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THE PROTECTIVE EFFECT OF *MORINGA OLEIFERA* LEAF EXTRACT ON MERCURY INDUCED TESTICULAR TOXICITY IN ADULT WISTAR RATS

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ABSTRACT

Exposure of the body systems and organs such as testis to heavy metals including mercury chloride has been found to course adverse effect which affects their functionality and may result to high rate of infertility to the population. Hence, this study was aimed at determining the ability, potency and nature of *Moringa oleifera* leaf extract in protecting the testis against mercury induced testicular toxicity. Thirty two male rats weighing between (100 – 280g) were used for the study. Animals were divided into four main groups. 1st group served as control, 2nd group were given varying doses of mercury chloride, 3rd group received a fixed dose of *Moringa oleifera* leaf extract and 4th group received both mercury and *Moringa oleifera* leaf extract. The group that received mercury only showed a histological evidence of testicular damage, necrosis of seminiferous tubules and interstitial tissues, loss of spermatid and presence of multi nucleated giant cells with sperm cell depletion. Animal treated with *Moringa oleifera* leaf extract only had some histological appearance as the control. In those treated with both mercury and *Moringa oleifera* leaf extract, the toxic effect of mercury was not marked. From this finding, *Moringa oleifera* leaf extract may have protective potential over the toxic effect of mercury on the testis.

Key Words: Moringa, Mercury, Toxicity, Testis, Wistar rats.

INTRODUCTION

Moringa oleifera is the most widely cultivated species of the genus Moringa, which is the only genus in the family Moringaceae. Its English names include moringa, drumstick tree, horseradish and ben oil tree or benzoil tree [1]. It is a fast-growing drought-resistant tree native to the southern foothills of the Himalayas in northwestern India and widely cultivated in tropical and sub-tropical areas [2]. *Moringa oleifera* is a tree brought from God to the hands of man. It was recognized by the Hands of man. It was recognized by the National Institutes of Health as the Botanical of the year for 2007 and praised again in 2011 and 2012. It is valued worldwide for its ability to treat over 300 diseases [3]. *Moringa oleifera* provides a rare combination of zeatin (a poteng antioxidant), quercetin (a flavonoid known for its ability to neutralize free radicals and relieve inflammation), betasitostrel (a nutrient superstar that blocks cholesterol formation or build up and is an anti-inflammatory agent for the body), caffeoylquinic acid (another powerful anti-inflammatory compound) and kaempferol (a key nutrient that promotes healthy body cellular function). All in all, enzymaticallly active and bioavailable *Moringa oleifera* provides 36 natural anti-inflammatory agents [4-7].

The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as betacarotene, vitamin K, manganese and protein among other essential nutrients [8,9]. In developing countries, *Moringa oleifera* leaves have potential to improve nutrition, boost food security, foster rural development and support landcare [10]. It may be used as forage for livestock, a micronutrient liquid, a natural antihelmimintic and possible adjuvant [11]. In Ajurvedic traditional medicine, the leaves are believed to affect blood pressure and glucose levels [12]. Abundant reports on the toxicity of certain heavy metals on human organs have been on the rise due to pervasiveness of these metals in the environment. Arsenic, Lead, Mercury, Chromium and their

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inorganic compounds are possibly the most potentially toxic metals in the environment. These metals have many industrial uses which increases the possibility of human exposure resulting to various distinctive [13]. Mercury is a heavy metal known toxicity widely dispersed in nature and noted for inducing public health disaster in nature [14]. Toxicity by heavy metals (mercury) can lead to reproductive damages/ impairment resulting to high rate of infertility in the society.

In view that *Moringa oleifera* has been shown to have an ameliorative effect on the Lead and Chromium testicular toxicity in rats. There is indication that it could have ameliorative effects against mercury induced testicular toxicity but we do not have satisfactory conviction on that. Therefore the search on the protective efficacy of *Moringa oleifera* leaf extract on mercury induced testicular toxicity is of vital importance for clarication and confirmation for future knowledge of its usefulness in protecting the reproductive organs and tissues against mercury toxicity.

