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THE EFFECT OF ETHANOLIC EXTRACT OF BRYOPHYLLUM PINNATUM ON THE MICRO ANATOMY OF THE TESTES OF ADULT MALES WISTER RATS

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ABSTRACT: *The main aim of this research study is to determine the effect of ethanolic fractions of bryophyllum pinnatum leaves on the male testes using adult male wistar rats. Thirty (30) adult male wistar rats were divided into three groups of ten rats each. Group (A) serve as the control group and was administered with distilled water, Group (B) was treated with 100mg/kg/body weight of the bryophyllum pinnatum extract. Group (C) received 200mg/kgbw of the extract. The treatment lasted for a duration of 8weeks. The rats were sacrificed the day after the last dose and the testes were removed and processed for histological studies. The results from the treated groups showed increase intercellular spaces within the seminiferous epithelium, shrunken and increase lumen, suggesting cells disintegration. This is indicative that bryophyllum pinnatum extract may have adverse effect on the testes of the treated rats*.

KEYWORDS: B*ryophyllum Pinnatum*, Micro anatomy, Ethanolic, Testes, Wistar rats

INTRODUCTION

Bryophyllum pinnatum popularly known as "Resurrection plant" is a perennial herb used in folkloric medicine in tropical Africa. It is classified as weed and the plant flourishes throughout the southern part of Nigeria (Gill, 1992). The divine herb contains a wide range of active compounds, including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids, have been isolated from this species. The plant is widely used in traditional medicine for the treatment of variety of ailments and well known for its haemostatic and wound healing properties. The pharmacological studies are reviewed and discussed, focussing on that different extracts from this plant have been found to possess pharmacological activities as immunomodulator, CNS depressant, analgesic, antimicrobial, antiinflammatory, antiallergic, antianaphylactic, antileishmanial, antitumorous, antiulcerous, antibacterial, antifungal, antihistamine, antiviral, febrifuge, gastroprotective, immunosuppressive, insecticidal, muscle relaxant, sedative.

It is astringent, sour in taste, sweet in the post digestive effect and has hot potency. It is well known for its haemostatic and wound healing properties. The plant have considerable attention for their medicinal properties and find application in folk medicine, as well as in the contemporary medicine. The present review highlights the various ethnopharmacological uses, phytochemical and pharmacological studies conducted on *B. Pinnatum* and also pinpoints unexplored potential of it. (Variers, 2002; Naude, 1977; Steyn *et al*, 1998) Various species of *B.Pinnatum* are used medicinally in IndoChina, China and Philippines Islands. It is naturalized throughout the hot and moist parts of India. The leaves and bark are bitter tonic, astringent to the bowels, analgesic, carminative, useful in diarrhoea and vomiting (Kirtikar, 1975). It is applied externally and taken internally for all types of pains and inflammations, various bacterial, viral and fungal infections, leishmaniasis, earaches, upper respiratory infections, stomach ulcers, flu and fever (Silva *et al.*, 1995)

In traditional medicine, the leaves of this plant have been used for antimicrobial (Oliver-Bever, 1983; Mehta *et al.* 1952; Akinpelu 2000). , antifungal (Misra and Bhat 1979) , antiulcer (Pal *et al,* 1991), antiinflammatory, analgesic (Pal *et al.* 1989 and 1992), antihypertensive (Ojewole, 2002), potent anti-histamine and anti-allergic activity (Pal *et al.,* 1999). The Creoles use the lightly roasted leaves for cancer, inflammations, and a leaf infusion for fevers. The Palikur mix the leaf juice with coconut oil or andiroba oil and then rub it on the forehead for migraines and headaches. To the Siona indigenous peoples heat the leaves and apply them topically to boils and skin ulcers. Along the Rio Pastaza in Ecuador, natives use a leaf infusion for broken bones and internal bruises. In Peru, indigenous tribes mix the leaf with aguardiente (sugar cane rum) and apply the mixture to the temples for headaches; they soak the leaves and stems overnight in cold water and then drink it for heartburn, urethritis, fevers and for all sorts of respiratory conditions. The root infusion is also used in epilepsy. Other tribes in the Amazon squeeze the juice from fresh leaves and mix it with mother's milk for earaches.

