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RESEARCH PAPER

HISTOLOGICAL CHANGES IN THE TESTIS OF RATS TREATED WITH ALOMO BITTER

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ABSTRACT

Considering the increasing demand for herbal aphrodisiacs, this study investigates the effect of Alomo bitters on the histology of testis in adult rats. 36 male rats of comparable weight (151.67 ± 2.89 grams) and sizes were involved in this study. The animals were assigned into four groups; a control group (A) and three test groups (B, C and D). For 3 weeks, group A received growers mash and water, while test groups B1-B3, C1-C3 and D1-D3 representing experimental durations of 1 week, 2 weeks and 3 weeks respectively; received growers mash and graded doses of Alomo bitters (7.5, 15, 22.5ml) daily. Histologically, micrographs from the test groups presented changes that included interstitial space exudates, cellular pyknosis, cellular degeneration, cell population reduction, and vacuolations. These changes were dosage-duration dependent suggesting that Alomo bitters can induce testicular damage and by implication, infertility in males. Thus, there is an urgent need to regulate the consumption of Alomo bitters as well as other herbal products considered to possess aphrodisiac potentials.

Keywords: Alomo-bitters, Herbs, Male, Testis, Histology.

INTRODUCTION

Current trends show that the inability to afford modern medical healthcare in developing countries has forced patients to seek traditional medical attentions (Watcho *et al.*, 2007). In this regard, the World Health Organisation (WHO) has estimated that about eighty percent of the world population rely chiefly on traditional medicines (Agaie *et al.*, 2007). Of the 119 plant derived drug listed by WHO study, 74% were discovered as a result of chemical studies to isolate the active compounds responsible for the use of original plant in traditional medicine (Farnsworth *et al.*, 1985). In Nigeria several plants have been claimed traditionally, to have medicinal potentials for the treatment of various ailments in both man and animals (Nwude and Ibrahim, 1980; Akinniyi and Sultanbawa, 1983), although their efficacy and safety remain doubtful as only a few of these have been properly identified and documented (Mbaya *et al.*, 2007; Nwosu *et al.*, 2004).

On the other hand, it is a known fact that healthy sexual functioning contributes significantly to one's sense of well-being and quality of life (Salonia *et al.*, 2004). While early report has revealed a high incidence of sexual problems in the general population (Alain, 1999), recent available records show male impotence (Erectile Dysfunction, ED) as the common medical condition affecting the sexual life of millions of men worldwide (Montorsi *et al.*, 2003; Shabsigh and Anastasiadis, 2003). To achieve sexual quality, many orthodox therapeutic approaches have been employed for quite a long time (Sagraves *et al.*, 2003). However, they present with limited efficacy, unpleasant side effects and contraindications in certain conditions (Lue *et al.*, 2003).

Similarly, many plants have been reported to be employed as remedies for such disorders, while others have been used as aphrodisiacs (Yakubu *et al.*, 2005; Tajuddin *et al.*, 2005; Dhawan *et al.*, 2003; Sumalata *et al.*, 2010). In fact, a number of plant extracts have been acclaimed to have aphrodisiac potentials (Noumi *et al.*, 1998) and used traditionally to improve sexual performances (Kamtchouing *et al.*, 2002; Carro, 1999). Adjanohoun *et al.*, (1996) and Noumi *et al.*, (1998) listed a variety of plants used in traditional medicine for fertility regulation. Also, several other medicinal plants are known for male contraceptive potentials by suppressing spermatogenesis or by spermicidal action (Paul *et al.*, 2006).

One of such herbal preparations with acclaimed aphrodisiac potentials is Alomo bitters -an alcoholic drink comprising seven herbal ingredients. The ingredients include *Khaya invorensis*, *Capparis erythrocarpus*, *Mondia whitei*, *Lecaniodiscus-cupanoïdes*, *Dialium guineense*, *Treulia Africana*, *Crytolepis-sanguinolenta*. Considering the rate of consumption, this study therefore, investigates the effects of Alomo Bitters on the histology of the testis.

MATERIALS AND METHODS

Substance of Study: Several bottles of “Alomo bitters” was purchased from main market in Ekpoma, Edo State, Nigeria with NAFDAC registration number: A1-8029; manufactured date: 14/10/2010; and batch number: ALM 287101. No expiry date was found on the bottles. These details were checked to ensure the authenticity of the products purchased.

