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Histopathological Effects of Prophylactic Consumption of *Sphenocentrum Jollyanum* (SJ) Ethanolic Root Extract on Select Organs of Male Albino (*Rattus novergicus*) Rats.

Blessing Emosho Atoigwe[1,2], Peter Uwadiegwu Achukwu[2], Bolaji Efosa Odigie[1,2]

[1]Department of Medical Laboratory Science, School of Basic Medical Science, College of Medical Science, University of Benin, Nigeria.
[2]Medical Laboratory Science, Faculty of Health Science and Technology, College of Medicine, University of Nigeria, Enugu, Nigeria.

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ABSTRACT

Background: *Sphenocentrum jollyanum* (SJ) belongs to the family, Menispermaceae, which possess varying healing properties including boosting appetite and sexual drives. In lieu of the indiscriminate abuses of the root of SJ for sexual performances; this study examines the histopathological effects from prophylactic consumption of ethanolic root extract of SJ on the testis, liver and kidney of male albino rats. **Methods:** An experimental study was conducted using thirty in-bred male albino rats of about 3-6 months (176-201g) body weight. They were selected randomly into five groups of 6 rats per cage. The rats were housed in wire gauze cages with saw dust used as beddings and adequate supply of food and water. The root of SJ was identified and authenticated. The preparation was crude, while, the ethanol extraction was scientific using Soxhlet apparatus. The administered dosage (400 to 1000mg/kg b.w.) was extrapolated to exclude the lethal dose from an existing work. The rats were sacrificed at the end of the experiment that lasted 72 days. Grossing was done after excision of the select organs and was rinsed in normal saline before fixing in 10% neutral buffered formalin. The organs were processed histologically, later examined microscopically and reviewed by 2 or more consultant pathologist. **Results:** A significant weight gain was recorded in the treated rats compared to the control. Histopathological effects observed were; periportal lymphocytosis and kupffer cell activation in the liver, testicular degeneration in the testis, and congested glomeruli and tubules in the kidney. **Conclusion:** Prophylactic consumption of SJ root may be harmful to the testis, liver and kidney at the cellular levels. Nonetheless, sufferers of erectile dysfunction may consult the physicians for proper guidance (after standardization of dose regimen) as it has been proven to alleviate the sufferings from poor erection in men.

Keywords: *Sphenocentrum jollyanum,* Prophylaxis, Burantashi, erectile dysfunction.

INTRODUCTION

Sphenocentrum jollyanum (SJ) is a plant that belongs to the family, Menispermaceae. It is a deciduous shrub up to 1.5m tall with grey bark and spirally arranged leaves, which are smooth on both sides.[1]It has been shown to possess antihypertensive, antioxidant, antinociceptive, antiviral and anti- angiogenic effects in animals.[1, 2]

Name & Address of Corresponding Author
Blessing Emosho Atoigwe
Department of Medical Laboratory Science,
University of Benin, Nigeria.

The plant is documented for its use against chronic coughs, worms and other inflammatory conditions as well as tumors.[3] It is also believed to be an emetic and purgative, while; the sap is believed to relieve stomach ache and constipation, and to boost appetite and sexual drive.[3]In Nigeria, a decoction of the root is applied to treat topical ulcer and the edible fruit is taken against fatigue.[4] However,[5] suggested that the plant may be a potential source of a new anti-diabetic agent. Detailed information on the description[2], distribution[4], cultivation[5], and the medicinal use of the plant has been documented.[1, 2, 4-6].

Conversely, the use of natural products with therapeutic properties is as ancient as human civilization; using it as the major source of treatment for different ailments.[7] In regions with rich diversity of flora spread, it forms an important component of their natural wealth.[6] Herbs and herbal formulations for the treatment of ailments have continued to receive increased attention due to a very strong belief that the products are safe.[8] The assumption to a large extent may have influenced the indiscriminate use of these

formulations by many, particularly amongst the rural dwellers.[9] The incidence of adverse effects and sometimes life-threatening conditions allegedly emanating from these herbal medicines has been reported among various ethnic groups.[9, 10] Hence, it has become impetus to ascertain the histopathology of some vital organs of human, which are concurrently and indiscriminately exposed to these medicinal herbs.[6]In response to the ongoing, it is important to examine the side effects that may be associated with the indiscriminate consumption of SJ as treatment option, which is administered as a self-prescribing herbal therapy without a safe-dose regimen, and may also be without a standard preparatory procedure.[11, 12] Consequently, with respect to the multifunctional morphological makeup of the liver and kidney as well as the testes (for sexual performance and enhancement); it becomes imperative to censoriously examine these specialized organs that is constantly being exposed to an indiscriminate intake / or prophylactic abuse of herbal preparations too frequently.[11]