In Mexico and Nicaragua it is also used to promote menstruation and assist in childbirth. In Nigeria and other West African countries, its fleshy leaves are frequently used as herbal remedy for an array of human disorders, including: hypertension, diabetes mellitus, bruises, wounds, boils, abscesses, insect bites, arthritis, rheumatism, joint pains, headaches and body pains. The leaves of the plant have great medicinal value and are used both, internally as well externally. The leaves possess various properties like haemostatic, refrigerant, emollient, mucilaginous, vulnerary, depurative, anti-inflammatory, disinfectant and tonic. They are useful in vitiated conditions of vata and pitta, cuts, wounds, hemorrhoids, menorrhagia, discoloration of the skin, boils, sloughing ulcers, ophthalmic, burns, scalds, corn, diarrhea, dysentery (Dalzeil, 1937), headaches (Sofowora, 1993), vomiting, acute inflammations and bronchitis. It is also employed for kidney stones, gastric ulcers, skin disorders and edema of the legs. Externally, the pulp of the leaves or the juice is applied on traumatic injuries to arrest the bleeding as it contract the minute arterioles and promote the healing of wounds. It is also used for headaches, toothaches, earaches, eye infections, wounds, ulcers, boils, burns and insect bites. On traumatic wounds, the heated leaves are crushed and applied. It reduces the edema and promotes the wound healing without leaving a scar. Internally, the leaves juice and cumin seeds are given along with the double amount of ghee in dysentery. The herb is highly recommended in bleeding disorders, piles and menorrhagia. With many of *Bryophyllum pinnatum's* traditional uses verified by plant and animal research, it is not unusual that it continues to be a popular remedy throughout the tropics where it grows. The plant is a good source of vitamins, Okwu & Okwu (2004) and contains bioactive substances such as alkaloids, flavonoids, saponins and tannis (Nwali *et al.,* 2012). The leaf is among the medicinal plants listed for use by the Yao people of Yunnan province in China, to treat rheumatoid arthritis, tummy bug, numbness of limb, bruises, burn, ulcer etc (Okwu & Okwu, 2004).

The anti-inflammatory property has been reported in rats in a formaldehyde induced oedema (Cypta *et al.,* 2010). Biochemically an increase in ALP and serum creatine were observed with a decrease in serum urea in rabbits (Ghasi, *et al.,* 2011). The plant shas anti-leshammanial activity (Da Silva *et al.,* 1999). It has been reported to have hepatoprotective and nephroprotective effective (Yadar & Dixit, 2003), (Hassiaka *et al.,* 2006) (Saladin & Yenitan, 2006), (Yemitan & Saladin, 2005). It has anobesic effect (Iswe & Aknyili, 2005). Antibacterial activity reported by Akinpelu, (2000) (Okwu & Nnamdi, 2011), (Okwu, 2001), (Fokansi *et al.,* 2005). (Schmitt *et al.,* 2003). Antidiabetic effect. This plant is commonly called a master herb or cure for all antimalarial effect (Chienepanskeda, 2010) by a large community of herbal

practitioners (Nyak, *et al.*, 2010). There is dearth of literature on the effect of *Bryophyllum pinnatum* as it affects the male gonad (testes), therefore this study is designed to investigate the effect of ethanolic extract of *Bryophyllum pinnatum* on the testes of adult male Wistar rats.

MATERIALS AND METHODS

Materials

Matured leaves of *Bryophyllum pinnatum* were obtained from Asabanka Farm in Akpabuyo Local Government Area of Cross River State, Nigeria. The plant was authenticated in the herbarium of Botany Department, University of Calabar, Calabar. Plant material was copiously washed with tap water to remove foreign matters, they were chopped, air dried and pulverized under shade after which it was powdered with mechanical grinder to obtain a coarse powder which was then subjected to extraction. Thirty (30) male albino Wistar rats weight 100-150g were obtained from animal house of Pharmacology Department, University of Calabar, Calabar. The animals were grouped into three (3) groups of 10 animals each and housed in cages and maintained under standard laboratory conditions. They were fed with standard animal pellet obtained from vital feed (Nig) Limited, Calabar and have free access to water *ad libitum* throughout the period of study.

