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RESEARCH PAPER

TESTICULO-PROTECTIVE EFFECT OF *MORINGA-OLEIFERA* SEED EXTRACT ON COPPER SULPHATE INDUCED INJURY IN WISTAR RATS

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ABSTRACT
The aim of this study was to observe the testiculo-protective effect of aqueous extract of Moringa oleifera seed also known as Moringa seed on copper sulphate induced injury in Wistar rats. Twenty adult male Wistar rats (200-300g) were randomly selected into four groups (5 rats per group). Group A served as control group and were given 2ml/kg of distilled water. Group B was administered aqueous extract* of *Moringa seed only (200mg/kg body weight). Group C was administered aqueous extract* of *Moringa seed (200mg/kg body weight) and copper sulphate (100mg/kg body weight) concurrently. Group D was administered copper sulphate only (100mg/kg body weight). All administrations lasted for three weeks. Results showed that in group D, there was proliferation of interstitial cells, degeneration of the seminiferous tubules as evidenced by hyalinization and blockage of the tubular lumen by immature spermatogenic series due to an arrest of spermatogenesis. There was also a significant decrease in testosterone level anda significant increase in lactate dehydrogenase level when compared with the control at p< 0.05. In group C rats, the results show mild interstitial connective tissue loosening and normal sequential maturation of spermatogenic series and normal levels of testosterone and lactate dehydrogenase. Conclusively, administration of copper sulphate causes testicular damage as evidenced by testicular hyalinization and arrest of spermatogenesis. However concurrent treatment of the rats with Moringa oleifera seed extract and copper sulphate have no effect on the testes, establishing the fact that Moringa oleifera seed extract offers a protective effect to the testes.

Keywords: *Testosterone, Moringa, copper sulphate, wistar rat, Moringa oleifera*

INTRODUCTION

Moringa oleifera, which is also called the drumstick tree, is a tree that grows in the foothills of the Himalayas in northern India. It is valued mainly for its edible fruits, leaves, flowers, roots, and seed oil, and it is used extensively in traditional medicine throughout its native country and other countries. Moringa (*Moringa oleifera*) has numerous medicinal uses, which have long been recognized in the

Ayurvedic and Unani systems of medicine (Toma and Deyno, 2014).

The testes produce the male gametes and the male sexual hormones (androgens). The term spermatogenesis describes and includes all the processes involved in the production of gametes, whereas steroidogenesis refers to the enzymatic reactions leading to the production of male steroid hormones. Spermatogenesis and steroidogenesis take place in two compartments morphologically and functionally distinguishable from each other. These are the tubular compartments, consisting of the seminiferous tubules and the interstitial compartment between the seminiferous tubules.

Copper is an important biological trace element which is necessary for different metabolic functions and activities of enzymes such as catalase, peroxidase and cytochrome oxidase. Copper is also important for the utilization of iron (Goyer, 1991). On the other hand, high amount of copper has been reported to be toxic. A study on workers exposed to electrical welding revealed a high increase in semen concentration of copper along with lowering of sperm count, sperm viability and semen volume (Wu *et al*., 1996). In adult male rats, long term ingestion of copper adversely affects fertility and testicular weight (Bataineh *et al*., 1998) and recently, deleterious effects of copper poisoning on sperm quality of rats have been reported (Sakhaee *et al*., 2012).

The aim of this investigation was to observe the testiculo-protective effect of aqueous extract of *Moringa* seed on copper sulphate induced injury in Wistar rats. Also, due to the widespread medicinal uses and beneficial effects of *Moringa oleifera*, it became necessary to assess or evaluate its specific effect on male testicular injury.

MATERIALS AND METHOD

Experimental animals

Twenty adult male Wistar rats (200-300g) were purchased at the Anatomy animal house and kept in the Biochemistry animal house, both located in the University of Benin, Nigeria, and allowed to acclimatize for two weeks (with 12 hours light: and 12 hours dark, respectively). The animals were fed with standard rodent pellets (Bendel Feeds and Flour Limited) and clean water *ad libitum.* The animals were handled according to standard protocols for the use of laboratory animals (National Institute of Health, USA: Public Health Service Policy on Human Care and Use of Laboratory Animals, 2002).

