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Histological changes in the testes and Epididymis of wistar rats following administration of varied doses of aqueous extract of *Aspilia africana* flowers

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Abstract

The use of medicinal plants to treat various ailments is fast becoming common place and their possible effects on the various organs of the body have also become a global public health concerns. Several plant materials have been claimed to be efficacious in modulating the activities of the male reproductive system, while some are used as aphrodisiacs, others are employed to regulate fertility. *Aspilia africana* have been claimed to have the propensity to exert some modulatory effects on the male reproductive system. This study was designed to elucidate the histological changes in the testes and epididymis of adult male *wistar* rats following administration of varied dosages of aqueous extract of *Aspilia africana* flowers. Twenty adult male *Wistar* rats weighing 250- 300g were divided into four groups (A-D) of five rats each. Group A served as control and was given 1ml of distilled water, while groups B, C, D were given 150mg/kg, 200mg/kg and 300mg/kg body weight respectively. Histological differences between selected organs of control and treatment were evaluated. The histological findings revealed a dose dependent increase or widening of the interstitial spaces and mild disruption of the spermatogenic series in the testes of the rats treated with 200 and 300mg of extract per kilogram body weight. The group treated with 300mg of the extract showed more marked disruption of germinal epithelium with shrunken seminiferous tubules. However, the group treated with 150mg/kg body weight of rat maintained the normal structural integrity but the interstitial spaces were evidently wider relative to the control. Studies on the epididymis revealed fairly normal tubules packed with mature spermatozoa, mildly decreased population of sperm cells and moderately decreased population of spermatozoa respectively with an intact epithelial lining across the groups treated with 150, 200 and 300mg of the extract per kilogram body weight of the rats when compared to the control. This shows that *A. africana* flowers may have a dose dependent deleterious effect on the histology of the testes and epididymis. However, Further study is therefore required to elucidate the mechanism by which the changes were achieved.

Keywords: Medicinal plants, Histology, Testis, Epididymis, *Aspilia africana*.

1. Introduction

Aspilia africana (Asteraceae) is a common herbaceous plant widely known for its ethno-medicinal values. It is a perennial plant with long history of agricultural uses as Farmers usually graze their cattle, sheep, and feed their rabbits and hares with it in most African countries especially in West Africa. It is also known as the iodine or haemorrhage plant as it is commonly used to stop bleeding even from large arteries. In the eastern part of Nigeria, it is used in snail farming hence the name 'oranjila'. It is polymorphic with at least 4 varieties occurring on wasteland of savanna & forested zones throughout Africa.

Aspilia africana have been reported to have several uses and properties and is widely used in Africa and Asia. Reports show that this traditional herb is used as an anti-inflammatory agent; it has astringent properties, acts as a bactericide and is very effective in wound healing [1]. Studies have shown that the leaves of *A. africana* is potent in the relief of febrile headache as well as cure ailments such as stomach disorders, sciatica and lumbago [2]. Another study reported that the ethanolic leaf extract of *A. africana* could increase vascular tone and also have *in vitro* gastro-protective properties [3].

Other abilities that have been credited to this plant include anti-bacterial, anti-fungal and anti-spasmodic properties. It is used to hasten delivery and it strengthens smooth muscle contraction [4, 5, 6, 7]. Reports from recent studies have revealed that the methanolic leaf extract administered intraperitoneally altered the estrus cycle and caused deleterious damages on uterine tissues [8, 9]. This plant is generally known as the hemorrhage plant, which is borne out of its ability to arrest bleeding even when a major artery is severed as revealed by a previous study [10]. Iwu [11] also reported its use in the treatment of anemia, corneal opacities and stomach ailments. A study carried out on the leaves revealed the presence of procenol in the purified aqueous extract of *A. africana* leaf, adding that sesquiterpenes and monoterpenes are the major essential lipid (oils) constituents of the leaves of *A. Africana* [12]. It was also reported that the leaves of *A. africana* contains abundance of micro and macro elements [13]. *Aspilia africana* leaf extract have been reported to decrease serum testosterone levels significantly, with histopathological studies of testes revealing varying degree of degeneration such as disorganized epithelial cells, fibrosis and cytoplasmic abnormalities [14].