Experimental protocol

The animals in the group A which served as control received distill water while the experimental Groups B and C received 16mg/kgbw and 32mg/kgbw of the dosage of the extract respectively. The plant extract was administered orally using orogastric tube after it was reconstituted in distilled water. The total duration of treatment with *Bryophyllum pinnatum* was eight weeks which is a complete duration for a single spermatogenic cycle. Animal individual body weight was checked twice a week before and after administration of extract.

RESULT

Histological observation

Group A (Control): The result showed spehnoidal cells in different shapes and sizes. These are the seminiferous tubules (S) delinated by connective tissue from each other. Between adjoining seminiferous tubular cells are the interstitium (I) which contains the interstitial cell (Ic). Each seminiferous tubule has a lumen (L) and several layers of ceels from the lumen to the basal membrane. These represent stages in the transformation of spermatozoa from germ cell to matured ones. Plate 1A and 2B\

Group B: This group received 100mg/kgbw. Histological section of testis from animals in this group showed intracellular spaces within the seminiferous epithelium. Plate 1B and 2B.

Group C: This group received 200mg/kgbw. Histological section of testes from animals in this group showed increased amount of intracellular spaces in the seminiferous epithelium with lumen increased. Plate 1C and 2C.

DISCUSSION

The effect of the extract of *Bryophyllum pinnatum* on the micro anatomy of the testis of adult Wistar rats was investigated. It was observed that the control group A showed intact normal histological features of the testes such as seminiferous epithelium (S), seminiferous lumen (L)

and the Interstitum (I). In the group B treated animals, it was observed that the tubules were shrunken and intracellular spaces were seen within the epithelium of the low dose group. The high dose group (Group C) showed marked intracellular spaces within the germinal epithelium and reduction of spermatozoa. The seminiferous tubules were moderately enlarged. The increased intracellular spaces suggest degeneration of the seminiferous epithelium and consequent reduction of spermatozoa in the lumen of the seminiferous tubule (Ueno and Mori, 1990; Abreu *et al.,* 1982).

Literature has shown that some of the phytochemical constituents such as bufadienoides, steroids, cardienolides, triterpenoids; saponin, tannin and phenol of the crude ethanolic leaf extrac of *Bryophyllum pinnatum* may possess cytotoxic properties (Kamboj and Saluja 2009; Ofokansi *et al.,* 2005). The observed effect from study of effect of *Bryophyllum pinnatum* on the testis may be as a result of the phytochemicals constituents of the plants which may have been cytotoxic to the cells of the testis. In this study however, the extract appeared to have no effect on the interstitial cells in the interstitium.