In view of the numerous works thought to have been carried out on the biochemical, haematological, phytochemical, histological and acute toxicity studies[1, 2, 5 & 6]; there remains a dearth of literature on the histopathological effects resulting from the prophylactic consumption of the ethanolic root extract of SJ on some organs of interest (liver, kidney and teste) in this study. Indeed there were apparent needs for the histopathology assessment of the liver and kidney of white rats being the power house for biochemical activities that may not be readily visible by evaluating for blood chemistry; while the deleterious effects of SJ could be very obvious at the cellular level, which is a vital point of focus for this study. Therefore, this study was designed to examine the histopathological effects from the prophylactic consumption of the crude ethanolic root extract of SJ at the cellular level on organs of albino rats. This study also believes that the deleterious effects observed may be applicable to human in a similar circumstance.

MATERIALS AND METHODS

Plant Collection, Authentication and Preparation

The root of *Sphenocentrum jollyanum* was obtained in the month of November 2014, from the Hausa Quaters at Ring Road, Benin City, Nigeria. The root specimen was identified and authenticated by Dr. H. A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria; while a voucher specimen was deposited in the Departmental herbarium (PBB/BEN13713). The roots of SJ were thoroughly washed with tap water and allowed to dry under normal atmospheric condition for a period of eight weeks. The dried roots were pounded using a wooden mortar and pestle and was further milled into fine powder using an electric blender (Kenwood 1.6L, BL480 Prestons, Australia). A total of 1, 450g of the powdered root was extracted with 70% (V/V) ethanol in three cycles using a Soxhlet apparatus. The mixture was filtered with a white muslin cloth and Whatman No 1 filter paper in two consecutive sieves. Thereafter the filtrate was evaporated with a Vacuum Rotary Evaporator under reduced pressure at 60^{o}C. The yellowish brown filtrate was then air-dried at room temperature (28^{0}C) yielding a total of 192g residue with a percentage yield of 12.2 % w/w and was stored in an air tight container, which was refrigerated at 4^{o}C.

Experimental Animals, Care and Management

The use of the animals and the experimental protocol was approved by the Experimental Ethics Committee on Animals Use at the Animal Care Unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Upon the collection of the approval, thirty in-bred, acclimatized, healthy male albino rats (*Rattus norvergicus*) of about 3-6 months old, weighing (176-201g) were used for this study at the Animal House of the College of Medicine (old site), University of Nigeria, Enugu-Nigeria. They were randomly selected into five groups of six rats and were labelled as (A_1, A_2, A_3, A_4, and A_5).in a finely prepared wire gauze cages using saw dust as beddings in an ambient humidity and temperature (21±2.8 0C) with 12 h dark and 12 h light circle. They were also provided with standard rodent chow from Livestock Feeds PLC, Lagos and water*ad libitum*.

Weight Determination and Conduct of Experiment

Empirical and physical measurements were carried out on the rats before commencement and after termination of the study. The body weights of the experimental animals were determined using the method adopted by[13]; which resulted to an average weight of 187.54g. However,[14] method was used for physical measurement according to behavioural signs of acute toxicity by close monitoring and observations. Based on the indiscriminate consumption of SJ in Nigeria to boost sex performance and vitality, and also, considering the acute toxicity studies of the leaves of SJ conducted in an earlier study by[6], and was found to be 1,445 mg/kg. Doses up to 1000 mg/kg body weight in rats were assumed to be safe and were then extrapolated to exclude the lethal dose that was used for the present study.

Treatment of Animals

A sterile five (5ml) syringe (without the needle) was used to administer the appropriate

concentration of the extract in the order of 400, 600, 800 and 1000mg/kg to the rats in cages A_1, to A_4 whereas cage A_5 served as the control group without experimental dose. The extract was administered for 72 days at the intervals of 2 days (Sub-chronic administration).

Animal Sacrifices and Grossing

At termination, all animals were sacrificed by cervical dislocation; organs of interest (liver, kidney and Testes) were excised and grossed properly by drying with cotton wool and weighed on a sensitive balance. Each weighed organ was standardized for 100g body weight of each rat and comparing with the control counterparts, which was according to the method by[6]. They were carefully assessed while dissecting each organ from sacrificed animal and also observing for necrotized regions, lesions, redness, change in colour, odour, and consistency.