The testes functions in spermatozoa production and testosterone synthesis. The above functions are controlled by the pituitary gland, which is in turn influenced by the hypothalamus. The pulsatile release of gonadotropin releasing hormone (GnRH) of the hypothalamus triggers the secretion of the pituitary gonadotropins, thus representing an elegant feedback loop with modulations by testicular hormones [15]. Medicinal plants have gained global acceptance and are now being used in the management of diseases, infections reproductive and public health concerns.

2. Materials and methods

2.1 Reagents/Chemicals

All reagents used were of analytical grade and products of British Drug House (BDH) England, E. Merck, Darmstadt, Germany and Aldrich Chemical Company

Collection and Identification of Plants

The fresh flowers of *Aspilia africana* were collected between September and November 2019 from Iguomo village, near Okada, Benin City, Edo State. It was identified and authentication was done at the college of Natural and applied sciences, Igbinedion University, Okada.

3. Methods

3.1 Extraction of Plant material

The fresh flowers were destalked, pooled, thoroughly rinsed with distilled water and left to drain at room temperature, air-dried, pulverized and stored for subsequent use. The powdered plant materials were macerated with distilled water for 24 hours. The mixture was then filtered using Whatman's (No. 1) filter paper and then lyophilized to give the crude aqueous extracts.

3.2 Animal study

A total of twenty adult male Wistar rats that weighed 250-300g were obtained from the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Edo State, Nigeria, and housed in the same facility for the study. The animals enjoyed natural light and dark cycles; food and water were available ad libitum. For the animal studies, the animals were divided

into groups A, B, C and D of five rats each. Group A served as control and was given 1ml of distilled water, while groups B, C, D were given 150mg/kg, 200mg/kg and 300mg/kg body weight respectively.

3.3 Histological Studies

The testes and epididymis were excised from the test and control rats and were immediately preserved in Bouin's fluid after being weighed and examined for gross and pathological changes. Principle: Tissues were fixed in Bouin's fluid and dehydrated using graded concentrations of absolute alcohol. The tissues were cleared by passing through xylene baths, followed by impregnation in paraffin wax. Sections were cut using a microtome and stained with Erlich's Haematoxylin (H) and Eosin (E). The Haematoxylin usually stains the cell nuclei bluish-black and eosin stain cell cytoplasm and most connective tissue fibres pink. The slides were examined microscopically, using a light microscope with digital camera and micrograph of each figure was taken.

4. Results

4.1 Histological changes in the testis

Histological studies on the testes of Wistar rats in the control group showed normal structural appearance with closely packed seminiferous tubules showing all cells of the spermatogenic lineage undergoing spermatogenic cycle of maturation composed of spermatogonia, spermatocytes, spermatids and spermatozoa. The interstitial spaces were well defined with evidence of Leydig cells within the interstitium. The results of the groups administered different doses of aqueous flower extract of *A. africana* showed a dose dependent increase or widening of the interstitial space, mild disruption of the spermatogenic series in the groups administered 200 and 300mg of extract per kilogram body weight of the rats and prominent spaces were detected within the germinal epithelia of the same dose groups. The group treated with 300mg of the extract showed more marked disruption of germinal epithelium with shrunken seminiferous tubules. However, the group treated with 150mg/kg body weight of rat maintained the normal structural integrity in terms of seminiferous tubules and spermatogenic cells but the interstitial spaces were evidently wider relative to the control.

