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The effect of ethanolic extract of *Moringa oleifera* on alcohol-induced testicular histopathologies in pre-pubertal albino Wistar rats

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Abstract
Excessive use of alcoholic beverages has identified alcoholism as one of modern society's major problems. This study was carried out to investigate the effect of *Moringa oleifera* on alcohol-induced testicular toxicities in pre-pubertal Wistar rats. Forty pre-pubertal Wistar rats were divided into 10 groups. Group 1–control, Group 2–*M. oleifera* only, Group 3–alcohol and then *M. oleifera*, Group 4–alcohol and *M. oleifera*, Group 5–*M. oleifera* and then alcohol, Group 6–alcohol only, Group 7–alcohol and then vitamin C, Group 8–vitamin C and alcohol, Group 9–vitamin C and then alcohol, and Group 10–vitamin C only. Alcohol caused numerous atrophies in the testes and damaged spermatogenic cells. *M. oleifera* and vitamin C however exhibited protective and reversibility effects. Results showed significant effect of *M. oleifera* on the testicular weight without any significant difference in body weight. In conclusion, *M. oleifera* ameliorates alcohol-induced testicular toxicities with its antioxidant properties comparable to vitamin C.

Keywords: *Moringa oleifera*; alcohol; puberty; testis; vitamin C.

Introduction

Infertility affects more than 80 million people around the globe. It is a ubiquitous phenomenon that transcends race and nationality (Anate and Akeredolu, 1991). Each male and female factor infertility accounts for about 40% cases of infertility, the remaining 20% is as a combination of male and female (Randolph, 2004).

Excessive use of alcoholic beverages results in a variety of medical and psychosociological disturbances that identify alcoholism as one of modern society's major problems (Cebral *et al.*, 1997). Most studies of ethanol-induced fertility alterations have been conducted with the male gender of both man and laboratory animals. The effects of ethanol on pubertal processes are poorly understood and only a few studies have been conducted in this respect. Some studies have however reported that ethanol delays certain aspects of sexual maturation (Cebral *et al.*, 1997).

Moringa oleifera, commonly known as drumstick tree has been used as antiulcer, diuretic, antiinflammatory, and for wound healing (Cáceres *et al.*, 1991; Udupa *et al.*, 1994; Pal *et al.*, 1995). Its leaves are also used as nutritional supplement and growth promoters due to significant presence of protein, selenium, calcium, phosphorus, β-carotene, and γ-tocopherol (Nambiar and Seshadri, 2001; Lakshminarayana *et al.*, 2005; Sánchez-Machado *et al.*, 2006).

This study was therefore designed to investigate the effect of *M. oleifera* on alcohol-induced testicular histopathologies in pre-pubertal Wistar rats.

Materials and Methods

Animals
Forty male albino Wistar rats aging between 20 and 35 days were obtained from a private farm in Port-Harcourt, Rivers State, Nigeria and allowed

to acclimatize in the Animal House, Basic Medical Sciences, University of Uyo, Town Campus Annex, Uyo, Akwa Ibom State, Nigeria for a period of 10–20 days for the animals to attain an average age range of 40–45 days old. They were then put into wire net-covered, wooden cages. Food and water was provided *ad libitum*. The Animal House was well ventilated throughout the course of the experiment.

Collection of plant material

The *moringa* leaves were obtained from Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria and authenticated at the Department of Botany, University of Uyo, Uyo, Nigeria.

Extract preparation

M. oleifera leaves were air-dried under the sun and powered using pestle and mortar. The powder was placed in a thimble and the soxhlet extractor was set up. The maceration process lasted for about 6 hours. The crude liquid extract was separated from the marc by filtration. The filtrate was then concentrated to dryness in a hot water bath at 40°C and stored in a refrigerator.

Experimental protocol

Before administration, the rats were weighed and their weights ranged between 105 and 165 g. They were then randomly divided into 10 groups consisting of four rats each. Group 1 served as control and the other 9 groups served as experimental groups.

