

## Original Research Article

# Effect of aqueous ginger (*Zingiber officinale*) extract on sperm quality and haematology in lead acetate-treated male albino rats

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### Abstract

**Purpose:** To evaluate the ameliorative effects of aqueous ginger extract on sperm quality and haematology in lead acetate-treated male albino rats.

**Methods:** Twenty-four male albino rats used for this study were randomly assigned to four groups ( $n = 6$ ). Group 1 was the normal control. Lead acetate (4 mg/kg) was given to group 2 for 6 weeks. Lead acetate (4 mg/kg) and ginger (300 mg/kg) were given to group 4 simultaneously for 6 weeks, while group 4 was given lead acetate (4 mg/kg) for 6 weeks, and then, ginger (300 mg/kg) for another 6 weeks. Haematological parameters, including packed cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) counts, hemoglobin (Hb) concentration, and differential leukocyte count were evaluated in blood obtained from the retrobulbar plexus of the rats.

**Results:** Sperm motility and viability were markedly lower ( $p < 0.05$ ) in group 2, in comparison to groups 1 and 3, while sperm motility and viability in group 3 were similar, but differed from group 1 ( $p < 0.05$ ). The sperm motility and sperm viability of groups 4 and 2 were comparable. Haematological profile showed a marked reduction in the RBC of rats in group 2, when compared to those in groups 1 and 3. The WBC of group 2 was significantly higher than those of groups 1, 3 and 4 ( $p < 0.05$ ).

**Conclusion:** The results indicate that aqueous ginger extract has the potential to protect sperm quality and haematology in lead acetate treated male rats.

**Keywords:** Ginger, Lead acetate, Sperm quality, Haematology

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## INTRODUCTION

Lead (Pb) is a pollutant, that is abundant in the environment, and it is known to be harmful, and poisonous [1]. Pollution of the environment with lead has resulted to toxicities in the kidney, liver, nerves, blood, testis and ovary in man and animals [2]. Prolonged environmental pollution with lead, results to its accumulation, in the

organs mentioned above, hence, causing many metabolic and histological changes in them [3,4].

Research has shown that lead toxicity causes severe damage to the reproductive system, interfering with spermatogenesis, hence, decreases sperm production and quality [5,6]. The mechanism by which lead toxicity causes testicular damage to exposed males is by

induction of oxidative stress, and inhibition of the body's defence to oxidants. [7]. Ginger (*Zingiber officinale*) is a spicy, and a medicinal plant. Its folkloric use in the treatment of inflammation of the joints, cold symptoms, sore throats, pyrexia, cramps and constipation has been reported [8]. It has also been reported to be an antioxidant, with cyto-protective effects, used in the treatment of microbial infections, inflammatory conditions and emesis [9]. *Zingiber officinale* contains zingerone, gingerdiol, zingibrene, gingerols and shogaols, and these constituents are antioxidants [10].

Ginger has been reported to have a preventive role on ischemia/reperfusion (I/R) injury in the rat's kidney [11]. It has also been shown to have inhibitory effect on cisplatin-induced nephrotoxicity in rats [11]. Hence, the objective of this study was to evaluate the ameliorative effects of aqueous ginger extract on the quality of epididymal sperm and haematology in lead acetate treated male albino rats.

## EXPERIMENTAL

### Materials

The lead (Pb) acetate (El-Gomhoria company, Egypt) used was bought from Joe Chem Ventures Ltd, University of Nigeria, Nsukka, Enugu State. Ginger rhizome was purchased from Ogige Market in Nsukka, Nigeria in March, 2018. It was authenticated as *Zingiber officinale* by Mr, AO Ozioko, a taxonomist of the Bioresources Development and Conservation Programme, Aku Road, Nsukka, Enugu State, Nigeria. A voucher specimen was kept for future reference in INTERCEDD (International Center for Ethnomedicine and Drug Development) herbarium (no. INTERCEDD/1788).

### Extraction of ginger (*Zingiber officinale*) extract

Ceramic mortar and pestle were used to crush the ginger rhizome, and Whatman no. 1 filter paper was used for filtration. Hot air oven was used to concentrate the filtrate at 37°C, and the filtrate was then, kept at 4°C during its use.

### Animals

Twenty-four male albino rats (*Rattus norvegicus*) weighing between 180 to 200 g (12 weeks of age) were used in this study. The animals were sourced from the Department of Veterinary Anatomy, University of Nigeria, Nsukka and housed in clean cages at room temperature at the laboratory animal unit of Department of

Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized for two (2) weeks, before the experiment started, during which they were fed *ad libitum* with feed and water.

### Ethical approval

Handling of the rats was in accordance with principles of laboratory animal care, and all the protocols and procedures were approved by the Ethical Committee of Faculty of Veterinary Medicine, University of Nigeria, Nsukka (approval ref no. 20170526). The handling is in accordance with Directive 1997/73/WE on the use of animals for experiment [12].

