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Studies on the Effect of Aqueous Seed Extracts of Parkia Biglobosa on The Histology and Glycogen Profile of The Epididymis of Wistar Rats

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ABSTRACT

This study was aimed at determining the effect of aqueous seed extract of Parkia biglobosa on the histology of the epidydimis of male wistar rats was studied. Twenty-one adult wistar rats weighing about 90-120g were use for this research work and were divided into three groups of 7 animals each. The control group was giving normal rat feed and water, the low dose group was administered (300mg/kgBw) of Parkia biglobosa extract and the high dose group was administered (500mg/kg) of the test substance. All extract were given daily by oral gavage method for thirty-one days. Twenty-four hours after the last administration, the animals in all the groups were sacrificed using cervical dislocation. The epidydimis were harvested, preserved, and fix in 10% buffer formalin and processed for hematoxylin and eosin (H&E) staining and periodic acid-Schiff (PAS) staining methods for glycogen distribution. The result of the study shows a ($P<0.05$) significant increase in the final body weight of the treated animals when compared with their initial body weight. The histological observation showed normal cytoarchitecture of the epididymis in the Control group. There was observable pathological appearance in the epididymis of the low dose group. However, there were prominent distortions in the high dose animals that received 500mg/kgBw of the extract. Histochemical observation shows a moderate PAS staining in the control group. While the treated groups reveals mildly stained cytoarchitechture of the epididymis.

Keywords

Parkia Biglobosa, Epidydimiscytoarchitechture, PAS staining.

Introduction

African locust bean tree (*Parkia biglobosa*) is a perennial tree, which belongs to sub family mimosideae and family leguminosae (now family fabacceae) [1]. Locust been tree is a leguminous crop peculiar to the tropics. The tree is not normally cultivated but can be seen in population of two or more in the savannah region of West Africa [2]. It grows in savannah region of West Africa up to the edge of Sahel zone [3]. Dalziel and Keay reported that locust bean extend from Senegal to Sudan and it habitat is in savannah land and it characteristics of transition areas from Sahelian to Sudanian eco zone locally on farmlands [4,5]. A matured Africa locust bean tree of 20-30 years can be about a ton and above of harvested fruits. The tree is about 7 to 20 meters high and bears pods that occur in large bunches and vary from 120 to 300mm in length. The seed has a tough dark or brown coat with a hard golden-yellow cotyledon [6]. The seed are encased in a tough, elastic and relatively thick coat that has a very low permeability. The seeds are rich in protein, lipids, carbohydrate, soluble sugars and ascorbic acid and when fermented are rich in lysine. The fat in the bean is nutritionally useful (approximately 60% unsaturated). The fermented locust bean seed (*Parkia biglobosa*) are commonly used in soups and stews [7]. It is a culinary product that can be used to enhance or intensify meatiness in soup, Sauces and other prepared dishes [8]. The high cost of animal protein has directed interest towards several leguminous seed among the plant species,

legumes are considered as the major source of dietary protein [9]. The foundations of typical traditional systems of medicine for thousands of years that have been in existence have formed from plants. The plants remain to offer mankind with new medicines. Some of the beneficial properties ascribed to plants have recognized to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years. The earliest reports carved on clay tablets in cuneiform date from about 2600 BC are from Mesopotamia; among the materials that were used were oils of Commiphora species (Myrrh), Cedrus species (Cedar), Glycyrrhiza glabra (Licorice), Papaver somniferum (Poppy juice) and Cupressus sempervirens (Cypress) are still used today for the cure of diseases extending from colds and coughs to inflammation and parasitic infections. Also, Fructus Agni Casti is used orally for the symptomatic treatment of gynecological disorders including corpus luteum insufficiency and hyperprolactinemia premenstrual syndrome, menstrual irregularities, cyclic mastalgia and also to treat hormonally-induced acne. Cortex Berberidis used orally for the treatment of cystitis, dysmenorrheal, eczema, fever, haemorrhoids, inflammation, menorrhagia, nasal congestion, rheumatism, tinnitus and vaginitis. Also used as a cholagogue, diuretic, emmenagogue, hemostat, laxative and a tonic. Moreover much report from different researchers has shown that *Parkia biglobosa* is use for the treatment of malaria, diarrhea and pains, but no definite report has been given about the studies of plant extract of *Parkia biglobosa* on the on the Epidydimis.

Materials and Method

Extract preparation

The *Parkia biglobosa* powder was dispensed in 1500mls of distilled water in a plastic rubber container after washing; the mixture was vigorously stirred intermittently with a stick and then allowed to stand for 24 hours before it was filtered with a what man filter paper tinned funnel into a conical flask. The filtrate was evaporated at 45°C with water bath to obtain the crude solid extract for three weeks and the extract obtained was stored in the refrigerator until the commencement of the administration.

Experimental animals

The twenty-one animals were allotted into three groups consisting of eight in the control group, seven low and six high dose groups. Animals In group 1 served as control and was fed with normal rat chew and normal saline, Group2 which served as low dose were treated with 300mg/kgBw of seed extract of *Parkia biglobosa* while group 3 animals which served as high dose were treated with 500mg/kgBw of the extract.

