Ameliorative Effects of the Methanolic Extract of the Rind of *Citrullus lanatus* on Lead Acetate Induced Toxicity on Semen Parameters and Reproductive Hormones of Male Albino Wistar Rats

T. A. Kolawole¹, D. V. Dapper²* and S. O. Ojeka²

¹Department of Physiology, Madonna University Elele Campus, Rivers State, Nigeria. ²Hemorheology and Immunology Research Unit, Department of Human Physiology, College of Health Sciences, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria.

Authors’ contributions

This study was carried out in collaboration between all authors. Author TAK designed the study, performed the statistical analysis, designed the protocol, and wrote the first draft of the manuscript. Author DVD supervised and managed the analyses of the study and wrote the final draft manuscript. Author SOO managed the literature searches and validated all the references. All authors read and approved the final manuscript.

ABSTRACT

Aims: The present study investigated the effects of the methanolic extract of the rind of *Citrullus lanatus* on lead acetate induced toxicity on semen parameters, reproductive hormone assay and testicular histology in male albino Wistar rats.

Study Design: Controlled experimental study using randomly assigned laboratory animals.

Place and Duration of Study: Department of Human Physiology, College of Health Sciences, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria and Department of Physiology, Madonna University, Elele Campus, Rivers State, Nigeria between January 2013 and February 2014.

Methodology: Twenty male rats were assigned into four groups: Group A to D of five rats each. Group A served as control and received 2ml/kg bw of 10% extract vehicle; Group B...
received 200mg/kg bw of the methanolic extract of the rind of Citrullus lanatus; Group C received 2.25mg/kg bw of lead acetate; and Group D were co-administered with 2.25 mg/kg bw lead acetate and 200 mg/kg bw of the methanolic extract of the rind of Citrullus lanatus. The drugs and extracts were administered orally to the rats for 35 days. On day 36, blood samples were collected from anaesthetized rats by cardiac puncture for reproductive hormone assay and the testes harvested for determination of semen parameters and histological studies. Semen parameters: count, motility, viability, and morphology were determined and assay for plasma levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone done.

**Results:** Results obtained showed that, compared to control rats, administration of the methanolic extract of the rind Citrullus lanatus significantly enhanced sperm count and all reproductive hormone levels ($P<0.05$); and also caused non-significant increases in sperm motility, percentage of spermatocytes with normal morphology and percentage of live spermatocytes, but decreased percentage of dead spermatocytes ($P>0.05$). Treatment with lead acetate caused a significant reduction in levels of all reproductive hormones and significant diminution of sperm motility, morphology, viability; with increases in percentage of dead spermatocytes ($P<0.05$). Expectedly, co-administration of the methanolic extract of the rind of Citrullus lanatus with lead acetate ameliorated the deleterious effects of lead acetate resulting in significant increases in sperm count and all reproductive hormones ($P<0.05$) and non-significant increases in motility, morphology and live spermatocytes ($P>0.05$); however, the percentage of spermatocytes with abnormal heads were significantly increased. The results suggest that the methanolic extract of the rind of Citrullus lanatus exerts a possible ameliorative effect on lead acetate induced toxicity on some reproductive parameters in male albino Wistar rats.

**Conclusion:** The findings suggest that the methanolic extract of the rind of Citrullus lanatus exerts a possible beneficial effect on male reproductive parameters in albino Wistar rats and validates anecdotal reports of the beneficial effect of watermelon consumption from our environment. We however, recommend further studies in this regard.

**Keywords:** Citrullus lanatus; lead acetate; semen; reproductive hormones.

### 1. INTRODUCTION

Watermelon (Citrullus lanatus) is a tropical plant that belongs to the family of cucumber (Cucurbitaceae); it grows in almost all part of Africa and South East Asia [1]. The fruit is a berry with a thick smooth exterior rind [exocarp] and a sweet, edible, juicy and fleshy center [mesocarp and endocarp]. The rind is usually discarded, it may be applied to feeds or used as fertilizer; but it is alsoedible and may be used as a vegetable [2]. The rinds can also be fermented, blended and consumed as juice. The rind can be stir fried, stewed or more often pickled. Pickled Citrullus lanatus rind is also commonly consumed in the southern United States [3]. The inner portions of the rind which is usually light green or white contains many hidden nutrients and is also edible; however, most times it is avoided due to its unappealing flavor. It contains mainly citrulline which is a known stimulator of nitricoxide [4]. The rind has been shown to contain alkaloids, saponin, cardiac glycosides, flavonoids, phenol, moisture, lipid, protein, fiber and carbohydrates [5].