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Figures

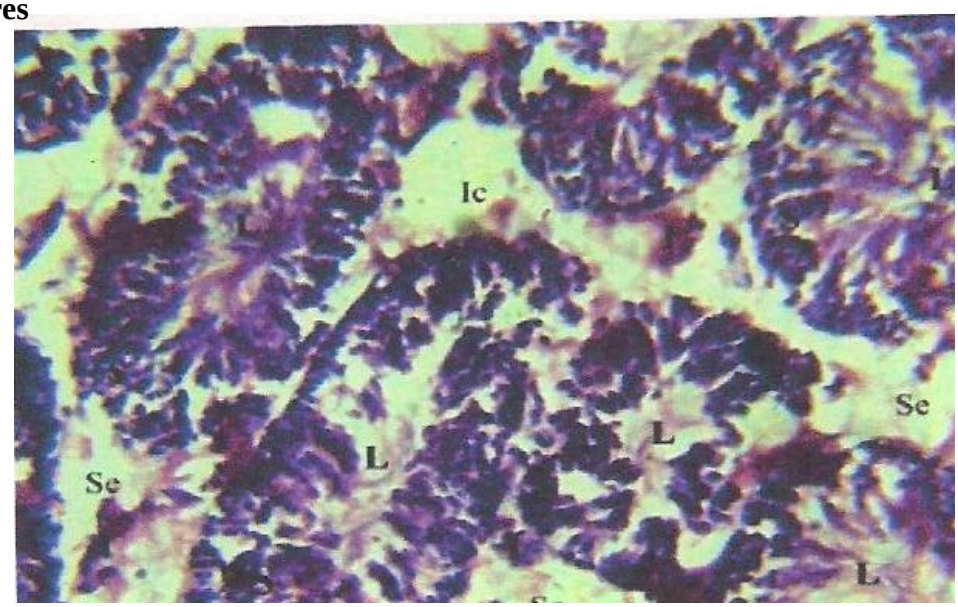


Plate 1A: Photomicrograph of the testis from a control group showing section of seminiferous tubule (S) with Intersitium (I), Lumen (L) and matured spermatozoa, seminiferous eptithelium (S.E) indicating successive stages in the development of spermatozoa (H&E X 100)

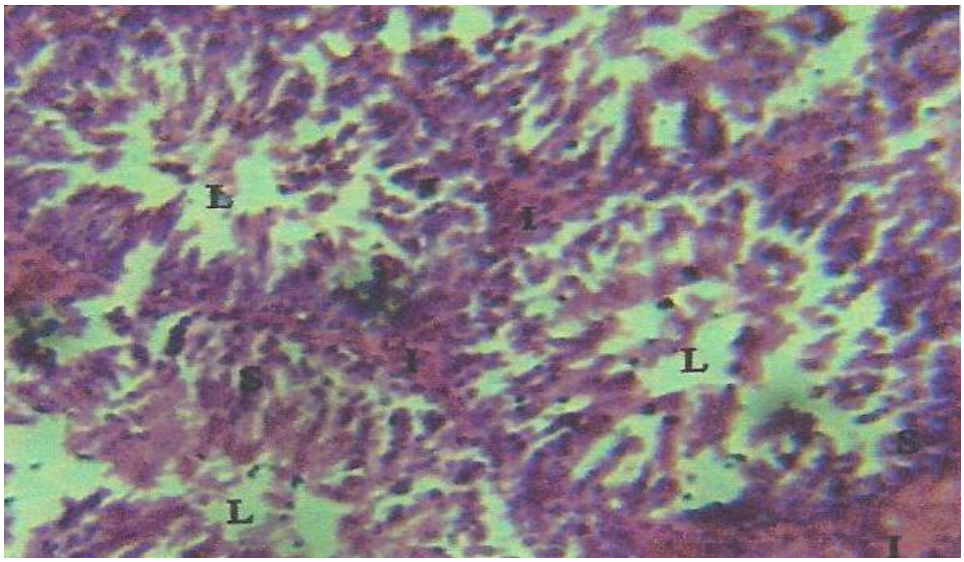
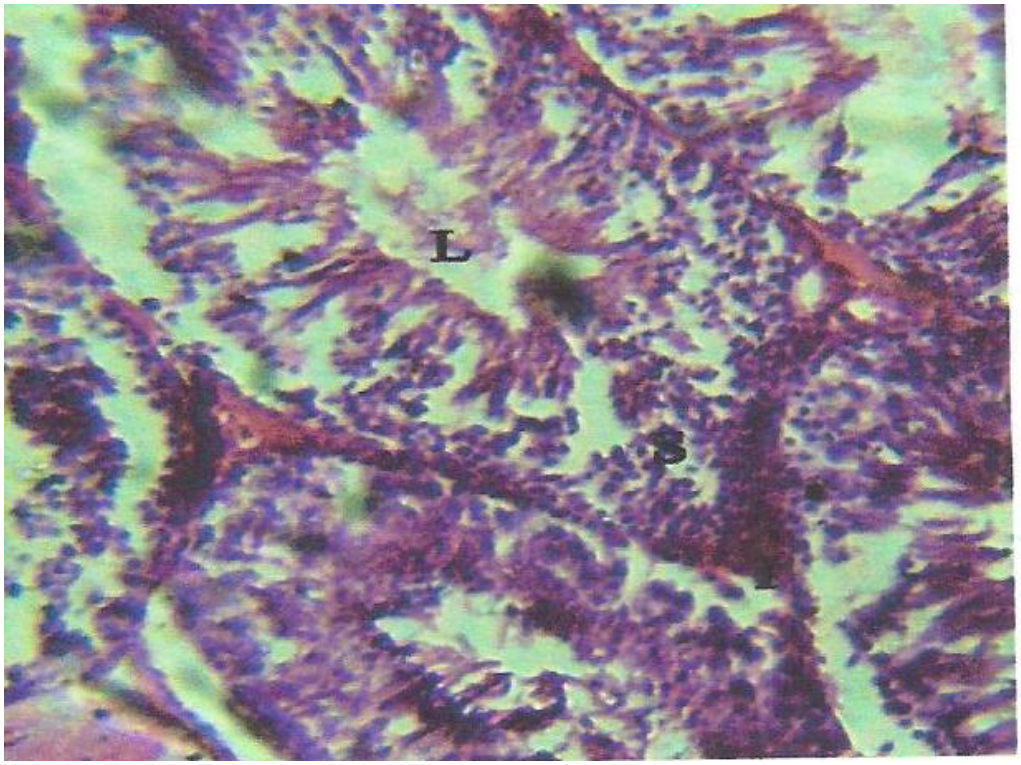