Experimental Animals: Thirty-two adult male Wister rats of comparable weights and sizes were procured from the animal farm of the Department of Physiology, College of Medicine, Ambrose Alli University, Ekpoma, and moved to the animal house of Anthonio Research Centre, Ekpoma, Edo State, Nigeria, where they were housed in wooden cages.

The animals were assigned into four groups: a control group (A) and three test groups (B, C and D) subdivided into B1-D1, B2-D2, and B3-D3 representing experimental durations of 1 week, 2 weeks and 3 weeks respectively. The rats were allowed to acclimatize for two weeks and were fed *ad libitum* during this period, with water and grower mash from Bendel Feeds and Flour Mills, Ewu, Edo State, Nigeria.

Substance Administration: All the animal groups were fed with feed (growers mash) plus water given *ad libitum*. However, as group A (control) received distilled water only, test groups B to D received water mixed with graded quantities of Alomo Bitters: B (7.5ml/kg), C (15ml/kg) and D (22.5ml/kg). Appropriate quantities of Alomo bitters were administered into the drinking water of the animals in each the groups respectively, using a sterile syringe of 20ml gauge convenience.

Study Duration: This study lasted between October, 2011 - December, 2011. However, the actual experiment lasted for five weeks (2weeks for acclimatization and 3 weeks for substance administration). During the five weeks study period, the animals were fed and monitored between the hours of 8:00am – 12:00 noon daily.

Sample Collection: At the end of each week, 3 rats from each of the groups respectively were sacrificed by administering chloroform as anaesthesia. The rats were then dissected to harvest the testes which were then fixed immediately in 10% formalin.

For descriptive purposes, the animals sacrificed at the end of week one from the test groups were designated as B1—D1 rats, while B2 – D2 and B3 - D3 represents the rats sacrificed at the end of week two and three respectively.

Tissue Processing: The tissues were processed using automatic tissue processor according to stand histological processing schedule as described by David (2004). Microscopy was done using a binocular light microscope at magnification x400.

RESULTS

Histological observations showed that the issue micrographs obtained from tissue section of the testes in group A (control) presented normal histological architecture (Plate 1). However, those from test group B1- treated with 7.5 ml of alomo bitter for 1 week presented interstitial space exudates (ISE), cellular pyknosis (P), vacuolation (V), cellular degeneration (CD) (Plate 4.2). Micrographs from group B2 -treated with 7.5ml for 2 weeks, presented seminiferous tubule wall degeneration (STD), cellular degeneration (CD) with congested vessels and vacuolation

(V) (Plate 4.3), while those from group B3 - treated with 7.5 ml for 3 weeks presented interstitial space congestion (ISC) with exudates (E) and vacuolation (V), seminiferous tubule wall degeneration (STD) with mild cellular vacuolations (V) and pale staining cells (Plate 4.4).

Similarly, tissue micrographs from group C1-treated with 15.0ml of alomo bitter for 1 week presented severe cellular degeneration (CD) with cell-population reduction, interstitial space enlargement (ISE) with exudates (E), seminiferous tubule degeneration (STD) with vacuolation (V), cellular pyknosis (P) (Plate 4.5). Micrographs from group C2 -treated with 15.0ml of alomo bitter for 2 weeks presented cellular degeneration (CD), interstitial space enlargement (ISE) and sminferous tubular wall degeneration (STD) with pale staining cells, cellular pyknosis (P) and vacuolation (V) (Plate 4.6). Those from group C3 -treated with 15.0ml of alomo bitter for 3 weeks presented cellular degeneration (CD), interstitial space enlargement and congestion (ISC), cellular necrosis (N) with vacuolation (V), seminiferous tubules wall degeneration (STD), and reduction in cell population (Plate 4.7).

Also, micrographs from group D1-treated with 22.5ml of alomo bitter for 1 week presented severe cellular necrosis (N) with vacuolations (V), exudations (E), interstitial space enlargement (N), and seminiferous tubule wall degeneration (Plate 4.8). Micrographs from group D2 -treated with 22.5ml of alomo bitter for 2 weeks presented vacuolation (V), pyknosis (P), and interstitial space exudation (E) with cells staining pale and “ghost-like” in appearance (Plate 4.9). Group D3; treated with 22.5ml of alomo bitter for 3 weeks (Plate 4.10) presented cellular necrosis (N), interstitial space congestion (ISC), vacuolation (V), seminiferous tubules degeneration (STD), pale staining cells with “ghost-like” appearance.