Several environmental factors have been shown to have adverse effects on male reproductive function [6,7]. Perhaps the progressive decline in male reproductive health and fertility over the past 30 years may be linked to these environmental toxicants and xenobiotic agents [8]. One of these toxicants with reported adverse effects on male reproductive
function is lead [9]. Lead is an abundant heavy toxic metal which is known to induce a broad range of physiological and behavioral dysfunction in humans [10]. Lead poisoning still remains an important health problem associated with several clinical symptoms with limited molecular mechanism underlying the toxicity [11]. Recent studies suggests that oxidative stress is a potential contributor to lead toxicity and that lead directly and or indirectly changes the pro-oxidant and antioxidant balance in biological tissues and all toxic metals have in common the ability to cause oxidative damage [12]. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage [13].

There is therefore the need to identify naturally occurring nutritional agents that could possibly help ameliorate the toxic effects of lead on the reproductive system. Anecdotal reports from our environment suggests that consumption of watermelon fruits possibly enhances sexual activity. The present study therefore attempts to explore the possible ameliorative effects of consumption of the rind of watermelon on lead acetate induced toxicity on the reproductive system using male albino Wistar rats as models.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh plant and fruits of watermelon were obtained from a local market in Rivers State, Nigeria. Lead acetate (C4H6O4Pb.H2O) was obtained from Sigma-Aldrich, United Kingdom.

2.2 Preparation of Extracts

The rinds were peeled off from the whole fruit washed thoroughly, sun-dried and milled into a fine powder. The method of extraction employed is percolation [14]. 24g of the powdered sample was soaked in a beaker containing 100ml of 98% methanol for a period of 48 hours and then filtered with a Whatman No. 1 filter paper size. The volume of filtrate obtained was 150ml before concentration; the filtrate was subsequently concentrated using a rotary evaporator. The weight of residue obtained was 8.5g.

2.3 Determination of Lethal Dose (LD50)

Acute toxicity study (LD50) was determined using the method described by Lorke in 1989 [15]. The (LD50) of the extract was found to be greater than 2000mg/kg body weight.

2.4 Experimental Procedure

Twenty male albino Wistar rats were used for this study. The rats were aged between 8 to 10 weeks and weighed between 170 and 200g. They were divided into four groups (Groups A to D) of 5 rats each. Rats in each group were numbered 1 to 5 and placed in separate cages in the Animal House of Madonna University, Nigeria under natural day and night cycles. The rats had free access to normal rat chow and tap water ad libitum. They were allowed two weeks of acclimatization to their environment and subsequently treated as follows:

a. Group A: Control Group
   Rats in this group were given 2ml/kg body weight 10% of extract vehicle being 98% methanol.
b. Group B: Extract Group
Rats in this group were given 200mg/kg body weight of the methanolic extract of the rind of *Citrus lanatus*.

c. Group C: Lead Group
Rats in this group were treated with 2.25mg/kg body weight of lead acetate as described by Falana and Oyeyipo [11].

d. Group D: Lead and Extract Group
Rats in this group were treated simultaneously with 2.25mg/kg of lead acetate and 200mg/kg body weight of the methanol extract of the rind of *Citrus lanatus*.

The methanolic extract of the rind of *Citrus lanatus*, the lead acetate and extract vehicles were administered to the rats daily using an oral cannula. All the rats were treated for a total of 35 days. On the 36th day, blood samples were collected from the anesthetized rats through cardiac puncture. The blood samples were placed in heparinized samples bottles and subsequently centrifuged at 1500rpm for 5 minutes and plasma obtained for assay of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone. The animals were then sacrificed and the testes immediately harvested for determination of semen parameters: sperm motility, sperm count, sperm viability and sperm morphology.

### 2.5 Determination of Semen Parameters

#### 2.5.1 Motility

As described previously by Kaur and Bansal [16], the caudal epididymis was identified and its content squeezed into 1ml of normal saline at room temperature. One drop of the semen suspension was charged into a Makler counting chamber and the number of motile and non-motile spermatocytes was counted in ten random fields. The number of motile spermatocytes was then expressed as a percentage of the total number of the counted spermatocytes [17].

#### 2.5.2 Sperm morphology

This was determined by smearing a drop of the stained semen suspension obtained during determination of sperm count on a glass slide; the smear was allowed to dry and subsequently examined under the light microscope at X400 magnification. For each sample, 200 spermatocytes were carefully observed and the percentage of total abnormalities of the spermatocyte head and total abnormalities of the spermatocyte tails were determined as described by Narayana et al. [18].