MATERIALS AND METHOD

Breeding of Animals

Thirty two adult male wistar rats weighing between 100-280g were used in this study. They were obtained from a private farm in Anambra State, Nigeria. The animals were kept in the research section of the animal house of the department of Anatomy, Faculty of Basic Medical Science Nnamdi Azikiwe University, Nigeria and allowed to acclimatise under normal temperature.The animals were fed ad libitum with standard diet (ratchow). The cages were cleaned every day to prevent infections.

Collection of Plant Materials

The leaves of *Moringa oleifera* was procured from a private farm at Okofia Otolo Nnewi Anambra State, Nigeria and authenticated in the herbarium unit of Department of Botany, Nnamdi Azikiwe University.

Preparation of Extracts

Fresh leaves of Moringa oleifera were collected, shade dried and pounded into powder before extraction. The aqueous extract was obtained by socking it in distilled water. 300mg/kg of body weight of the extract was administered to the animals.

Chemicals

Mercury chloride was obtained from the Department of Biochemistry, Nnamdi Azikiwe University, Anambra state, Nigeria and was dissolved in 2mls of distilled water at a concentration of (0.5mg/kg, 0.25mg/kg, 0.125mg/kg) and administered to the animals.

Experimental Protocol

Before the administration, the rats were weighed and recorded. They were divided randomly into eight groups of four animals each. Group H served as the control and were orally administered with 2.5ml/kg of distilled water. The experimental groups A, B, C, D, E, F and G received different doses of drug as follow; Group A received 0.13ml of mercury chloride [HgCl2], Group B received 0.07ml of mercury, Group C received 0.03ml of mercury chloride. Group D received 0.11ml of mercury chloride + 0.6ml of *Moringa oleifera* leave extract. Group E received 0.05ml of HgCl2 + 0.5ml of the *Moringa oleifera* leaf extract. Group F received 0.02ml of mercury Chloride + 0.4ml 0f *Moringa oleifera* leave extract. Group G received only *Moringa oleifera* leave extract of 0.3ml each. The drug where administered once in a day between the hours of 6am to 6pm daily for twenty one days. Administration was done orally using intubation method. Twenty four hours after the last administration, the animals were weighed, sacrificed under chloroform vapour and dissected. The abdominal cavity was opened up through a midline abdominal incision to expose the reproductive organs, the testis were detached and cleared free of the surrounding tissues, the testis was weighed with an electronic analytical and precision balance and were recorded. The organ was washed and cleared free of blood, it was trimmed to a size of 3mm x3mm thick and then fixed in bowen's fluid temporally for four hours for histological studies.

Tissue Processing

For easy study of section under microscope, the tissue passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in zenkers fluid for four hours after which the tissues were washed overnight under a stream tap water. Dehydration of the fixed tissues was carried out in different percentages of alcohol 50%, 70% and 90% absolute. After dehydration, the tissues were cleared in xylene for two hours. Infiltration was done in molten paraffin wax at a temperature of 60 for two hours each in two changes and then section.

RESULTS

Physical and Behavioural Changes

At the beginning of the experiments, all the animals looked healthy and agile. During the one week of acclimatization, their stools were normal. On administration of mercury, varying gradations of toxicity were observed. Generally, the signs of toxicity observed include: laboured breathing, staggering / loss of balance and decreased food intake. These signs were not observed following administration of *Moringa oleifera* leave extracts in groups (D, E, F, &G) and group H (control).

The final body weight for group A, B showed a statistically significant decrease ($P<0.05$). The final body weight for groups D & E treated with mercury chloride plus *Moringa oleifera* leave extracts showed slightly decrease unlike A&B that decrease more. The final body

weight of group F showed no change. The final body weight groups G, H & C were higher than other groups. The weight change of group C showed statistically increased compared with groups A & B.

Morphometric Analysis of testis Weight

DISCUSSION

Reproductive toxicity resulting from exposure to heavy metals in males is one of the areas of concern in toxicity today.

Reports have shown over the years that heavy metals including mercury intoxicate the testis by generating reactive radicals thereby resulting into celluler damage like diminution of enzyme activity, damage to lipid bilayer and DNA resulting to increase oxidative stress damages in sperm membranes protein and DNA [15].