Group 1–control, Group 2–*M. oleifera* only (2 weeks), Group 3–alcohol (2 weeks) and then *M. oleifera* (2 weeks), Group 4–alcohol and *M. oleifera* (2 weeks), Group 5–*M. oleifera* (2 weeks) and then alcohol (2 weeks), Group 6–alcohol only (2 weeks), Group 7–alcohol (2 weeks) and then vitamin C (2 weeks), Group 8–vitamin C and alcohol (2 weeks), Group 9–vitamin C (2 weeks) and then alcohol (2 weeks), and Group 10–vitamin C only (2 weeks).

Dosage

Administration of *M. oleifera* extract, vitamin C, and alcohol was by gastric gavage. The LD_{50} of ethanol extract is 5000 mg/kg in acute oral toxicity testing (Rathi *et al.*, 2006). Standard dose of *M. oleifera* is calculated at 400 mg/kg body weight. 30% ethanol was calculated at 2 ml/kg body weight (Akang *et al.*, 2011) and vitamin C was calculated at 10 mg/kg body weight.

Preliminary phytochemical analysis

Phytochemical screening was also carried out to check the presence of bioactive agent of the ethanolic extract of *M. oleifera*. This was carried out using the standard method of chemical analysis (Trease and Evans, 1989). All materials required for the screening tests were washed, rinsed with distilled water, and dried before the test.

The confirmatory tests were on alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, flavonoids, and terpenes.

Sacrifice

At the end of the experimental period, the rats were sacrificed after anaesthetizing with chloroform. The testes were removed and fixed in 10% buffered formalin in preparation for tissue processing.

Tissue preparation for histology

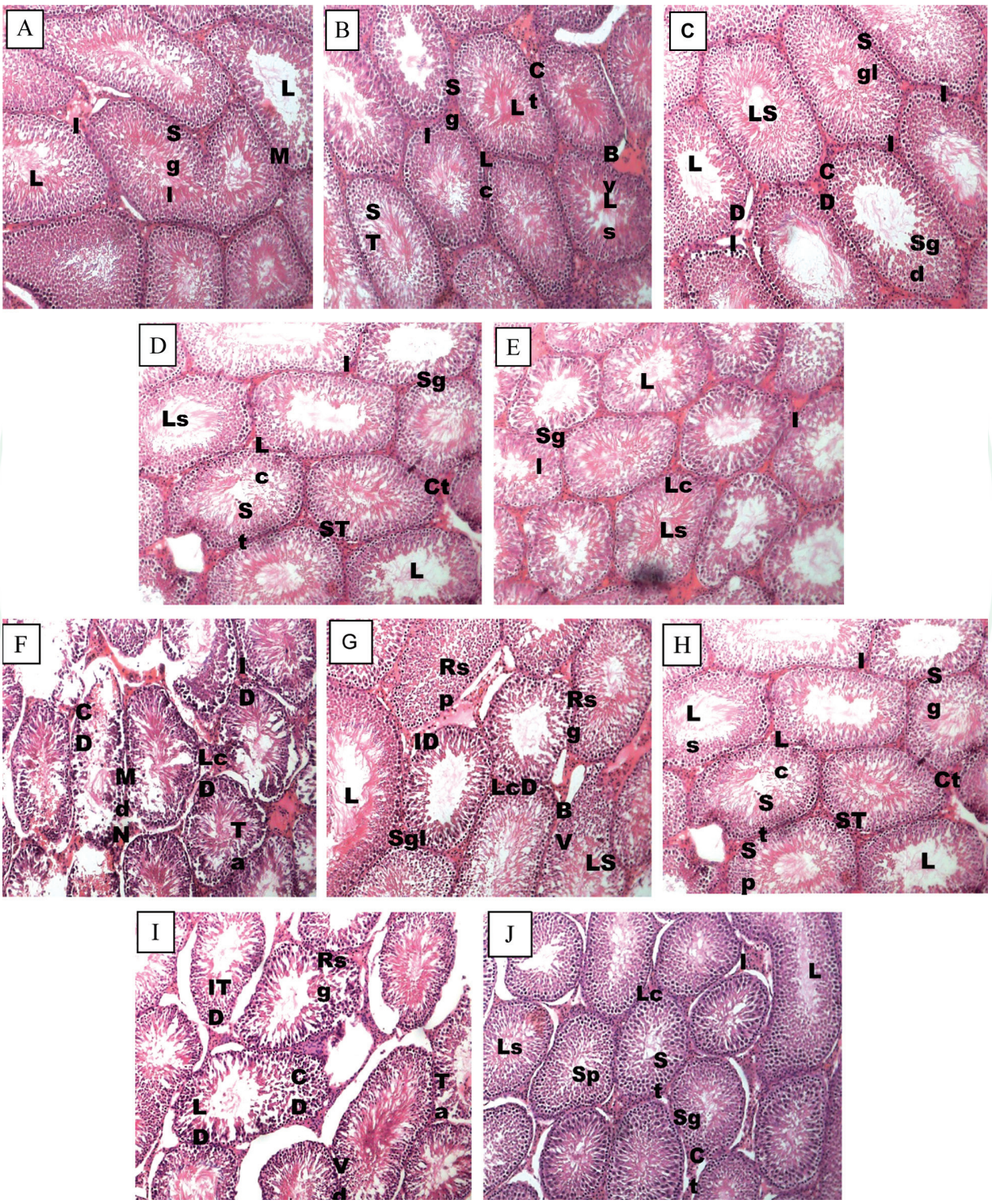
The organs were processed for histological work as follows: one testis from each animal was fixed in 10% formol saline. The fixed tissues were transferred to a graded series of ethanol and then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Serial sections of 5 LM thickness were obtained from a solid block of tissue, cleared, fixed in clean slides, stained with Haematoxylin and Eosin stains, and examined under a light microscope.