#### 2.5.3 Sperm viability

To determine viability, fluid from the caudal epididymis was carefully dropped on a slide and mixed with a drop of 0.5% eosin solution. After 2 minutes, the slide was examined under a light microscope at X40 magnification. The percentage of viable (unstained) and non-viable spermatocytes (stained red) was determined as described by Cheesbrough [19].

#### 2.5.4 Sperm count

This was determined as described earlier by Narayana et al. [18] with minor modifications. Briefly, the caudal epididymis was carefully separated from the testis and minced in 2ml of normal saline followed by filtration through a nylon mesh. The suspension was then stained with 2% eosin in normal saline. The spermatocytes heads were counted using a Neubauer
haematocytometer. Chamber counts for the sperm head in eight chambers (except the central chamber) were averaged and expressed as the number of sperm per caudalepididymis [17].

2.5.5 Testicular Histology

The testes of all the rats were fixed in 10% formalin, dehydrated stepwise in graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5µ thickness paraffin section was taken from the mid portion of each testicular tissue and stained with hematoxylin and eosin, followed by examination under a light microscope at X200 magnification. Photomicrographs was taken as appropriate and analyzed by a pathologist with requisite experience.

2.6 Determination of Plasma Concentrations of the Reproductive Hormones

The plasma concentrations of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone were determined for all experimental rats by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits. The hormonal kits used for the assay was produced by Monobind Inc. Lake Forest, CA, USA. Samples were run in the same assay to avoid inter-assay variations. Hormone levels were determined on the same day of collection of blood samples.

2.7 Statistical Analysis

Statistical analysis was performed using SPSS 17.0 version. Significant differences were determined using the one way analysis of variance (ANOVA) test followed by the LSD post hoc tests. A p value <0.05 was considered statistically significant. The results are presented in Tables 1 and 2. All data were expressed as Mean±Standard Error of mean (SEM).

3. RESULTS

Table 1 shows the effects of the methanolic extract of the rind of Citrullus lanatus on some semen parameters of male albino Wistar rats when administered alone (Group B) and when co-administered with lead acetate (Group D). It also shows the values of the semen parameters in control rats (Group A) and in rats administered with lead acetate alone (Group C). Results obtained indicate that administration of lead acetate in Group C rats caused a general diminution of all semen parameters with significant reductions in percentage motility, percentage of spermatocytes with normal morphology and percentage of live spermatocytes and corresponding increases in the percentage of spermatocytes with abnormal heads and tails and percentage of dead spermatocytes (P<0.05) as compared to Control (Group A) rats. Although, co-administration of lead acetate and methanolic extract of the rind of Citrullus lanatus in Group D rats caused a general improvement in all the semen parameters; however, significant improvements were seen only for sperm count (P<0.05) when compared to Control (Group A) rats. Similarly, significant difference was observed only in the sperm count amongst Group B rats treated with the methanolic extracts of the rind of Citrullus lanatus compared to Control (Group A) rats (P<0.05). Values of these parameters are as shown in the Table 1.

Table 2 shows the results of the assay for Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone for all the rat groups. Administration of lead acetate to rats in Group C caused a significant reduction in the values of each hormone under assessment, as compared to Control (Group A) rats (P<0.05). However, administration of the methanolic
extracts of the rind of *Citrullus lanatus* to rats in Group B caused significant increases in the values of each of the hormone under assessment (*P*<0.05). Furthermore, co-administration of both lead acetate and the methanolic extract of the rind of *Citrullus lanatus* to rats in Group D caused significant increase in the plasma levels of all the reproductive hormones under investigation, as compared to Control (Group A) rats (*P*<0.05), suggesting a possible amelioration of the deleterious effects of the administration of lead acetate seen amongst rats in Group C. Values of all the reproductive hormones are as shown in Table 2.

Table 1. Effect of methanolic extract of the rind of *Citrullus lanatus* extract on some semen parameters of male albino Wistar rats following exposure to lead acetate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Motility [%]</th>
<th>Normal sperm morphology [%]</th>
<th>Abnormal sperm morphology [%]</th>
<th>Viability of sperm [%]</th>
<th>Sperm count [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>66.0±6.78</td>
<td>74.0±4.85</td>
<td>2.0±0.55</td>
<td>6.8±1.39</td>
<td>44.0±6.59</td>
</tr>
<tr>
<td>Group B</td>
<td>78.0±7.18</td>
<td>85.2±3.56</td>
<td>6.0±1.18</td>
<td>7.0±2.24</td>
<td>52.0±5.64</td>
</tr>
<tr>
<td>Group C</td>
<td>24.0±6.78*</td>
<td>49.0±3.32*</td>
<td>24.2±4.02*</td>
<td>26.0±1.87*</td>
<td>16.0±4.58*</td>
</tr>
<tr>
<td>Group D</td>
<td>80.0±6.52</td>
<td>79.2±4.10</td>
<td>24.2±4.02*</td>
<td>6.4±0.51</td>
<td>53.3±5.93</td>
</tr>
</tbody>
</table>