Collection and preparation of extract of Moringa seeds

The *Moringa oleifera* seeds were purchased at a supermarket along the Benin-Lagos road, Ugbowo, Benin City, Nigeria. The seeds were harvested from their pods, washed and air dried for a week in the Department of Pharmacognosy, University of Benin, Nigeria. The seeds were powdered using pestle and mortar. Two hundred grammes of the powdered seeds were soaked in 4 litres of distilled water for 72 hours. Stirring was done every six hours. The powdered seeds were filtered and the filtrate was then concentrated to dryness over a hot water bath at 40°C. The extract was stored in a glass container and kept in the refrigerator prior to use.

Experimental design

Twenty (20) animals were randomly selected into four groups (5 rats per group). Group A served as the control group and were given 2ml/kg of distilled water. Group B was administered aqueous extract of Moringa seed only (200mg/kg body weight). Group C was administered aqueous extract Moringa seed (200mg/kg body weight) and copper sulphate (100mg/kg body weight) concurrently. Group D was administered copper sulphate only (100mg/kg body weight). All administration lasted for three weeks and they were done using orogastric tube.

Collection of blood samples

After three weeks of administration, the animal

were anaesthetized using chloroform. Blood was collected through cardiac puncture using 5ml syringe and needle. The blood was transferred into EDTA bottle and centrifuge at 10,000 g for 10 min. The serum was collected and analyzed for the hormone, testosterone using a suitable testosterone kit (Accu Bind Elisa Microwells, ISO 13485 and 9001).

Tissue homogenate

The right testis of each animal was weighed, placed in a mortar with 0.1g acid washed sand and ground using pestle. Cold normal saline (5ml) was added and the content was transferred into a clean 5ml container. The homogenate in the container was centrifuged at 1000g for 15 min and was stored in a deep freezer at -2 to -4°C prior to the analysis (Weisshaar *et al*., 1975). Levels of lactate dehydrogenase were measured spectrophotometrically with Randox commercially prepared kit.

Tissue preparation for histology

The left testis of the animals from each group was fixed in Bouin's solution. The fixed tissue was transferred to a graded series of ethanol and then cleared in xylene. Once cleared, the tissues were embedded in a paraffin wax in the oven at 58°C. Serial sections of 5µm thickness were obtained in solid block of tissue, cleared, fixed in clean slides, stained with haematoxylin and eosin and examined blindly under light microscope (Homayoon et *al*., 2012).

Statistical analyses

Statistical analyses were done using GraphPad Instat3 software. Comparison between the control and the treated groups were done using student's t- test. Results were expressed as Mean ±SEM (Standard Error of Mean) and values were considered to be statistically significant at $p < 0.05$.

RESULTS

The results of histopathology of the testes and blood concentrations of the serum levels of Testosterone and Lactate dehydrogenase are presented in Figs 1, 2, 3, 4 and Tables 1 and 2.