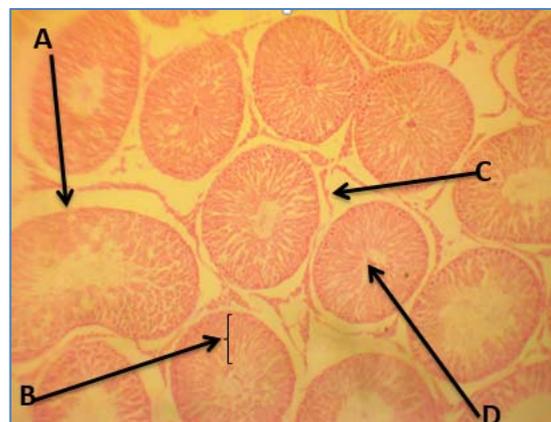


Plate 1: Rat control testis composed of seminiferous tubules-A, with seminiferous epithelia lined by cells of the spermatogenic series in their various developmental stages- B, interstitial space-C, and tubular lumen filled with spermatozoa-D (H & E \times 100)

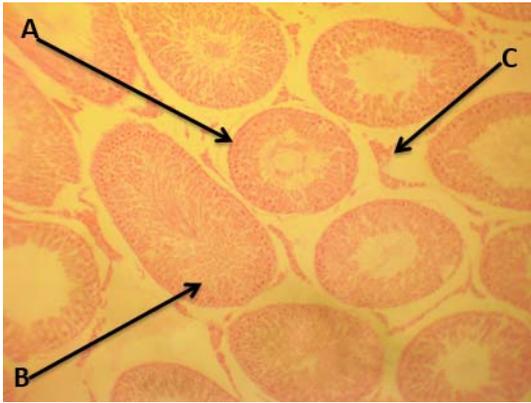


Plate 2: Rat testis given 150mg/kg showing tubules-A, lined by spermatogenic series-B, with normal sequential maturation, prominent leydig cells-C, interstitial oedema and mild reduction in luminal spermatozoa (H & E ×100).

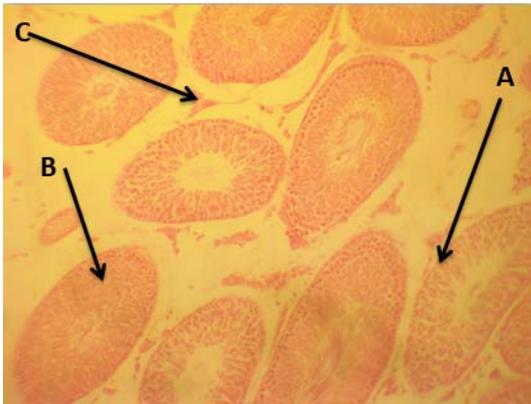


Plate 3: Rat testis given 200mg/kg showing fairly normal seminiferous tubules-A, spermatogenic series-B, prominent leydig cells-C, moderate interstitial oedema and mild disruption of the germinal epithelia (H & E × 100).

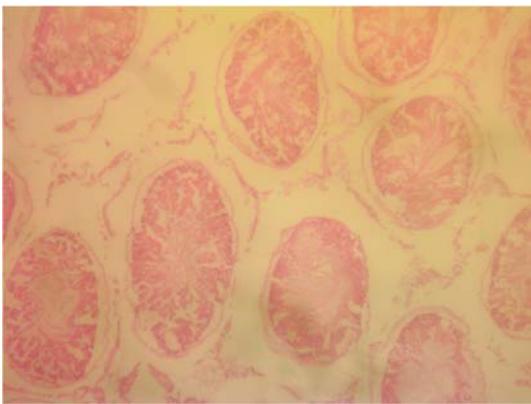


Plate 4: Rat testis given 300mg/kg of *A. africana* showing degeneration of the spermatogenic series-A, shrunken seminiferous tubules-B, mild degeneration of leydig cells and moderate interstitial oedema (H & E × 100).