Results

Effects on testicular histology

The control (Group 1) showed normal histological architecture of the testis (Figure 1A). Group 6 treated with alcohol showed numerous atrophied and damaged seminiferous living cells, spermatogonia, spermatocytes, spermatids, spermatozoa and lumen filled with semen, degenerated interstitial leydig cells, and interstitium against background of connective tissues with marked area of necrosis (Figure 1F). Group 5 treated with *M. oleifera* and then alcohol showed areas of numerous seminiferous tubules containing myoid living cells, spermatogenic living cells, spermatogonia, spermatocytes, spermatids, and spermatozoa and lumen filled with semen. In between the seminiferous tubules are cells of leydig and interstitium against background of connective tissues with slight area of cellular alteration. Hence the alcohol could not have much effect on the testicular histology (Figure 1E).

Figure 1: Photomicrographs showing histological testes sections of (A) Group 1 served as control, (B) Group 2 treated with *M. oleifera* only, (C) Group 3 treated with alcohol and then *M. oleifera*, (D) Group 4 treated with alcohol and *M. oleifera*, (E) Group 5 treated with *M. oleifera* and then alcohol, (F) Group 6 treated with alcohol only, (G) Group 7 treated with alcohol and then vitamin C, (H) Group 8 treated with vitamin C and alcohol, (I) Group 9 treated with vitamin C and then alcohol, and (J) Group 10 treated with vitamin C only at magnification of 100X stained with Haematoxylin and Eosin.



Group 9 treated with vitamin C and then alcohol showed numerous reversible, atrophied, and damaged seminiferous tubules containing swollen myoid living cells, swollen spermatogenic living cells, spermatogonia, spermatocytes, spermatids, and spermatozoa and lumen filled with semen, in between the seminiferous tubules are the interstitial leydig cells, interstitium against background of connective tissues (Figure 1I).