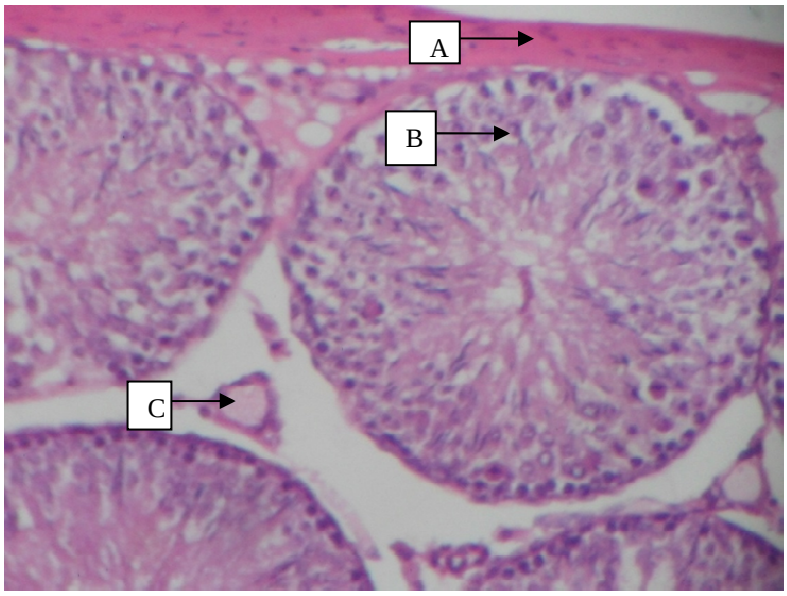

Fig. 1: Group A (Control): Rat testis composed of tunica albuginea A, seminiferous tubules with normal sequential maturation of sperm cells B and interstitial cell C (H&E x 100)

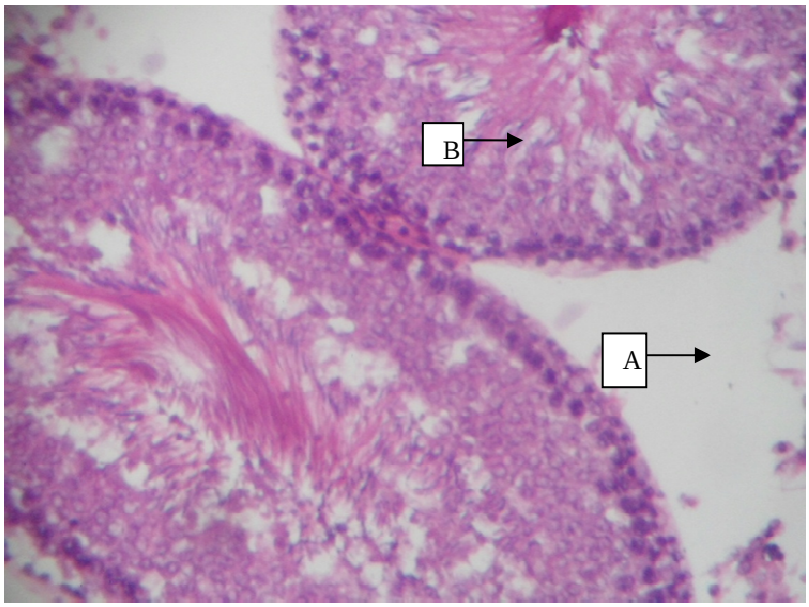

Fig. 2: (Group B): Rat testis treated with only 200mg/kg body weight of Moringa seed extract for 3 weeks showing mild interstitial connective tissue loosening A and normal sequential maturation in the tubules B (H&E x 100)

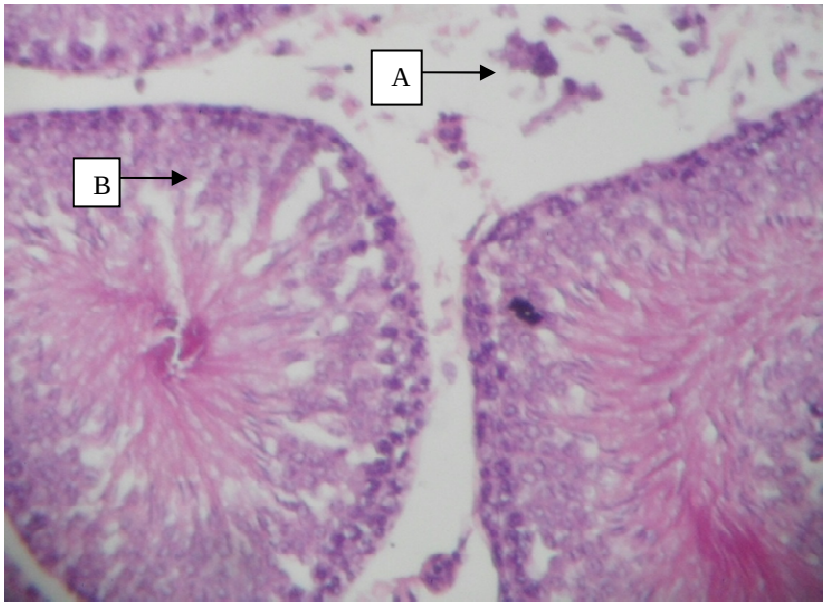

Fig. 3: (Group C): Rat testis treated with 200mg/kg body weight of Moringa seed extract and 100mg/kg of $CuSO_4$ for 3 weeks showing mild interstitial connective tissue loosening A and normal sequential maturation of spermatogenic series B (H&E x 100)