4.2 Histological changes in the epididymis

The results of the histological studies on the caudal epididymis of the control group revealed regular epididymal ducts with pseudostratified columnar epithelia and its stereocilia. The epithelia are surrounded by basement membrane containing basal cells. The lumens of the tubules were packed with mature spermatozoa [16]. The histological

examination of the cross sections obtained from the groups treated with 150, 200 and 300mg of the extract per kilogram body weight of the rats showed fairly normal tubules packed with mature spermatozoa, mildly decreased population of sperm cells and moderately decreased population of spermatozoa respectively with an intact epithelial lining across the groups.

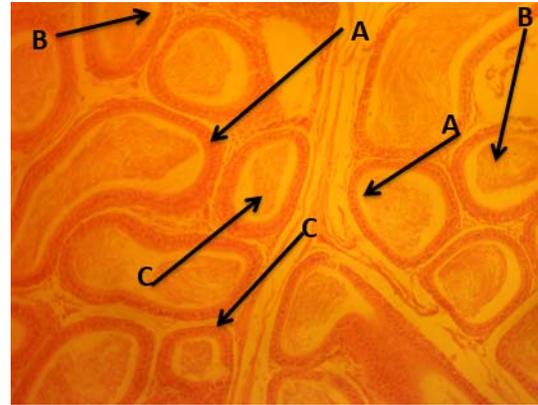


Plate 5: Rat control epididymis showing epididymal tubules-A, lined by low pseudostratified columnar epithelium, lumen packed with mature spermatozoa-B and interstitial space-C (H & E × 100).

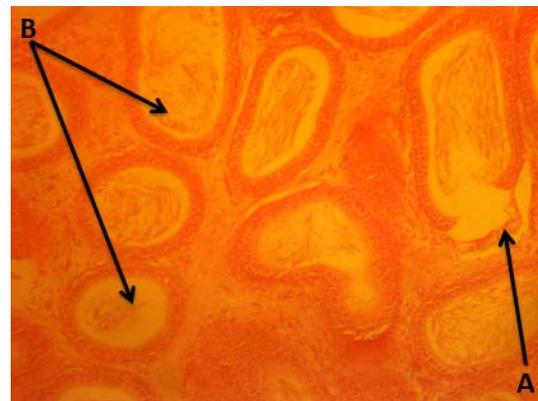


Plate 6: Rat epididymis given 150mg of *A. africana* showing fairly normal architecture with a focal area of epididymal tubule disruption-A and mild reduction in the population of mature spermatozoa in the lumen-B (H & E × 100).

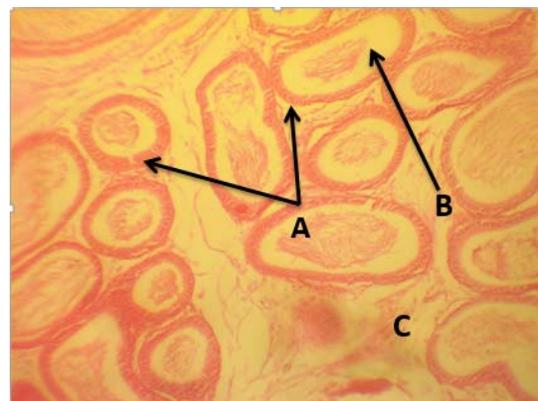


Plate 7: Rat epididymis given 200mg/kg of *A. africana* showing fairly normal epididymal tubules lined by low pseudostratified columnar epithelium-A, lumen showing decreased population of spermatozoa-B, and sparse interstitial tissue-C (H & E × 100)

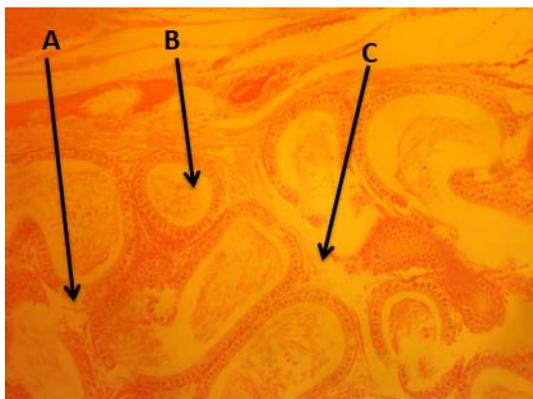


Plate 8b: Rat epididymis given 300mg/kg of *A. africana* showing fairly disrupted epididymal architecture with distorted tubules-A, decreased luminal spermatogenic population-B and sparse interstitial tissue-C (H & E \times 100).

