and systems and thus their functions.

**Aim.** In this study, the toxic effect of lead on the testicular system was investigated, and Aqueous Extract of *Juglans Nigra* (JnE) (a well known antioxidant) was administered orally to prevent this effect.

**Materials and Methods.** Twenty-four adult male Wister rats, randomly divided into four groups (n = 6), were used for this study. Group B and Group D were given 3g of JnE/Kg Body Weight/Day (orally) and 1% sodium acetate and Lead acetate respectively, while group A (control) and group C were given sodium acetate and lead acetate respectively. All treatments were for eight weeks. The animals were sacrificed twenty-four hours after the last treatment. Sperm count, motility, morphology, and testosterone level were determined. The control and “test” groups were compared using independent-sample t-test.

**Results.** The results obtained showed that lead caused a significant decrease in epididymal weight, sperm count, sperm mortality and testosterone level; and significant increase in abnormal structure of spermatozoa. These (abnormalities) were however, prevented in the JnE treated groups.

**Conclusion.** It is therefore concluded that oral administration of JnE promote fertility and annuls lead induced testicular toxicity.

**Introduction**

Lead is one of the first metals to be smelted and used [1]. It probably was first mined in Turkey around 3500 BC [2]. Its density, workability and corrosion resistance were among the metals attractions [3].

Lead poisoning (plumbism or painter’s Colic [4]) is one of the oldest known environmental hazards [3]. But the level of Lead found today in most people is in order of magnitude greater than those of pre-industrial times [5, 6]. Humans in one way or the other get exposed to lead through air, water, soil, food and consumer products [7]. Still, the modern understanding of their full extent and the small amount of Lead necessary to produce the hazards is relatively new [5]. In fact, blood lead levels once considered safe are now considered hazardous. Also, no level of lead in the body below which no harm can occur has been discovered [5].

Lead interferes with a variety of body processes and is toxic to the body systems including the cardiovas-
cular, reproductive, haematopoietic, gastrointestinal, renal and nervous systems [4], renal functions [8], release of glutamate learning [9].

In the humans, lead inhibits porphobilinogen synthase and ferrochelatase preventing both porphobilinogen formation and the incorporation of iron into protoporphyrin XI, the final stage in heme synthesis. This causes ineffective heme synthesis and subsequent microcytic anemia [10]. Also, research has shown that lead affects both male and female reproductive system mainly because of its ability to cause lipid peroxidation [8]. And a growing body of evidence suggest that metal cation of Lead as well as Iron, Aluminum stimulate free radical formation. Also, Quinlan et al., [10] observed that even when lead ions alone do not induce peroxidation, they do accelerate the rate of peroxidation caused by iron ions.

These oxidation properties of lead suggest that a source of antioxidant such as Walnut (*Juglans nigra*) should have the tendency to prevent the adverse effect of Lead on the body. *Juglans nigra* contains a high amount of copper which is believed to take part in the removal of free radicals from the body, hence preventing cell structure damage [11]. Studies funded by the California Walnut Commission [12] show the effectiveness of walnut against condition ranging from heart diseases to diabetes, osteoporosis, lung cancer and other diseases [13].

Since humans are inseparable from their environment and almost entirely not free from exposure to Lead, it is very necessary to figure out ways by which our body can be made to still maintain homeostasis (basis of good health) even on exposure to relatively high level of lead exposure. This research work, therefore, aims at knowing whether or not oral administration of *Juglans nigra* will prevent Lead induced testicular toxicity base on the knowledge that *Juglans nigra* has very strong antioxidant components. This research could, therefore, propose another way by which lead toxicity could be tackled.

### Materials and Methods

Twenty four adult male Wister rats [average Body Weight (BW) 140.75 ± 4.41 g] obtained from the animal house section of Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Nigeria, were used for this study. The animals were kept in the animal house section of Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria, and allowed to acclimate over a period of ten days.

### Plant Materials

The nuts of *Juglans nigra* were bought from Zaria, Kaduna state, Nigeria and authenticated at the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria.