All values=Mean±SEM; * significantly different from values of Group A control at *P*<0.05

![Fig. 1. Photomicrograph of transverse section of the testis of rats in Group A control rats.](image)

*Arrow indicates normal architecture of seminiferous tubule (H&E, 200X)*

Figs. 1 to 4 are the photomicrographs of sections of testicular tissues from the various corresponding rat groups respectively. Figs.1 and 2 are sections obtained from Group A control and Group B extract treated groups respectively. Both slides show a normal testicular architecture and presence of several normal spermatocytes.
Table 2. Effect of methanolic extract of the rind of *Citrullus lanatus* extract on reproductive hormone assay of male albino Wistar rats following exposure to lead acetate

<table>
<thead>
<tr>
<th>Groups</th>
<th>Follicle stimulating hormone [FSH] [mIU/ml]</th>
<th>Luteinizing hormone [LH] [mIU/ml]</th>
<th>Testosterone [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: Control group</td>
<td>0.12±0.02</td>
<td>0.10±0.01</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>Group B: Extract group</td>
<td>0.32±0.07*</td>
<td>0.38±0.08*</td>
<td>2.54±0.02*</td>
</tr>
<tr>
<td>Group C: Lead acetate group</td>
<td>0.04±0.02*</td>
<td>0.04±0.02*</td>
<td>0.06±0.02*</td>
</tr>
<tr>
<td>Group D: Lead acetate and Extract group</td>
<td>0.28±0.04*</td>
<td>0.32±0.06*</td>
<td>1.70±0.09*</td>
</tr>
</tbody>
</table>

All values=Mean±SEM; * significantly different from values of Group A control at P<0.05

Fig. 2. Photomicrograph of transverse section of the testis of rats in Group B extract treated rats. (H&E, 200X)

Fig. 3 is a photomicrograph of testicular tissue obtained from rats in Group 3: lead acetate treated group. It shows abnormal architecture of the seminiferous tubules with some disorganization of the outer fibroblast capsule layer and a reduced spermatocyte cells population as compared to both Figs. 1 and 2.

Fig. 4 is the photomicrograph obtained for Group D: lead acetate and extract treated rats. It shows an attempt at the reorganization in the architecture of the seminiferous tubule with a fairly normal fibroblast layer.
Fig. 3. Photomicrograph of transverse section of the testis of rats in Group C: lead acetate treated rats. Arrow areas of abnormal architecture of the seminiferous tubules. (H&E, 200X)

Fig. 4. Photomicrograph of transverse section of the testis of rats in Group D: lead acetate and extract treated rats. Arrow areas of attempt at repair of testicular tissue. (H&E, 200X)
4. DISCUSSION

The present study describes the possible beneficial effects of the administration of the methanolic extract of the rind of *Citrullus lanatus* on semen parameters and reproductive hormones of male albino Wistar rats; furthermore, a possible ameliorative effect of the extract following co-administration with lead acetate is also described.

The diminution of semen parameters and reduction in the levels of male reproductive hormones following lead acetate administration, seen in the present study, is consistent with previous reports of possible impairment of reproductive capacity induced by lead [20]. Sperm parameters such as count, motility and morphology are reportedly key indices of male fertility; being fair markers of spermatogenesis and epididymal maturation of spermatocytes [21]. These parameters along with sperm viability were all found to be adversely affected by lead acetate administration in the present study. Several mechanisms have been proposed to account for the deleterious effect of lead on male reproductive function: A reduction of plasma testosterone concentration observed in Group C rats in the present study is one such possible mechanism: normal levels of testosterone is critical for spermatogenesis and the proper function of the seminiferous tubules [22]. Exerting a direct cytotoxic effect on maturing or matured spermatocytes in the epididymis is another possible mechanism [23]. The most common morphological abnormalities of the spermatocyte observed in lead acetate treated rats in the present study was the presence of either a bent or a curved tail. Considering the physiology of spermatogenesis, this abnormality most likely occurs during spermatocyte transport through the epididymis for maturation and storage. It is during this stage that the developing spermatooza acquires motility [24]. This could also explain the reduced sperm motility observed in the present study because a functional spermatocyte tail is critical for motility. The reduced spermatocyte viability and increased percentage of dead spermatocytes amongst Group C rats is consistent with the observed reduction in sperm motility; on microscopic examination non motile spermatocytes would invariably be counted as dead. Further, it has been reported that most heavy metals by increasing the production of reactive oxygen species and by increasing the generation of testicular hydrogen peroxide and hydroxyl radicals in experimental rats [25,26] could exert a detrimental effect on spermatogenesis and thus semen parameters [27].