Group 2 treated with *M. oleifera* only (Figure 1B), Group 3 treated with alcohol and then *M. oleifera* (Figure 1C), Group 4 treated with alcohol and *M. oleifera* (Figure 1D), Group 7 treated with alcohol and then vitamin C (Figure 1G), Group 8 treated with vitamin C and alcohol (Figure 1H), and Group 10 treated with vitamin C only (Figure 1J) showed normal histological architecture of the testis when compared with control such as numerous seminiferous tubules containing swollen myoid living cells, spermatogenic living cells, spermatogonia, spermatocytes, spermatids, and spermatozoa and lumen filled with semen, in between the seminiferous tubules are the interstitial leydig cells, interstitium against background of connective tissues. These showed the protective action of *M. oleifera*; along with its preventive and reversibility of alcohol-induced testicular injuries.

Phytochemical constituents of M. oleifera

The results of the preliminary phytochemical screening showed that *M. oleifera* was positive for the presence of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, flavonoids, and terpenes.

Discussion

Many agents shown to have deleterious effects on the spermatozoa or the cyto-architectural pattern of the testes. Some of these agents include caffeine, nicotine, steroids, alcohol, anesthetic agents, and insecticides. Researchers have expressed their concern about the rising cases of male spermatozoa abnormalities (Kuku and Osegbe, 1989).

In this study, testicular atrophy and distortions in spermatogenic cells were observed in groups treated with alcohol. These findings were however ameliorated in groups treated with *M. oleifera* and vitamin C, as they had the same morphological status as the control. Numerous studies have indicated that alcohol abuse in men can cause impaired testosterone production and testicular atrophy (Adler, 1992). Those changes can result in impotence, infertility, and reduced male secondary sexual characteristics. Testicular atrophy results primarily from the loss of sperm cells and decreased diameter of the seminiferous tubules (Van Thiel *et al.*, 1974). Spermatogenic cells occupy 95% of testicular volume. Therefore, failure of spermatogenesis may be characterized by testicular atrophy associated with oligospermia or azoospermia (Wright *et al.*, 1991). Increased ethanol consumption in human teenagers has led to concerns of significant hormonal changes during puberty. In rats, acute ethanol administration prior to puberty profoundly decreases serum LH levels, which decreases testosterone secretion and testicular weights (Emanuele *et al.*, 1998).

Ethanol promotes oxidative stress both by increased formation of reactive oxygen species and depletion of antioxidant status (Wu and Cederbaum, 2003). These reactive oxygen species cause destructive and irreversible damage to cellular components, such as lipids, proteins, and DNA (Catalá, 2009). Deficiencies of vitamin C lead to a state of oxidative stress in the testes that disrupts both spermatogenesis and the production of testosterone (Johnson, 1979). Conversely, ascorbate administration to normal animals stimulates both sperm production and testosterone secretion (Sönmez *et al*., 2005). This vitamin also counteracts the testicular oxidative stress-induced by exposure to pro-oxidants such as arsenic, PCBs (Aroclor 1254), cadmium, endosulfan, and alcohol (Sen Gupta *et al.*, 2004; Senthil *et al.*, 2004; Maneesh *et al.*, 2005; Rao *et al.*, 2005; Chang *et al.*, 2007).

Sreelatha and Padma (2009) in their study revealed that *M. oleifera* leaves bear a potent antioxidant activity. Their constituents scavenge free radicals and exert a protective effect against oxidative damage induced to cellular macromolecules. Natural antioxidants that are present in herbs are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Herbs contain free radical scavengers like polyphenols, flavonoids, and phenolic compounds. A number of scientific reports indicate certain terpenoids, steroids, and phenolic compounds such as tannins, coumarins, and flavonoids have protective effects due to its antioxidant properties (Chandrasekar *et al.*, 2006).

Conclusion

M. oleifera possess tremendous antioxidant properties that ameliorate the deleterious effect of alcohol on pre-pubertal testes.

Ethical Approval

The study was approved on rats by the ethics committee of Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Conflict of Interests

None declared.

Authors' Contributions

All authors contributed equally to this work.

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