Generally, the histological findings suggests alomo-bitters-induced testicular damage and the observed changes were dosage-duration dependent.

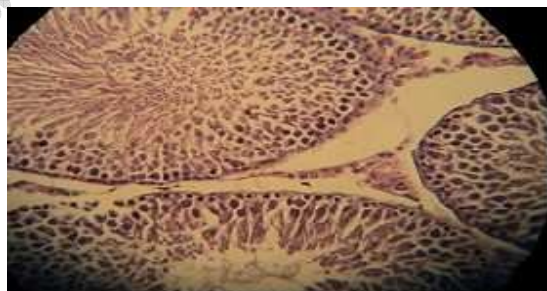


Plate 1: Testis (H&E X400) showing normal histological features of the Testis

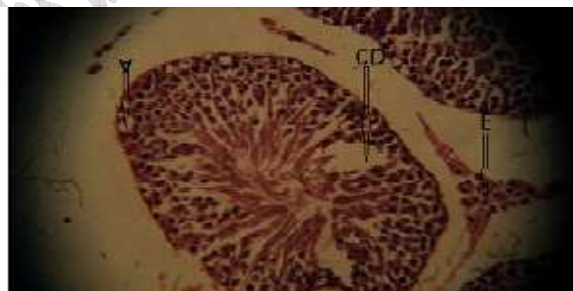


Plate 2: Testis (H&E X400) showing cellular degeneration (CD), interstitial space exudate (E), and Vacuolation (V)

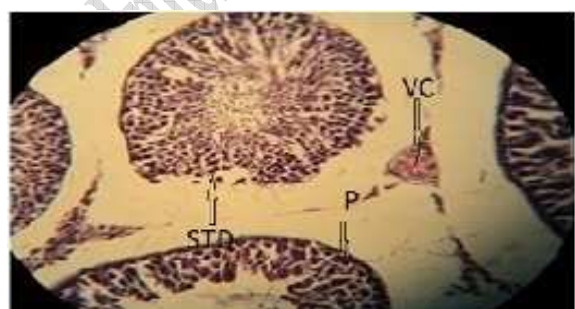


Plate 3: Testis (H&E X400) showing Seminiferous tubules degeneration (STD), Pyknosis (P), Vascular congestion (VC)

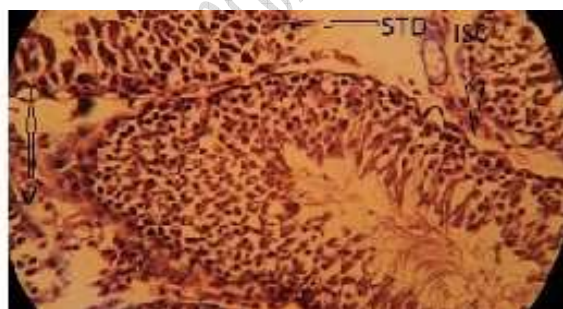


Plate 4: Testis (H&E X400) showing Interstitial space congestion (ISC), Seminiferous tubule degeneration (STD), Vacuolation (V)

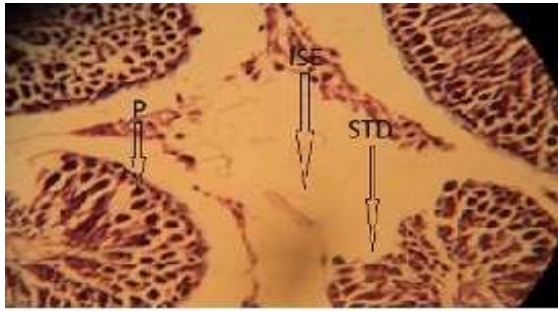


Plate 5: Testis (H&E X400) showing Pyknosis (P), Interstitial space enlargement (ISE), Seminiferous tubules degeneration (STD)



Plate 6: Testis (H&E X400) showing Pyknosis (P), Interstitial space enlargement (ISE), Seminiferous tubules degeneration (STD)

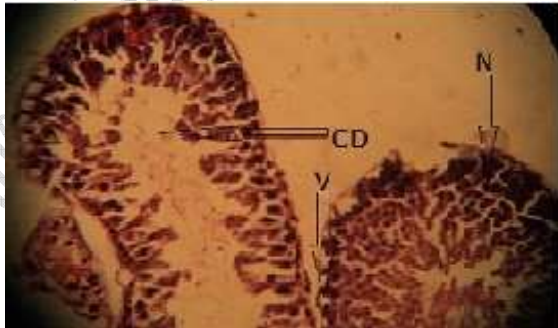


Plate 7: Testis (H&E X400) showing Cellular degeneration (CD), Necrosis (N), Vacuolation (V)