The pituitary-gonadal axis is of immensely importance in the regulation of male reproductive function. The anterior pituitary secretes both Follicle Stimulating Hormone and Luteinizing Hormone; the latter via regulating testosterone secretion plays an important role in the final maturation of spermatocytes, while the former is needed for the maintenance of gametogenesis [28]. Assay of gonadotrophic hormones amongst Group C rats exposed to lead acetate in the present study showed significant reduction of the plasma levels of both Follicle Stimulating Hormone and Luteinizing Hormone. The reduced plasma testosterone level also observed could be attributed to the reduction in the level of Luteinizing Hormone. Luteinizing Hormone is responsible for maintaining testosterone concentration [29,30]. Perhaps the reduction of gonadotropin secretion by lead acetate in the present study may be on account of a possible depressive effect on the hypothalamic neural mechanisms essential for the release of Gonadotropin Releasing Hormone [31,32]. This eventually will lead to disturbances in the secretion of pituitary gonadotropins an essential for both spermatogenesis and steroidogenesis [33].

Noteworthy, co treatment of lead acetate with the methanolic extract of the rind of *Citrullus lanatus* ameliorated the effect of lead acetate on most of the parameters under investigation as seen in Group D rats; with the return of the sperm count, motility, morphology and hormones levels towards fairly normal values comparable to values seen amongst Group A control rats. This possible ameliorative effect of the methanolic extract of the rind of *Citrullus*
lanatus maybe attributed to its contents of phenols and flavonoids [5]. The best described property of almost every group of flavonoids is their capacity to act as antioxidants [5]. The flavones and catechins are perhaps the most powerful flavonoids for protecting the body against damage by reactive oxygen species [34]. The relationship between the total phenol content and antioxidant activity has been widely studied in different foodstuffs: Antioxidant activity of foodstuff significantly increases with the presence of a high concentration of total phenol and flavonoid [35]. Therefore, the high phenolic and flavonoid contents of the watermelon rinds suggests possible high antioxidant potential.

The ameliorative effect of the methanolic extract of the rind of Citrullus lanatus described in the present study may also be attributed to its ascorbic acid content [36]. Although, low ascorbic acid levels have been reported to be associated with low sperm counts, an increased number of abnormal sperm, reduced motility and agglutination of spermatocytes [37,38], dietary supplementation with ascorbic acid have been reported to improve sperm quality [39]. Ascorbic acid also has antioxidant properties and the ability to remove free radicals presumably generated by lead acetate which may otherwise cause oxidative stress [37,40]. The results of the present study suggests that the methanolic extract of the rind of Citrullus lanatus significantly increased the plasma level of Follicle Stimulating Hormone, Luteinizing Hormone and testosterone. The significant increase in testosterone level may perhaps be the effect of ascorbic acid enhancing the testosterone synthesis [41,42]. The elevation in both Follicle Stimulating Hormone and Luteinizing Hormone levels by the methanolic extract of the rind of Citrullus lanatus in rats exposed to lead acetate may be due to the fact that ascorbic acid is a possible vitaminergic transmitter activating release of both Follicle Stimulating Hormone and Luteinizing Hormone from the anterior pituitary gland [43]. Our results are in agreement with previous reports that supplementation with ascorbic acid reverted to normal levels of Follicle Stimulating Hormone, Luteinizing Hormone and testosterone following cadmium induced toxicity in male rats [44] and in hyperglycemic rats [45,46].

Results obtained from histological analysis in the present study provides a histological basis for the beneficial effects of the extracts of rind of Citrullus lanatus on reproductive functions in male albino Wistar rats.

5. CONCLUSION

In conclusion, the present study reports that administration of the methanolic extract of the rind of Citrullus lanatus ameliorated the effects of lead acetate administration on semen parameters and reproductive hormone levels of male albino Wistar rats. Our findings suggests a possible beneficial effect of Watermelon rind on male reproductive function, at least in albino Wistar rats, and likely validates previous anecdotal suggestions and observations from our environment in this regard. However, we suggest studies to further determine these effects of Watermelon.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No 85-23 revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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