The result in this present study showed that exposure to mercury in the rats caused damage to testes resulting in the obstruction of the spermatogenesis. This result in conformity with the report by [15] which showed that sodium chromate in rats caused shrinking of nuclear size of testicular cells and decline in cell population of spermatogenic cells. Other report reported that testicular toxicity of other heavy metals eg,copper, iron, manganese results to male subfecundity,spermatogenic and steriodogenic impairment [16-18]. From the result of the present study, it showed that concurrent treatment with Moringa oliefera slightly prevented the toxicity brought about by mercury exposure and thus is evident on the histoachitecture of groups D,E and F administered mercury and *Moringa oleifera* leaf extract. This result agrees with [19], who reported that toxic actions of metals are oxidative in nature and it is indicative *Moringa oleifera* was able to attenuate the toxicity of lead due to its anti-oxidative potential. This is also in agreement with [20] which have shown the anti-oxidative properties of *Moringa oleifera* and its ability to elevate a variety of anti-oxidant enzymes and testicular bio-markers.

Table 1. Comparison of mean initial and final body weight and weight change in all the groups (A, B, C, D, E, F, G & H)

Groups	A	B	C	D	E	F	G	H	P.Sig.
Initial Body Wt	265±0.913	260±0.913	230±0.913	210±0.913	180±0.913	125±0.913	100±0.913	87.5±0.913	0.000
Final Body Wt	240±0.913	240±0.913	245±0.913	197.5±0.913	177.5±0.913	125±0.913	117.5±0.913	117.5±0.913	0.000
Wt change	25±0.913	20±0.913	15±0.913	12.5±0.913	2.5±0.913	-	17.5±0.913	30±0.913	0.000

Mean ± SEM given for each measurement

Table 2. Comparison of mean relative testis weight in all the groups (A, B, C, D,E,F,G & H)

GROUPS	GP A	GP B	GP C	GP D	GP E	GP F	GP G	GP H
TESTICULAR WEIGHT	1.31±0.00000	1.46±0.00000	1.48±0.00000	1.46±0.00000	1.48±0.00000	1.02±0.00000	0.97±0.00000	0.93±0.00000

(Mean ± SEM given for each Measurement). The relative testis weight for all the groups varies as their dosage varies. Group C and E is higher than other groups but that of Group G Moringa treated are almost similar to Group H

Figure 1. Bar chat representation of weight of animal before administration.

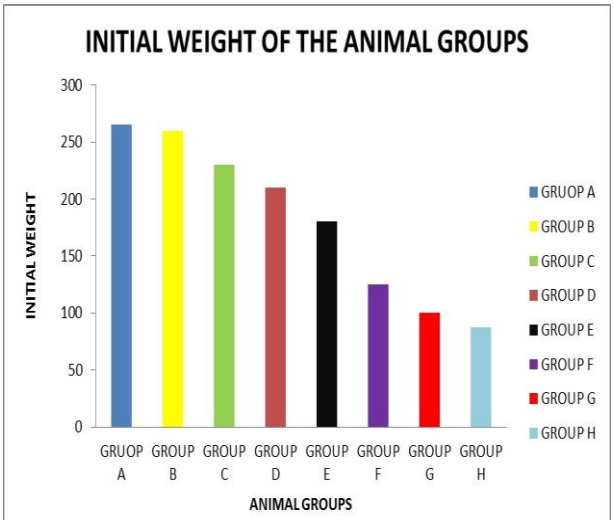


Figure 2. Bar chat representation of weight of animal after administration

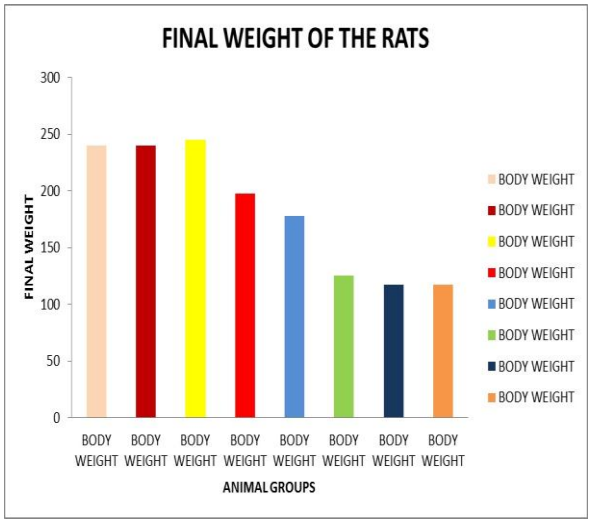


Figure 2. Bar chart representation of organ weight (testis)