Histological Processing

After grossing, cut tissues from areas of interest was done at 3-5mm and was preserved in 10% neutral buffered formalin for tissue processing. Tissues were processed in an automatic tissue processor (Hestion -ATP7000 tissue processor-Germany) for dehydration, dealcoholisation, and impregnation or infiltration by molten paraffin wax. Also, embedding was done with the aid of the embedding machine(GMI SKU#: 8315-30-0004 Sakura Tissue-Tek 5, MN. USA). Sections of the tissues were obtained at 3-5 microns using the digital rotary microtome (Hestion ERM 4000 Germany) to produce serial ribbons. Staining of the sections was according to haematoxylin and eosin staining technique as described by [13].

Duration of Study

Collection and extraction of plant material, animal acclimatization, test administration, animal sacrifice, grossing, fixation, tissue processing, sectioning, staining, microscopic examination of histological sections and photomicrography lasted for about 6 months (November 2014 to May 2015).

Statistical Analysis

Data were presented as means ± Standard Error of Means (S.E.M) and analysed using one way Anova while significance was set at $p<0.005$ using the Graph Pad Prism version 6.1.

RESULTS

The results showed a significant weight gain in the albino rats after 72 days of prophylactic consumption of SJ ethanolic crude extract, which was dose dependent when compared with the control animals [Groups Mean Weight Before and After Experimentation: A=189.5±3.80kg (204.5±2.64kg), B= 189.8±3.84kg (211.5±1.32kg), C=191.8±4.99kg (226.5±2.23kg), D=196.5±4.56kg (233.5±2.12kg) and E=202.5±2.42kg (218.5±3.48kg)] without significant statistical difference $P< 0.005$; using one way Anova. The weight of the animals in group C and D (800mg/kg and 1000mg/kg respectively) increased more rapidly with increase in SJ extract consumption over the period of studies ($P<0.005$). Empirical studies showed an improved appetite in the earlier stages of the experiment compared to the last few days for animals in group (D=1000mg/kg). Histopathology of the testes showed mild interstitial congestion and tissue loosening (Oedema) [Plate 1]. There was no reduction in the weight of the testes of the rats after prophylactic administration of the extract. Sections of the liver revealed mild portal congestions and moderate peri-portal lymphocytosis as well as mild kupffer cell activation [Plate 2]. The kidneys however, showed constricted glomeruli and congested tubules [Plate 2 &3].

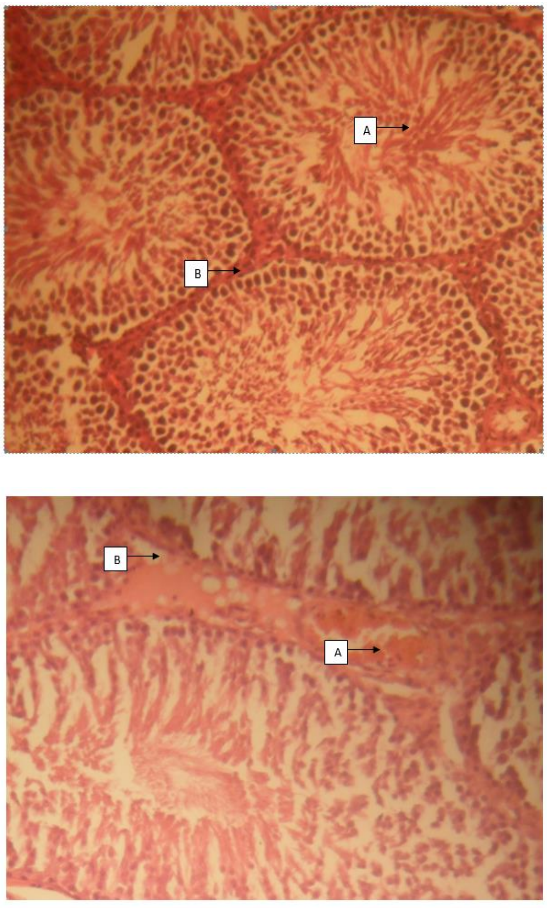
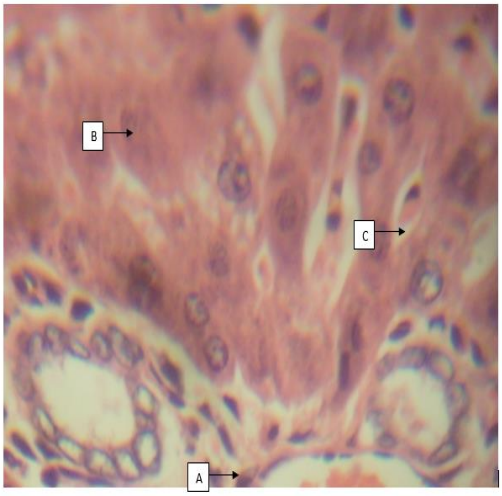