Experimental design and procedure

The rats were all weighed before administration. They were divided into 3 group high dose with 6, low dose with 7 and control with 8 rats appropriately depending on the weight of the rats. The extract weighing 157g was dissolved in 317mg of distilled water. The high dose was given 500ml of the extract, low dose 300ml of the extract and control 300mg/kg of normal saline.

Termination of the experiment

At the end of the one-month period of administration, animals in all the groups were weighed and then sacrificed using cervical dislocation. The epididymis were removed and weighed using sensitive weighing balance to check for the testicular weight and processed for testicular glycogen distribution and routine testicular histology. The caudal epididymis were separated from the testis and processed immediately for epididymal sperm parameters.

Histological analysis

The epididymis was removed and preserved in a container with 10% neutral buffer formalin. There were done for 72 hours to achieve good tissue penetration and effective fixation. After this, they were placed in ascending grade of ethanol for dehydration. First they were treated with two changes of 70% ethanol each lasting for one hour followed by 95% ethanol and then absolute alcohol for the same duration. Following dehydration, the tissues were cleared in three changes of xylene each lasting for fifteen minutes. Then impregnation in molten paraffin wax at 058°C was carried out overnight and following morning, the tissues were embedded in wax to form blocks. These tissues block was trimmed and sectioned at 3 to 5µm thickness using a microtome. The sections were floated in warm water (28°C) and then taken up on aluminized glass slide. They were air-dried and stained using the hematoxylin and eosin (Harris, 2011) staining methods.

Statistical Analysis

Statistical analysis was done using Statistical package for Social Sciences (SPSS) version 16 Chicago Inc. One way ANOVA, followed by Bonferroni's Multiple Data Comparison Test was used to perform the analysis. Result of descriptive statistics of the experimental data was presented as Mean standard error of the Mean (Mean + SEM). Paired sample T-test were considered statistically significant at $P<0.05$.

Results

Effect of aqueous extract of *Parkia biglobosa* on body weight

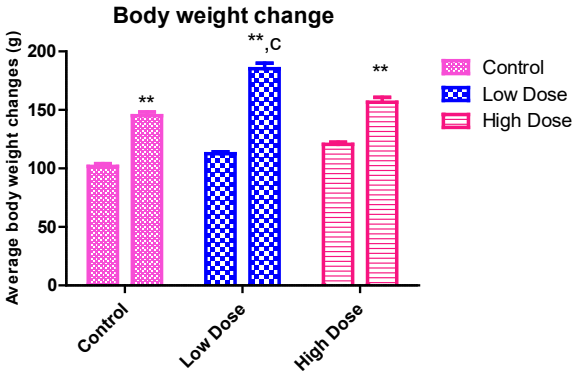
Table 1: Morphological analysis.

Body Weights		
Groups	Initial	Final
Control	101.8 ± 2.273g	145.0 ± 3.225
Low dose	112.5 ± 1.677g	185.2 ± 4.790g
High dose	120.5 ± 1.891g	156.6 ± 4.226g

Values are presented as Mean ± SEM

*=significantly different from the initial body weight at $p<0.01$

At the end of the research work, the mean body weight of the animals in the control group was 145.0 ± 3.225g as against its initial weight of 101.8 ± 2.273g, whereas the mean body weight of the treatment group (low dose and high dose) were 185.2 ± 4.790g and 156.6 ± 4.226g as against 112.5 ± 1.677g and 120.5 ± 1.891g respectively. The present study shows a significant difference between the control and high dose groups.



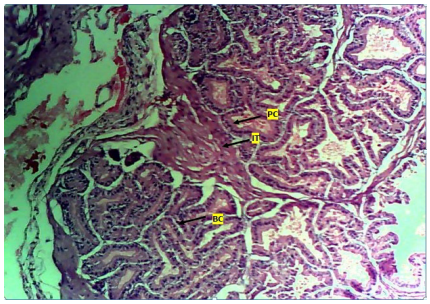


PLATE 1a: Photomicrograph of the control of the epididymis. Showing the principal and basal cells appearing normal. (H & E. X 10) stained.

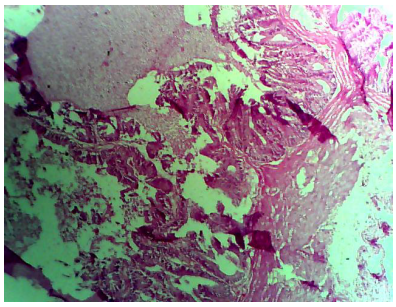


PLATE 1b: photomicrograph of the control. Group Epididymis architecture is moderately. Interstitial tissue appear normal. **(PAS staining**