### Preparation of Aqueous Extract of *Juglans nigra*

The preparation of aqueous extract of *Juglans nigra* was done in Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria following the method described by Henry et al., [14]. The shells were removed and the *Juglans nigra* were grounded to a fine powder. The fine powder was left to dry under shade, and after then soaked in distilled water (1:1, by volume) in a separating funnel plugged with cotton wool. It was drained after twenty-four hours and rewashed with more of the solvent. The filtrate was poured in an evaporating dish and placed on a water bath at 80 °C, and reduced pressure (70% atmospheric pressure) to remove the solvent. After all the solvent had evaporated, the extract was scrapped and stored in a dry glass container at a temperature of 0 – 4 °C. Yield was 5.67% w/w. Fresh solution of the extract was prepared in distilled water just before use to maintain its potency.

### Animal Treatment

The twenty four rats were randomly grouped into four (Group A, B, C and D, n = 6). Rats in group A served as the control and were neither exposed to lead nor treated with *Juglans nigra* extract (JNE), but they were allowed to drink 1% sodium acetate *ad libitum*. Group B rats were allowed to drink 1% sodium acetate *ad libitum* and also administered 200 mg of JNE/Kg BW/Day. Group C and D were allowed to drink 1% lead acetate *ad libitum*, while Group D rats were in addition administered 200 mg of JNE/Kg BW/Day. Groups A and B were allowed to drink 1% sodium acetate *ad libitum* so as to cancel out the potential effects of acetate, and to ensure that only the effects of lead were measured in the test “groups” (C and D). All treatments were for eight weeks.

### Animal Sacrifice and Collection of Samples

Twenty-four hours after the last treatment, the rats were weighed and then sacrificed by cervical dislocation and blood samples (4 ml/rat) were collected by cardiac puncture into a standard test tubes and allowed to clot, serum was collected by centrifugation at 5.1 x g
for 15 minutes (g = 9.821 m/s/s). Epidydymes and testes were excised.

Collection of Data and Statistical Analysis
The epidydymes were dissected free of the surrounding tissues and were weighed. Testosterone level was assayed using radioimmunoassay as described by Odell et al. [15]. Strictly following the methods used by Salah et al. [16], sperm count and sperm motility were determined. Sperm morphology was accessed using the method described by Saalu et al. [17].

The control and “test groups” were compared using independent-sample t-test. The significant level was set to $P < 0.05$.

Results
The following results were obtained and are presented as mean ± SEM and level of significance is taken at “$P < 0.05$” (*), “$P < 0.001$” (**) and/or “$P < 0.0001$” (**).

Weight Gain and Epididymal Weight (g)
Gain in body weight of group C and group D were not significantly ($P > 0.05$) different from that of the control, however, no significant difference in the epididymal weight of group D and the control (Table 1).

Sperm Count and Serum Testosterone Level
Sperm count in group C was noted to be significantly ($P < 0.001$) lower, while those of group B and D were not significantly different from that of the control. A similar trend was found for serum testosterone level (Table 2).

Sperm Motility
Sperm motility was found to be significantly ($P < 0.01$) lower in group C compared to the control, while the sperm motility for group B and that of group D were not significantly different from that of the control (Table 3).

Sperm Morphology
Spermatozoa of group B were found to be of significant ($P < 0.05$) better morphology, those of group C were of significant ($P < 0.05$) poorer morphology, while those of group D were not significantly ($P > 0.05$) different from those of the control (Table 4).

Discussion
The significantly higher weight gain in group B compared to the control, and the non significant difference in weight gain of group C and group D rats with respect to the control prove that oral administration of aqueous extract of *Juglans nigra* for 8 weeks is able to
increase body weight gain while exposure to Lead acetate for 8 weeks did not significantly affect gain in body weight. This can be accounted for by the high fat component of Juglans nigra [18] and the anti-metabolic effect of Lead [19]. This is in support of the findings of Ronis et al. [20] that rats exposed to lead for 8 weeks showed significant impairment in neither growth nor weight gain. Longer period of exposure (for example 12 weeks and above) have been shown to significantly decrease gain in body weight [21].