Plate 1B: Photomicrograph of section of the testis from 100mg/kg group showing intercellular space within the seminiferous epithelium with shrunken lumen (H&EX100)



Place 1c: Photomicrograph of section of the testis from high 200mg/kg group showing an increase amount of intercellular spaces in the seminiferous epithulum with lumen increases (H&E X 100)

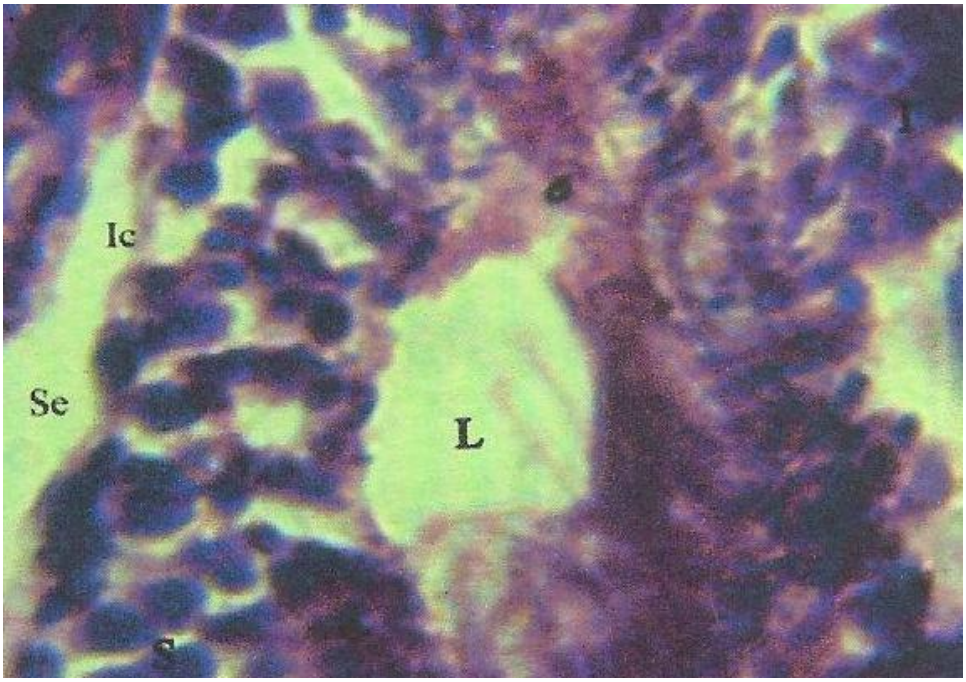


Plate 2A: Photomicrograph of the testis from a control group showing section of seminiferous tubule (S) with interstitiiu, Lumen (L) and matured spermatozoa, seminiferous epithelium (S.E) indicating successive stages in the development of spermatozoa (I) (H&E X 400)