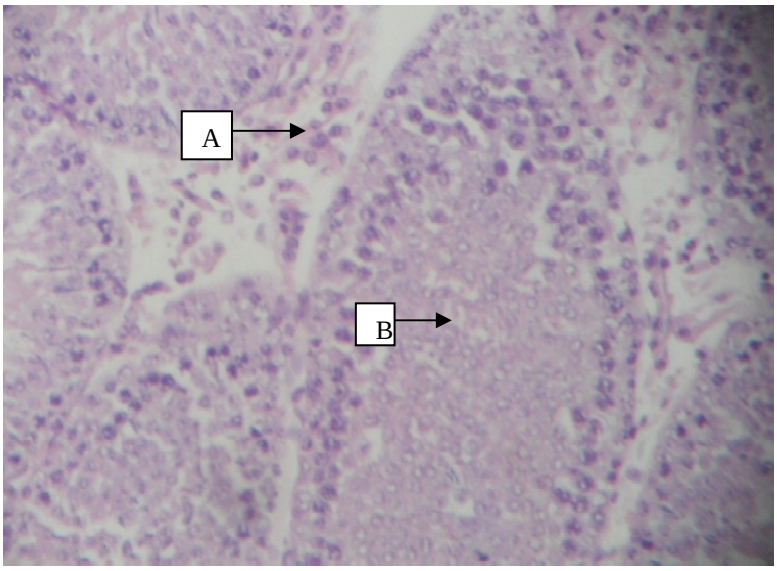

Fig. 4 :(Group D): Rat testis treated with 100mg/kg body weight of copper sulphate ($CuS0_4$) only for 3 weeks showing interstitial cell proliferation A in response to decrease in testosterone level, lumen packed with immature spermatogenic series B (H&E x 100)

Table 1: Testosterone levels of the experimental animals

GROUPS	MEAN ±SEM (ng/ml)
A (control)	1.58±0.24
B	1.10±0.28
C	1.78±1.13
D	0.70±0.14*

MEANS±SEM; n = 5

The result in Table 1 showed that there was a significant decrease in testosterone level in group D when compared with the control at $p<0.05$.
*= $p < 0.05$ VS control

The copper sulphate treated experimental animals had interstitial cell proliferation in response to decrease in serum testosterone level. The serum level of Lactate dehydrogenase was significantly increased showing evidence of tissue damage (Table 2).

DISCUSSION

Histological observations of the experimental animals in the control group that were given 2ml/kg of distilled water and the treatment group B that were given 200mg/kg of aqueous extract of *Moringa oleifera* seed for three

Table 2: Lactate dehydrogenase levels of the experimental animals

GROUPS	MEAN±SEM (µl/g/min)
A (control)	70.67±19.23
B	77.68±15.89
C	77.76±21.66
D	538.85±23.81*

MEANS±SEM; n=5

The result in Table 2 showed that there was a significant increase in lactate dehydrogenase level in group D animals that were treated with copper sulphate only compared with the control at $p< 0.05$. *= $p < 0.05$ VS control

weeks, showed normal testicular tissues stain, with normal, prominent and well defined seminiferous tubules as seen in Fig.1. There were also indications of normal sequential maturation of the different stages of spermatogenesis.