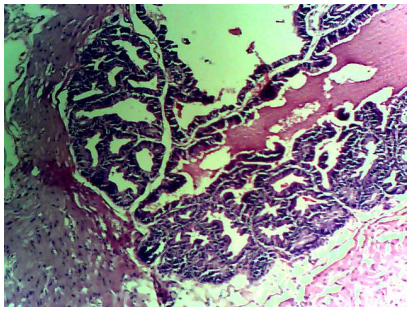


PLATE 2a: Photomicrograph of the low dose group of the epididymis showing areas of moderate dysplasia, exhibiting dense hyperchromasia and abnormal crowding of pseudo stratified epithelium. **(H&E × 10)**

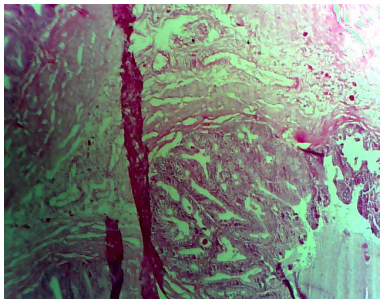


PLATE 2b: photomicrograph of the low dose group showing mild staining architecture of the epididymis **(PAS X10)**

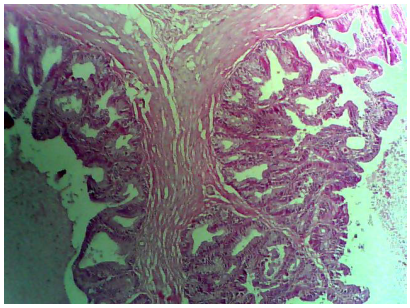


PLATE 3a: Photomicrograph of the high dose of the epididymis showing benign hyperplasia of the principal cells. **(H & E. X10)**

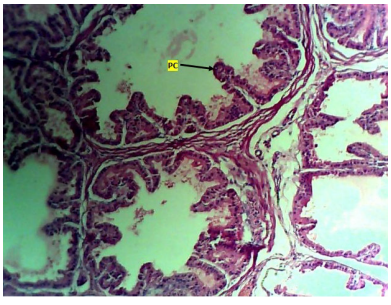


PLATE 3b: Photomicrograph of the high dose group showing mildly stained architecture. **(PAS staining X10)**

Discussion

The use of plant extracts as fertility enhancer in animals is on the increase because of the shifting of attention from synthetic drugs to natural plant products [10]. The increase in the number of users as opposed to the scarcity of scientific evidence on the safety of medicinal plants has raised concerns regarding toxicity and detrimental effects of these remedies. In rural communities, the exclusive use of herbal drugs prepared and dispensed by herbalists without formal training in the drug formulation and preparation for disease treatment is still very common, thus there is the need for screening methods to be established to ascertain the safety and efficacy of these herbal products [11].

The epididymis is a tube that connects the testicle to the vas deferens in the male reproductive system. It is present in all male reptiles, birds, and mammals [12]. Spermatozoa formed in the testis enter the caput epididymis, progress to the corpus, and finally reach the caudal region, where they are stored. Sperm entering the caput epididymis are incomplete; they lack the ability to swim forward (motility) and to fertilize an egg. It stores the sperm for 2–3 months. During their transit in the epididymis, sperm undergo maturation processes necessary for them to acquire these functions [13].

In the morphological study, the body weight of the control group were statistically significantly ($P<0.001$) increased. The experimental animals in the low dose groups that received 300mg of the extract per kilogram body weight showed significant increase in the final weight ($P<0.01$) respectively when compared to the control groups. However, the high dose experimental group showed significant ($P<0.01$) decrease in body weight compared to the control groups, indicating the toxic effect of aqueous extract of *Parkia biglobosa* at high a dose. This study is in line with the report from [14] that weight loss is a simple and sensitive index of toxicity after exposure to toxic substance. The decreased weight also could be due to decreased food intake in the rats due to the high tannin content in the high dose, which is one of the components of aqueous extract of *Parkia biglobosa*. High concentrations of tannins may be toxic, reducing voluntary feed intake and nutrient digestibility, however at low to moderate concentrations, tannin supplementation may shift site of protein degradation increasing metabolizable amino acid flow to the small intestine [15].

Histological results from this study reveals normal cytoarchitecture of the control and low dose groups while the high dose group treated with 500mg/kg of the extract showed cellular degradation as observed in the lumen which suggest benign hyperplasia reaction leading to the destruction of stored sperm cells. This reveals the cytotoxic property of aqueous extract of *Parkia biglobosa* on the epididymis, which causes the degeneration of epididymal epithelium, necrosis and exfoliation of principal cells. This effect is capable of reducing testosterone biosynthesis by the leydig cells [16].

In the histochemical study, the result reveals varying reduction in glycogen levels of the low dose and high dose groups indicating that the extract could have inhibited the glycogen synthesis, which may lead to decrease in spermatogenesis [17,18]. The marked color reduction observed in the high dose may also be due to interference in glycogenolysis, since glycogen is an energy source for general metabolism and constant supply of glucose is essential for proper functioning of Epididymis [19].

Conclusion

From the results obtained in this study, it could be suggested that aqueous seed extract of *Parkia biglobosa* may have an adverse effect on the epididymis when treated at high dose ($P<0.01$). Thus, intake of the aqueous seed extract of *Parkia biglobosa* at a high dose and long duration may affect proper sperm storage in males.

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