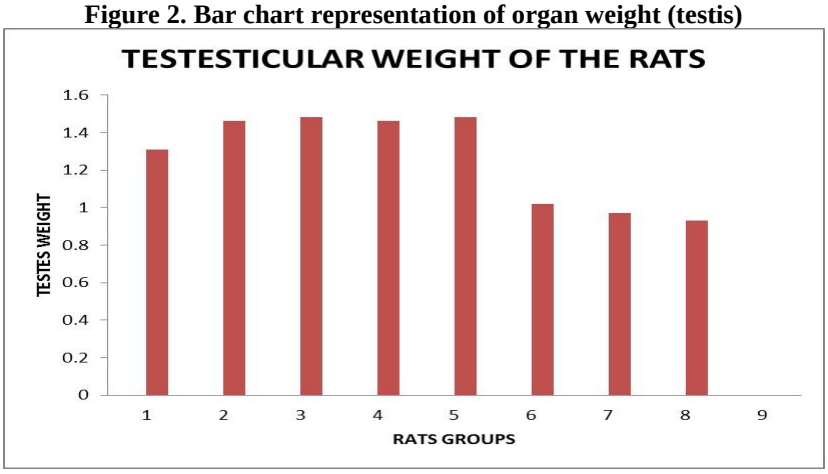
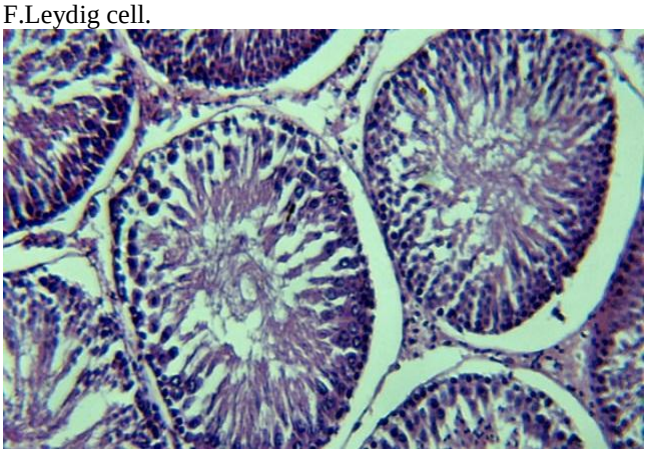
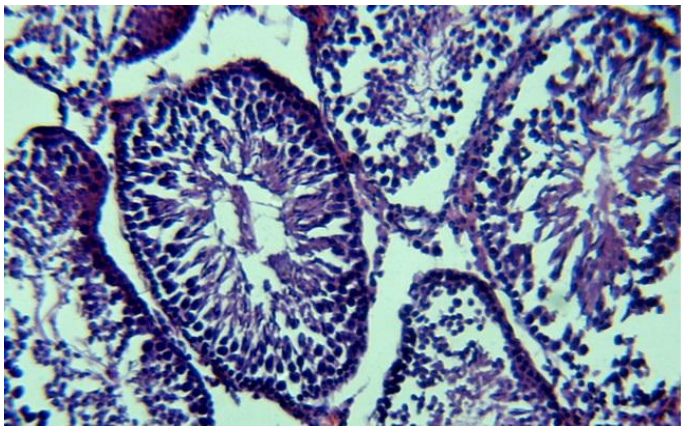


Plate 1. Histopathological Findings

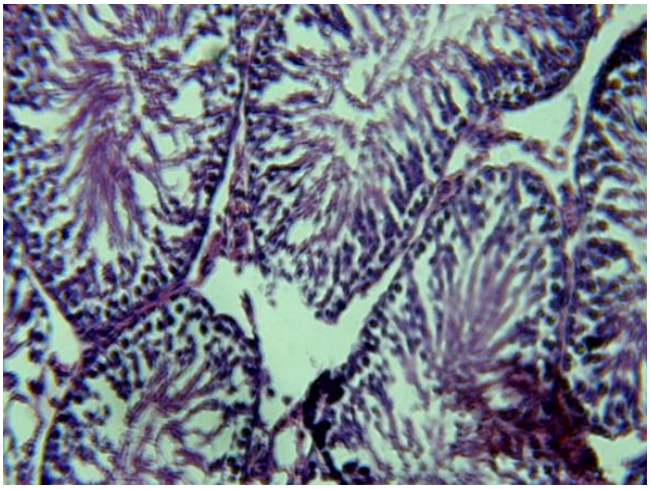
Group H, (control group), showing the normal arrangement of germinal cells, Sertoli cells, and Leydig cells. Stained by H & E technique, *x 200)* A. Myoid cell, B. Seprmatogonia, C. sertoli cell, D. Primary spermatocyte, E.Spermatozoa, F.Leydig cell.