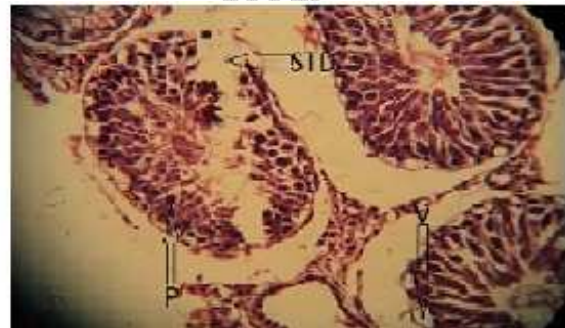


Plate 8: Testis (H&E X400) showing Pyknosis (P), Seminiferous tubule degeneration (STD), Vacuolation (V)

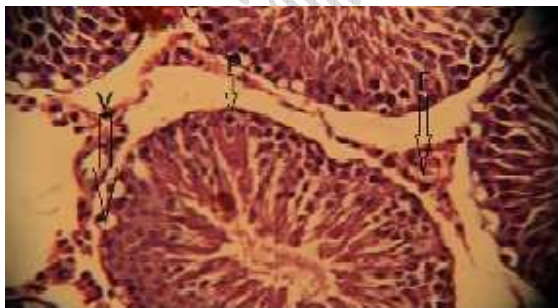


Plate 9: Testis (H&E X400) showing Pyknosis (P), interstitial space exudate (E), and Vacuolation (V)

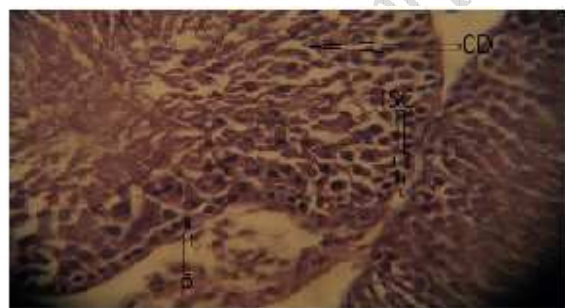


Plate 10: Testis (H&E X400) showing Pyknosis (P), interstitial space congestion (E), and cell degeneration (CD)

DISCUSSION

The observed histological changes in the testis, following the administration of Alomo Bitters, suggest that Alomo Bitters can induce male sterility. In fact, the findings reported by Lampio (2009), Watcho et al., (2007), Patman et al., (2005) that some of the constituents of Alomo Bitters - *Mondia whitei* and *Khaya ivorensis*, can cause several testicular damages in a dose depended manner surely supports this assertion.

Furthermore, the presence of interstitial space exudates, shrinkage of the seminiferous tubules, complete disorganization of the testicular tissue with destruction of spermatogonial cells and germinal cells, cellular pyknosis, vacuoles in the cytoplasm of the Sertoli cells, necrosis and degenerating cells in the present study, implies that Alomo Bitters is capable of inducing testicular cell damage like snake venom as previously reported by Penna-Videaú, *et al.* (2000).

Similarly, Russell (1999) had reported that such testicular lesions are the early morphological sign of testicular injury which was considered by Nolte *et al.* (1995) and Penna-Videaú *et al.* (2000) as the main testicular response to harmful stimuli. Thus, the histological findings in this study contradict the general belief that Alomo Bitters boost male fertility.

From the foregoing, it can be concluded that the structural integrity of the testes was compromised under the influence of Alomo Bitters in a dosage-duration dependent manner. Definitely, the observed changes would adversely affect the function of the testes and might particularly become worse, if the dosage is high and consumption prolonged. It is our opinion therefore, that prolonged or repeated ingestion of alomo bitters should be discouraged considering the increasing cases of male sterility hitherto tagged idiopathic.

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AUTHORS' CONTRIBUTIONS

Salisu A.A., performed this research work under the supervision of Dr. Ihongbe J.C., with assistance from Anyanwu R.A., and Uwuigbe M.. Izekor S., was involved with the daily activities during the experimental period and assisted Salisu AA.