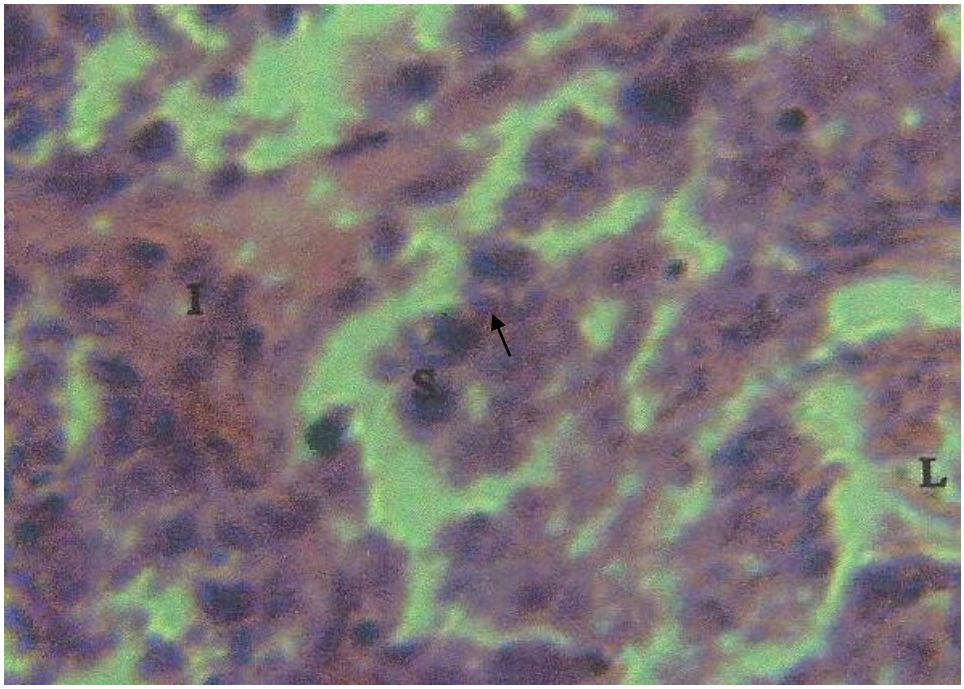


Plate 2B: Photomicrograph of the testis from a 100mg/kg group showing intercellular spaces within the seminiferous epithelium with shrunken lumen (H&E X 400)

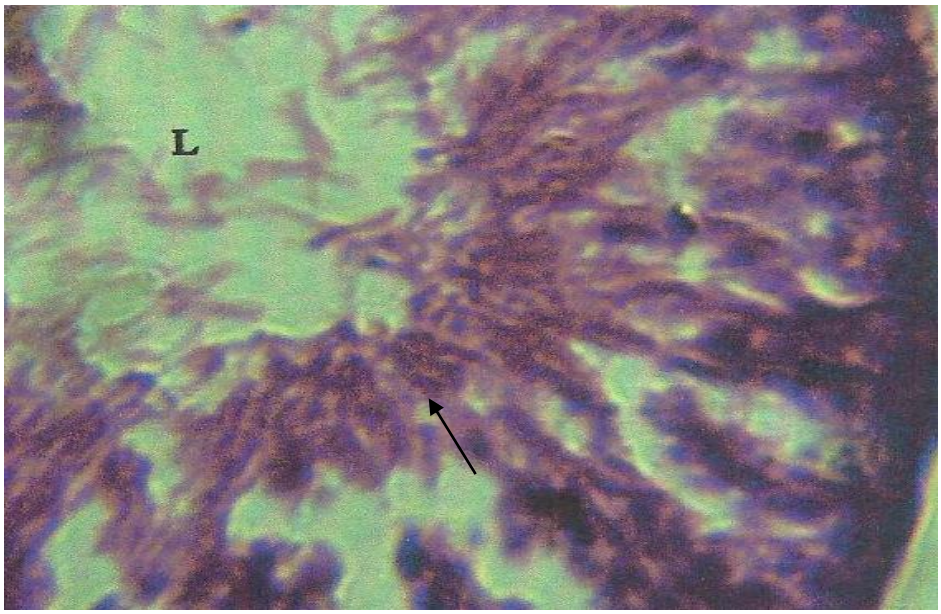


Plate 2C: Photomicrograph of the testis from 200mg/kg group showing an increase amount of intercellular space in the seminiferous epithelium with lumen increased (H&E X 400)