Plate 1: Control Section of rat left Testis (LT) composed of seminiferous tubules A, separated by interstitial space Band Section of the rat Right Testis (RT) treated with 1000mg/kg of the root extract of *Sphenocentrum jollyanum* for 72 days at 2 days interval; showing mild interstitial congestion A and tissue separation B (H&E staining x 10 objective).



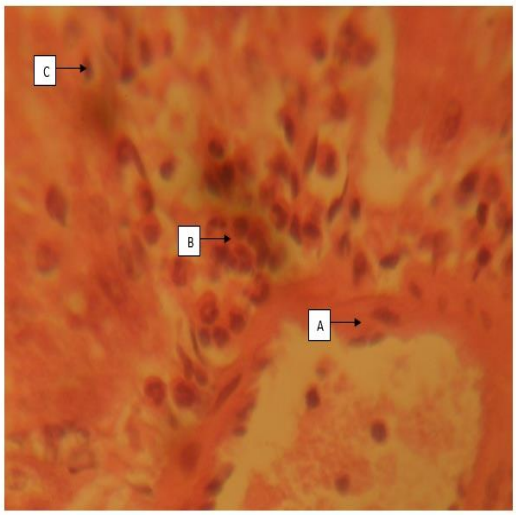
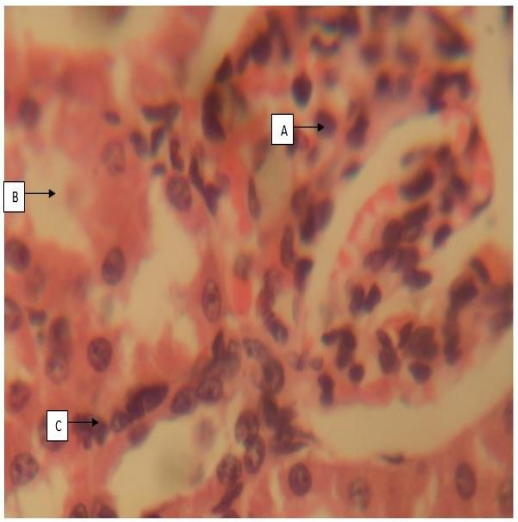


Plate 2: A section of the rat control liver (CL) composed of portal triad A, surrounded by hepatocytes B and sinusoids C; and a section of the rat treated liver (TL) with 1000mg/kg ethanolic root extract of SJ for 72 days showing mild portal congestion and dilatation A and moderate activation of periportal lymphocytes B with the kupffer cells C (H&E x 40).



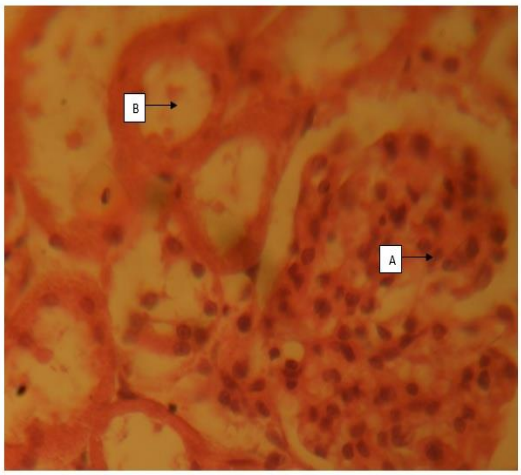


Plate 3: A section of the control rat kidney (CK) composed of cortical glomeruli A, tubules B and interstitial space C; and a section of the rat treated kidney (TK) with 1000mg/kg ethanolic root extract of SJ for 72 days showing congested glomeruli A and tubules B (H&E x40).