5. Discussion

Testis is the male gonad that is responsible for the production of spermatozoa in a cascade of processes collectively referred to as Spermatogenesis. The process of spermatogenesis is under the regulatory influence of Luteinizing Hormone, Follicle Stimulating Hormone and Testosterone [17]. Any alteration in the levels of circulating testosterone, LH and FSH and distortion of Leydig cells is likely to affect the process of spermatogenesis negatively. The result obtained from the histological study of the testes compares favorably with a previous report that shows a slight degeneration of the germinal epithelium of the seminiferous tubules, abnormal widening of the interstitial space and loss of Leydig cells [18]. In contrast, another study reported no histological alterations following exposure of adult male Wistar rats to ethanolic leaf extract of *chrysophyllum albidum* [19]. Evidently, the interstitial spaces across the treatment groups were wider relative to the controls, which may be the reason behind the shrinkage of the seminiferous tubules seen in the highest dose group. The presence of prominent spaces in the germinal epithelia and disruption of the spermatogenic series recorded in some of the treatment groups is also indicative of structural disorganization, signifying testicular cytotoxicity or necrosis [20]. The mechanism of action of the extract may be the permeation of the blood testes barrier [21] and/or the above effects may have resulted from a direct or an indirect effect on the germinal epithelium or hormones that aid spermatogenesis respectively. Since fertility is dependent on structurally and functionally normal germinal epithelia with normal spermatogenic cells, it is highly probable that there may have been a dose dependent gradual spermatogenic arrest, which may have impaired the fertility status of the treated groups.

The epididymis is the highly convoluted tubular link between the rete testis and the ductus deferens. Its main function is to convey sperm cells from the germinal epithelium of the seminiferous tubules, maturation and storage of spermatozoa. It is made up of three parts, the caput, corpus and cauda epididymis with the cauda serving as storage for mature spermatozoa. To acquire fertilizing capacity, spermatozoa have to be exposed to the epididymal environment and this environment is under the control of its epithelium and testicular androgens [22]. The result of the histology of the epididymis favorably competes with a

previous study that reported that oral administration of ethanol extract of *Carica papaya* did not affect the caudal epididymal histological profile [23]. Reports have shown that sperm maturation is usually controlled by the protein secreted by the epididymal principal cell [24, 25]. In this study, the caudal epididymal epithelium appeared relatively normal in the experimental groups when compared with the controls, which goes to show that the aqueous extract of *A. africana* flowers did not cause any harmful or deleterious changes in the epididymal epithelium and by extension, did not affect protein and sialic acid synthesis [26, 27]. However, the dose dependent depletion in the population of spermatozoa in caudal epididymal lumen in the extract-treated groups especially in the higher dose groups may have been as a result of spermatogenic arrest caused by probable reductions in the relevant hormonal levels, which may have been caused by the presence of high quantities of tannins in the extract in line with previous studies [28, 29, 30, 31].

6. Conclusion

From the results of this present study, administration of varied doses of aqueous flower extract of *A. africana* revealed slight changes on the normal histoarchitecture of both the testis and epididymis in a dose dependent manner. These deleterious effects including the possible spermatogenic arrest recorded in this studies are enough to reduce fertility potentials. We therefore recommend further studies to clarify the mechanism by which the recorded changes were achieved and the active principle responsible for the results recorded in a bid to develop it into a spermicidal agent that may be useful in fertility control to check population explosion.

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