The significant (P < 0.001) increase and decrease in epididymal weight of group B and group C respectively, and the non significant difference in epididymal weight of group D show that while Juglans nigra extract protects and promotes the cells and tissues of the epididymis, lead exposure does the opposite. These further show that the extract is able to effectively reduce or even annul the cyto-toxic effect of lead (in the case of group D). These results coincide with the findings of Marchlewicz et al. [22] that lead exposure (and toxicity) causes epididymal damage and degeneration of epithelial and interstitial tissues, and those of Batra et al. [23] that lead has spermicidal effect on high exposure. These suggest that oral administration of extracts of Juglans nigra promotes maintenance of normal epididymal and interstitial metabolic activities. Similar effects of Juglans nigra noted by Wang et al. [24] were linked to its high copper content which also forms its antioxidant basis.

The highly significant (P < 0.0001) decrease in sperm count in group C supports the finding of Xu et al. [9], that the testicular sperm count are important indicators of adverse effect of Lead on spermatogenesis, and this can be accounted for by direct influence of Lead on testicular tissues [23], these observations go in line with those of Salawu et al. [25] that exposure to Lead is capable of inducing infertility in male wister rats by significantly reducing sperm count. Meanwhile group B showed no significant difference in sperm count compared to the control. However, group D compared to the control also showed significant (P < 0.05) decrease in sperm count, but group D had significantly (P < 0.05) higher sperm count compared to group C. Thus, Juglans nigra although did not totally prevent the adverse effect of Lead on sperm count, it significantly reduce the Lead’s adverse effect on the sperm count and significantly (P < 0.05) increased Sperm count of Group D compared to Group C. This is a confirmation of the importance of Juglans nigra as a potent antioxidant and free radical scavenger [26, 27], which ameliorated the potential increase in free radicals generation from lead toxicity.

The non significant difference in testosterone level of group B and group D, and the significant decrease in testosterone level of group C compared to the control support the findings of Martin et al. [28] and Salawu et al. [25] that the impairment of spermatogenesis in lead toxicity is mainly a consequence of the decline in serum testosterone level. This further establishes that the normalcy found in group D despite their lead exposure must have resulted from the administered Juglans nigra. In this case, it could be proposed that Juglans nigra somehow affects the hypothalamic-pituitary-testicular axis, at least at a point in the axis.

The fact that groups B and D showed no significant difference in sperm motility from the control, Group C showed highly significant decrease in fast and slow motility relative to the control and a significant increase in non motile cells shows that Lead highly affect Sperm motility, a sign of infertility. This is in support of the findings of Jensen et al. [29], and Berry et al. [30] that administration of Lead acetate resulted in decline in sperm density, motility and viability. This could be due to the influence of lead acetate on sperm quality [23]. This result also gives evidence that Juglans nigra supports fertility in male rats. Based on its ability to improve motility, it is reasonable to predict that Juglans nigra has an effect on the mitochondria in the body of the tail of the spermatozoon, more so that the synthesis of energy (in the form of adenosine triphosphate) for sperm motility takes place in these mitochondria [31].

The significant decrease in normal and a significant increase in abnormal sperm morphology in group C rats compared to the control further confirms the anti-fertility effects of lead. These support the findings of Jensen et al. [29] and Berry et al. [30] that the administration of Lead acetate resulted in significant increase sperm abnormalities. This could be accounted for by the Lead’s ability to cross the cell membrane in various ways that causes structural abnormality [32]. The significant increase in normal sperm morphology and a significant decrease in abnormal sperm morphology in group B, and the non significant difference of group D from the control confirms that Juglans nigra is effective in retaining cell structural components. This observed cytoprotective effect of Juglans nigra is in support of the findings of Batra et al. [22], but could still be linked to its nutritional components as well as the electrolyte components which actively competes with lead and thus prevents lead’s adverse effects [23].

It can be concluded that exposure to Lead causes testicular toxicity by increasing abnormalities in sperm.
characteristics (sperm count, sperm morphology, sperm motility), decrease in epididymidal weight and testosterone level. *Juglans nigra*, however, prevented (significantly reduced) lead induced testicular toxicity, which is linked to its antioxidant activity.

## References


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