### Study design

Twenty-four mature male strain randomly assigned to four (4) groups of six rats each were used for the study. In group 1, (normal control) distilled water was given as *placebo*. Rats in group 2 were treated daily with lead acetate alone at 4 mg/kg per OS [6] from day one for six weeks. Rats in group 3 were treated daily with lead acetate at 4 mg/kg and ginger extract at 300 mg/kg orally [11] from day one for six weeks, while those in group 4 were treated for six weeks with 4 mg/kg of lead via the oral route, and then treated for more 6 weeks with 300 mg/kg of ginger extract via the same route.

### Measurement of testicular weight

After 30 days of treatment, rats from each group were sacrificed using chloroform. The testes from each rat were carefully removed, free of, extraneous tissues, and the testes were measured using a weighing balance [13].

### Evaluation of motile sperms

Wet mount of sperm from the cauda epididymis was made on a prewarmed slide, and a light microscope was used to view the proportion of spermatozoa, moving progressively in one direction on a slide per field [14]. Spermatozoa moving in one direction were counted, while those showing circular, pendulous or backward movements were not counted.

### Assessment of sperm viability

Eosin-nigrosin stain was mixed with sperm sample on a prewarmed slide [15]. Sperm-stain smear was made, and air dried. Live (white) and dead (pink/red) spermatozoa were counted

differently, under a microscope, and estimated in percentage [14].

### Evaluation of haematological parameters

On the 30<sup>th</sup> day of study, non-heparinized capillary tubes were inserted into the retrobulbal plexus of the rats, and blood samples were collected into EDTA sample bottles.

### Measurement of packed cell volume (PCV)

This was determined with the hematocrit method [16]. Values are expressed in percentage.

### Assessment of white blood cell (WBC) and red blood cell (RBC) counts

Improved Neubauer chamber was used for counting the cells [16]. Values were expressed as number of counts  $\times 10^6/\mu\text{L}$  of blood.

### Determination of hemoglobin (Hb) concentration

This was done using cyanomethaemoglobin method [16] and values recorded in g/dl

### Differential leukocyte count

Lieshmann Technique [16] was used. Every leucocyte type was evaluated as a percentage of the total count, and changed to the absolute value per microlitre of blood.

### Statistical analysis

Data obtained from the study were analysed using one-way analysis of variance (ANOVA). Duncan Multiple Range Test was used to separate the variant means. Marked difference was accepted at  $p < 0.05$ .

## RESULTS

### Testicular weight

The testicular weights among the groups are comparable ( $p > 0.05$ ) (Table 1).

**Table 1:** Testicular weight, and sperm motility and viability of rats

Parameter	Group			
	1 (Normal control)	2 (LA only)	3 (LA and ginger SML)	4 (LA 6 weeks, then ginger 6 weeks)
Testicular weight (g)	1.10 $\pm$ 0.00	0.90 $\pm$ 0.16	0.90 $\pm$ 0.10	1.07 $\pm$ 0.03
Sperm motility (%)	76.68 $\pm$ 8.82 <sup>a</sup>	30.00 $\pm$ 5.77 <sup>b</sup>	73.33 $\pm$ 3.33 <sup>a</sup>	40.00 $\pm$ 5.77 <sup>b</sup>
Sperm viability (%)	76.67 $\pm$ 8.82 <sup>a</sup>	40.00 $\pm$ 5.77 <sup>b</sup>	66.67 $\pm$ 3.33 <sup>a</sup>	46.67 $\pm$ 3.33 <sup>b</sup>

**Key:** a and b are different superscripts used to show statistical differences between the mean ( $p < 0.05$ ). LA= Lead acetate, SML= simultaneously

### Sperm motility and sperm viability

Sperm motility and viability of group 2 were markedly lower in comparison to groups 1 and 3 (Table 1). Sperm motility and viability in groups 3 and 1 are similar, and both are also comparable in groups 2 and 4 ( $p > 0.05$ ).

### Haematological profile

There were significant decreases in PCV and Hb in group 2, in comparison to groups 1, 3 and 4 (Table 2). The RBC of group 2 was markedly lower in comparison to groups 1 and 3. The WBC, was significantly higher in group 2 animals, when compared to groups 1, 3 and 4 (Table 3). There was also a significant increase in the number of lymphocytes of animals in groups 2 and 4, when compared to groups 1 and 3 but all the values fell within the normal range reported for this specie. The neutrophils in groups 2 and 4 were markedly higher, when compared to groups 1 and 3, however, all the values are normal for the specie.

## DISCUSSION

The similarity in testicular weight of lead acetate treated group and other groups was however, not in line with the study that reported atrophy of the testes of rats treated with lead acetate [17]. The significant decrease in sperm motility and viability observed in group 2 (lead acetate only) animals, when compared to animals in groups 1 (normal control) and 3 (lead acetate + ginger) may be due to reactive oxygen species attack caused by lead [18,19]. Spermatozoa are destroyed by oxidative stress. The oxidants damage the structure of lipid matrix in the membranes of sperm cells, and this affects the quality of sperm adversely [20].