In Fig. 4 (showing the experimental animals) where only 100mg/kg of copper sulphate was administered, there was proliferation of interstitial cells, degeneration of the seminiferous tubules as evidenced by hyalinization and blockage of the tubular lumen by immature spermatogenic series due to an arrest of spermatogenesis. The interstitial cells of Leydig, found adjacent to the seminiferous tubules in the testicle produce testosterone in the presence of luteinizing hormone. The proliferation of these cells is in response to decreased testosterone level. The possible explanation for these changes that were induced by copper sulphate, could be as a result of the participation of copper in the formation of Reactive Oxygen Species (ROS). Copper is a strong oxidant, which could bind to cell molecules during the high load (high amount) of 100mg/Kg body weight that was used in this study (Linder and Hazegh-Azam, 1996). Cupric ions coming from the corrosion of metallic copper have been reported to be reduced to cuprous ions in the presence of biological reductants (Bastidas *et al*., 2000). Cuprous ions are able to catalyse the formation of ROS through the decomposition of hydrogen peroxide (H_2O_2) using the Fenton/Haber-Weiss reaction (Gaetke *et al*., 2003). In addition, ions from metals such as copper exhibit high affinity for thiol groups and may therefore severely disturb many metabolic functions of cell (Hultberg *et al*., 1997). Consequently, oxidative stress that occurs as a result of the state of redox disequilibrium in which ROS production overwhelms the antioxidant defence capacity of the cell, may lead to adverse biological consequences such as damage to lipids, DNA or proteins resulting in excess cell proliferation (as evidenced by interstitial cell proliferation in this study) or mutagenesis (Kappus, 1987).

Mammalian spermatozoa membranes are very sensitive to damage that are mediated by lipid peroxidation, because they are rich in polyunsaturated fatty acids (Mishra and Acharya, 2004). Also these fatty acids are essential requirements for the male germ cell to maintain sperm functions (Ollero et al., 1998). In Fig. 3, showing the experimental animals in which *Moringa oleifera* seed extract (200mg/kg) and copper sulphate (100mg/kg) were administered concurrently, there were unremarkable seminiferous tubules sections with normal sequential maturation of sperm cells. Observation indicates that the toxic effect of copper sulphate

was blocked by *Moringa oleifera* seed extract. This may be due to the presence of flavonoids in *Moringa oleifera* seed. Flavonoids are well known antioxidants that can ameliorate oxidative stress-related testicular impairment in animal tissues (Ghosh *et al*., 2002). It also stimulates testicular androgenesis and is essential for testicular differentiation, integrity and steroidogenic functions (Salem *et al*., 2001).

Testosterone level was reduced in the animals that were treated with copper sulphate only, when compared with the controlas seen in Table 1. Copper sulphate results in shrinkage and collapse of tubules. Copper is known to produce a suppressive influence on male reproductive activity, mainly on testicular weight and steroidogenesis and accessory sex organ weight in a dose-dependent manner (Chattopadhyay *et al*., 1999).

Testosterone stimulates the conversion of round spermatids into elongated spermatids between stages VII and VIII of the spermatogenic cycle (Hammami *et al*., 2008). Lactate dehydrogenase (LDH) is an enzyme found in almost all body tissues. It plays an important role in cellular respiration. Its level is normally low in blood. However, when tissues are damaged by injury or disease, they release more lactate dehydrogenase into the blood stream (Butt *et al*., 2002). An abnormal increase in the level of this enzymeas seen in Table 2 in the animals that were administered copper sulphate only, which indicates an anomaly caused by copper sulphate induced damage. The lowering of LDH level in the group treated with *Moringa oleifera* seed extract and copper sulphate concurrently (Table II) is a positive pointer to the fact that *Moringa* phytochemicals (Faizi *et al*., 1994) acted to prevent the damaging effect of copper sulphate on the testes.

CONCLUSION

Administration of copper sulphate only to the experimental animals, resulted in testicular damage as evidenced by testicular hyalinization and arrest of spermatogenesis. However concurrent treatment of the rats with *Moringa oleifera* seed extract and copper sulphate did not show any adverse effect on the testes, establishing the fact that *Moringa oleifera* seed extract offers a protective effect to the testes and may help to reduce the risk of prostate cancer.

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