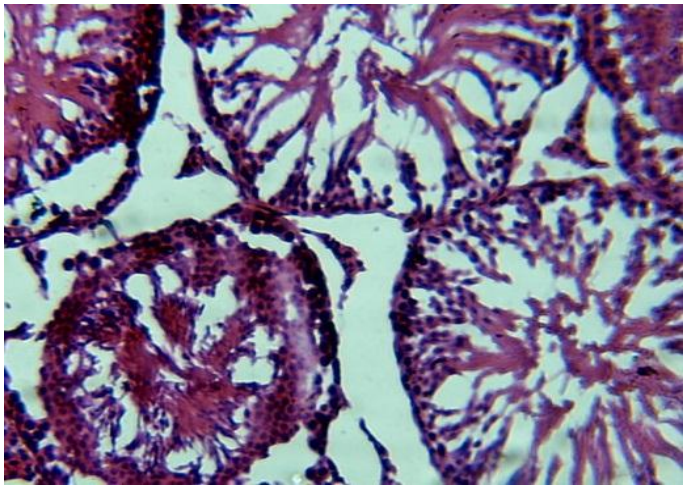
Group A, (treated with 0.13ml of mercury chloride), showing alteration in normal histological structure. Stained by H & E technique, x 200.



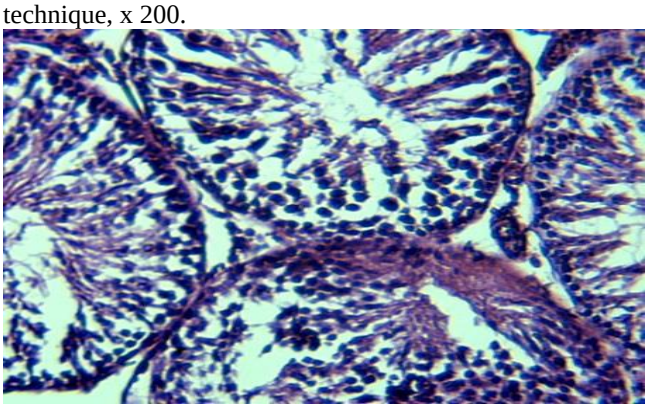
Group B,(treated with 0.07mlof mercury chloride) showing alteration in normal histological structure. Also, there is evidence of mild distortion of the spermatid cells. Stained by H & E technique, x 200.



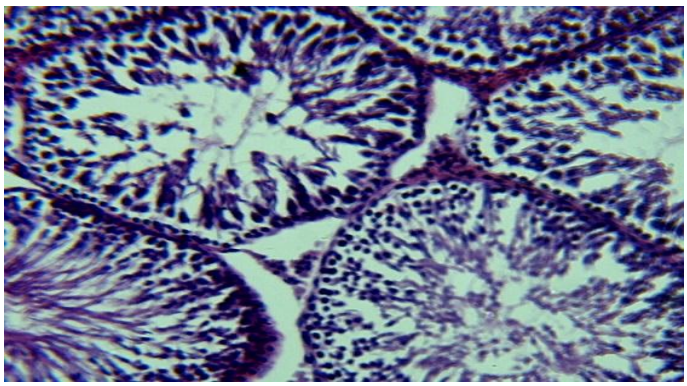
Group C, (treated with 0.03ml of mercury chloride), showing alteration in normal histological structure, distortion of the spermatid cells, with necrotic changes both in seminiferous tubules and the interstitial tissue. Stained by H & E technique, x 200.