The similarity in sperm motility and viability of animals in group 3 (lead + ginger), when compared to group 1 (normal control), may be due to antioxidant properties of ginger [21-23]. Vitamin C, an antioxidant is one of the constituents of ginger [24].

**Table 2:** Packed cell volume, haemoglobin concentration and red blood cell count of rats

Parameter	Group			
	1 (Normal control)	2 (LA only)	3 (LA and ginger SML)	4 (LA 6 weeks, then ginger 6 weeks)
Packed cell volume (%)	49.67 ± 2.03 <sup>a</sup>	41.67 ± 0.67 <sup>b</sup>	47.33 ± 1.45 <sup>a</sup>	46.33 ± 0.88 <sup>a</sup>
Haemoglobin conc. (g/dL)	10.70 ± 0.31 <sup>a</sup>	7.27 ± 0.88 <sup>b</sup>	9.10 ± 0.23 <sup>c</sup>	8.43 ± 0.26 <sup>c</sup>
Red blood cell count (10 <sup>6</sup> /µl)	28.17 ± 1.09 <sup>a</sup>	24.00 ± 0.58 <sup>b</sup>	26.00 ± 0.58 <sup>ac</sup>	24.33 ± 0.33 <sup>bc</sup>

**Key:** a, b, c are different superscripts showing differences between the means ( $p < 0.05$ ). LA= Lead acetate, SML = simultaneously

**Table 3:** Total white blood cell and differential leucocyte counts of rats

Parameter	Group			
	1 (Normal control)	2 (LA only)	3 (LA and ginger SML)	4 (LA 6 weeks, then ginger 6 weeks)
Total WBC	70.00 ± 1.15 <sup>a</sup>	82.00 ± 2.00 <sup>b</sup>	68.66 ± 0.67 <sup>a</sup>	72.00 ± 1.15 <sup>a</sup>
Neutrophils	40.67 ± 0.67 <sup>a</sup>	30.67 ± 0.67 <sup>b</sup>	46.00 ± 1.15 <sup>c</sup>	30.00 ± 0.00 <sup>b</sup>
Lymphocytes	58.00 ± 1.54 <sup>a</sup>	68.00 ± 1.15 <sup>b</sup>	52.00 ± 1.15 <sup>c</sup>	68.00 ± 1.15 <sup>b</sup>
Monocytes	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Eosinophils	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00

**Key:** a, b, c are different superscripts showing differences between the mean ( $p < 0.05$ ). LA = lead acetate, SML= simultaneously

The fact that the sperm motility and viability of rats in group 4 (lead acetate 6 weeks, then ginger 6 weeks) are comparable to group 2 (lead acetate only), may be because the toxic effect of lead had occurred before commencement of treatment with ginger. This suggests that it is better to prevent lead toxicity with consumption of ginger than allowing the toxicity to occur before treatment with ginger.

The significant decreases in PCV and Hb concentration of group 2 (lead acetate only) when compared to groups 1 (control), 3 (lead acetate + ginger) and 4 (lead acetate 6 weeks and then ginger 6 weeks) as well as the significant decrease in RBC of group 2, when compared to those in groups 1 and 3 are in line with previous studies [25]. Lead affects RBCs adversely, resulting to a destabilising effect on cellular membrane of RBCs, decreasing its fluidity and increasing the rate of RBC haemolysis. This appears to be the final effect of ROS-generation and lipid peroxidation in the RBC membrane [26].

The significant increase in WBC in group 2 rats, in comparison with groups 1, 3 and 4 may be as a result of the body's immune response to lead toxicity. The significant decrease in the number of neutrophils of rats in groups 2 and 4, in comparison with groups 1 and 3 may be due to defect in granulopoiesis in the bone marrow, caused by lead toxicity.

The significantly higher number of lymphocytes of rats in groups 2 and 4, in comparison with groups 1 and 3 may have contributed to the observed higher WBC in groups 2 and 4.

## CONCLUSION

The results from this study indicate that ginger extract has a marked protective effect on sperm quality (motility and viability) and haematological profile in lead acetate-treated male rats. The results also show that ginger may be more effective, when supplemented in animals diet rather than using it as a therapeutic agent after the occurrence of lead toxicity. The potential clinical significance of the findings of this study is that ginger is effective as a prophylactic agent, but has no curative effect.

## DECLARATIONS

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

This study was done by us, and we accept every liability pertaining to claims relating to the content of this article. RI Odo and EC Mbegbu designed the study, and supervised the experimentation. RI Odo and LN Anyanwu collected, and analysed

the data. The manuscript was written by RI Odo and EC Mbegbu, and all authors edited it, and approved it for publication.

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