DISCUSSION

Indiscriminate therapeutic abuse of plants and herbal products in Nigeria is alarming.[13] The ethnomedicinal practices by some local herbal practitioners that involve the use of plants and plants extract for treatment of some ailments without scientific proof of efficacies are of topmost importance.[15] In this study, it was observed that there was weight gain in the albino rats after 72 days of the prophylactic administration of the ethanolic root extract of *Sphenocentrum jollyanum* (SJ). This observation, thus, agrees with[6], who carried out similar studies on the toxicity of the ethanolic seed extract of SJ and thus reported a significant weight gain ($P<0.05$), which was comparably higher than the control groups. The empirical studies also showed an improved appetite in the earlier stages of the experiment. However, when compared to the last few weeks for animals in groups (C and D that received 800 and 1000 mg/kg of the SJ ethanolic extract) respectively, there was a decline in feeding habits and the quest for food by the animals, which was thought to be dose dependent. This observation, thus, buttress the claims by[3]; reporting that the plant SJ is an excellent means of boosting appetite in human. Nonetheless, the reasons for the withdrawal from feeds at a much later stage of the experiment may be due in parts to dose, concentration and duration of administration of SJ. This is however, a statement of fact and has been reported in an earlier and similar study on P. yohimbe used as burantashi.[11]

In this study, the histopathological effects showed that there was moderate periportal lymphocytosis as well as kupffer cell activation as a result of

hepatocellular degeneration; indicating that SJ root extract may be harmful to the liver [Plate 2]. This claim has been reported in an earlier study and therefore, corroborates the findings.[16] In respect to the testis, which showed mild interstitial congestion and tissue loosening [Plate 1], thus, indicating that the ethanolic extract of SJ may be harmful also to the testes. This information aligns with previous literature on the above subject matter.[16] However, the present finding is not in agreement with[17], whose reports revealed that the tissue morphology of the seed oil extract treated mice; showed no changes or lesions and therefore, concluded that the use of SJ seed oil at the doses employed had no deleterious effects and to a large extent provided more information on the therapeutic safety for herbal therapy. The disparity in the results shown in this study is in compares to the former[17] and may be attributed to the difference in the parts of the plant used for the various study. Recall that the present study made use of the root of SJ while the former[17] experimented with the seed oil of SJ plant. More so, it has been recorded that the different part of a plant may retain different phytochemical constituents for various reasons.[3, 13, 18]

A previous study has shown that Burantashi is a true aphrodisiac used as a possible treatment for organic, psychogenic and substance induced erectile impotence and other male sexual dysfunctions.[19] It functions primarily by increasing the amount of blood flow to erectile tissues and may in-turn increase testosterone levels. For this reasons, men tend to use it as sex enhancer or for vigour and sexual performance.[11] Our results also support an earlier report by[11] regarding the gross abnormalities that were not observed in select organs (testes, liver and kidney) of albino rats under the influence of burantashi. It may therefore be said that SJ used as Burantashi may not necessarily affect these specialized organs grossly, and the reasons for this claim may not be readily substantiated at the moment. This report is in agreement with the findings by[6]; revealing that the gross examination of vital organs (heart, lung, liver, kidney, pancreas, and testis) after the administration of SJ seed extract showed no detectable inflammation. However, at the cellular level (looking at the histopathology of organs under the microscope); it was stressed in an earlier report that prolong administration of higher doses of *Pausinystalia yohimbe*, which was used as burantashi resulted in an increased toxic effects on the testis, liver and kidney of rats exposed to yahimbine extracts.[11] Similarly, higher doses of SJ used as burantashi in the present study were used in a prolonged duration (72 days sub-chronic administration) and with a high concentration. Our study hereby corroborates the findings of [11], where it was shown that there is a correlation between dose rate, concentration and duration of administration of burantashi going by the degree of adverse effects of the herbal plant on the testis, liver and kidney respectively [Plate 1, 2 & 3].

CONCLUSION

Prophylactic consumption of SJ plant (without underlining pathological condition) may be harmful to the testes as well as affecting the liver and kidney at the cellular levels; especially when consumed without an appropriate safe dose regimen as shown in our study. Therefore, we advise that African men in particular should desist from the habit of using SJ root as Burantashi for the purpose of deriving sexual enhancement and boosting performance. Nonetheless, sufferers of erectile dysfunction may consult the physicians for proper guidance and dosage as it has been proven to alleviate the sufferings from poor erection in men. Notwithstanding, the oral administration of the root extract is recommended for further studies; to corroborate the present report.

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