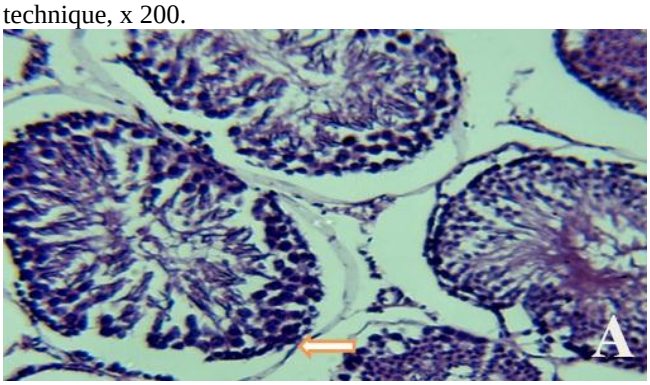
Group D (treated with 0.11ml of mercury chloride + 0.6ml of *Moringa oleifera* leave extract), showing normal arrangement of germinal cells, sertoli cells, and leydig cells with enhanced spermatid cells. Also, evidence of necrotic changes is seen in the interstitial tissue. Stained by H & E technique, x 200.



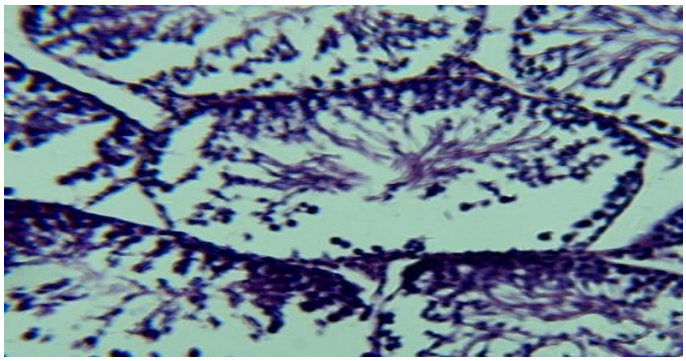
Group E (treated with 0.05ml of HgCl2 + 0.5ml of Moringa oliefera leaf extract), showing normal arrangement of germinal cells, sertoli cells, and leydig cells. There is necrotic changes in the interstitial tissue, loss of spermatids (*) and multinucleated giant cells (Arrow).Stained by H & E technique, x 200.



Group F, (treated with 0.02ml of mercury Chloride + 0.4ml 0f Moringa leave extract), showing normal arrangement of the cells(A), and also giant cells(Arrow). There is necrotic change in the seminiferous tubules. Stained by H & E technique, x 200.



Group G (treated with 0.3ml of *Moringa oleifera* leave extract), showing the normal arrangement of the histological structure of the testes with mild generalized germ cell depletion. Stained by H & E technique, x 200.



DISCUSSION

Reproductive toxicity resulting from exposure to heavy metals in males is one of the areas of concern in toxicity today. Reports have shown over the years that heavy metals including mercury intoxicate the testis by generating reactive radicals thereby resulting into celluler damage like diminution of enzyme activity, damage to lipid bilayer and DNA resulting to increase oxidative stress damages in sperm membranes protein and DNA [15]. The result in this present study showed that exposure to mercury in the rats caused damage to testes resulting in the obstruction of the spermatogenesis. This result in conformity with the report by [15] which showed that sodium chromate in rats caused shrinking of nuclear size of testicular cells and decline in cell population of spermatogenic cells. Other report reported that testicular toxicity of other heavy metals eg,copper, iron, manganese results to male subfecundity,spermatogenic and steriodogenic impairment [16-18]. From the result of the present study, it showed that concurrent treatment with *Moringa oliefera* slightly prevented the toxicity brought about by mercury exposure and thus is evident on the histoachitecture of groups D,E and F administered mercury and *Moringa oleifera* leaf extract. This result agrees with [19] who reported that toxic actions of metals are oxidative in nature and it is indicative *Moringa oleifera* was able to attenuate the toxicity of lead due to its anti-oxidative potential. This is also in agreement with [20] which have shown the anti-oxidative properties of *Moringa oleifera* and its ability to elevate a variety of anti-oxidant enzymes and testicular bio-markers.

CONCLUSION

Mercury caused disruption to testicular histo-architecture hence testicular metabolism of *Moringa oleifera* would reduce its deletrious effect. Though the ameliorative effects in this investigation is not absolute, variation in dosage especially increase in that of *Moringa oleifera* extract against mercury could possibly produce improved result.

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