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Investigation on the Effect of *Parkia biglobosa* Seed Extract on the Prostate of Male Wistar Rats

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Abstract: The research was directed at assessing the prostatic effect of the extract of *Parkia biglobosa* on the male Wistar rats. Twenty one (21) experimental rats weighing about 90-120 g and divided into three groups consisting of seven animals were used. The control group was fed with normal rat feed and water, while the experimental groups the low and high groups received (300 mg/kgBw) and (500 mg/kgBw) of the extract daily by oral gavage method for thirty one (31) days. All animals were sacrificed a day after the end of the extract administration by cervical dislocation. The prostate was removed, weighed and fix in 10% buffer formalin. Result showed that following administration of extract of *Parkia biglobosa*, a significant increase was observed in the test group. Histological observation show that administration of *Parkia biglobosa* at high dose and long duration could result to cystic changes in the epithelium. Study reveals that these focal areas of cystic changes are prognosis of benign prostatic hyperplasia. Other histological observation shows that administration of aqueous extract of *Parkia biglobosa* at high dose reveal congestion of blood vessels and functional hyperplasia characterized by increased in-folding of the glandular epithelium resulting in the decrease in glandular diameter. Histochemical observation reveals a weak PAS-positive epithelial nuclei and a weak to negative PAS- positive cytoplasm, studies reveal that this could cause atrophic tubules and reactive hyperplasia.

Keywords: *Parkia biglobosa*, Prostate, Functional Hyperplasia, Histochemical, PAS Stains

1. Introduction

The name *Parkia Biglobosa* was giving to African Locust beans by Robert Brown, a Scottish botanist in 1826 after Mongo Park, a Scottish surgeon who explored West Africa in 1790's. Mongo Park gave named it 'nitta' [1]. *Parkia biglobosa* tree have been known to be a native of Africa and is an important multipurpose tree of West African Savannah land [3], which is primarily grown for its pods that contain both a sweet pulp and valuable seeds. The pods are flat and have irregular cluster of up to 30 seeds [4]. Many part of the African locust bean tree are used for medicinal purposes and have high value commercially. *Parkia biglobosa* seed is calledIyere in Yoruba land while the fermented seed as Iru. Iruis the best of sources of plant protein in African diet which is also called as fermented vegetable protein [5]. Iru is acceptable in many African countries, especially Nigeria. Parkia Biglobosa has different name indifferent African countries. - kinda in Sierra Leone, in Nigeria and Ghana it is referred to asdawadawa or Iru [6, 7], in Benin Republic afintin and sonru; nététu in Senegal and Burkina Faso as soumbala; Japan as natto; and kinema in Nepal [8]. In Burkina Faso, *Parkiabiglobosa* (Jacq.) R. Br. ex G. Don f. (Fabaceae; Mimosoideae) is a tree of utmost importance as a source of edible products and income for the vast majority of rural households. [9, 10]. These are all produced by either natural fermentation or inoculated fermentation of African Locust bean seeds. Apart from serving as a rich source of plant protein to the poor,. It also serves as good source of

protein for animal feeds, chick and fish (Livestock) [1, 5, 11]. In some areas, due to extreme competition in accessing the resource, people harvest *P. biglobosa* pods before their complete maturity, and this is likely to affect the regeneration of the species and the quality (taste and nutritional properties) of the edible products derived from the pods. [12].

Parkia biglobosa serves as dietary protein in many rural areas in developing countries since some of them cannot afford animal protein because they are either too expensive or simply unavailable. This situation has made many people to depend mainly on carbohydrate diets; comprising of grains or starchy roots and tuber crops with low protein level or content, thus leading to high level of malnutrition. In the quest of rural dwellers to increase the protein level of their food, many wild fruits have been found to be good alternative. Consumption of mainly cereal grains or starchy roots and tuber crops leads to malnutrition and various health problems associated with protein and vitamin/ mineral deficiencies. In the search for plant protein and vitamin substitutes, the African locust bean (*Parkia biglobosa*) has found very popular use especially in the form of fermented 'Iru', which is a product of its seeds, [13]. One of the important part of the locust bean tree is the seed which is high in protein, carbohydrate and is a good source of fat and calcium for village settlers [14].

Plant extract are continually sort for as effective and cheaper sources of medication of all the word especially in advance countries. 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 prostate gland.

2. Materials and Method

2.1. Extract Preparation

Fresh dawadawa seed was gotten from a market in Makurdi, Benue State Nigeria. The dawadawa seed were verified and authenticated by the Herbarium unit of the department of Botany, University of Calabar. The seed were air-dried at a room temperature of about 27°C. The blended sample of *Parkia biglobosa* was weighed using digital weighing balance, and was found to be weighing 160 g. The blended seed was macerated with 1500 mg of distill water and the mixture was left for 48 hours at 20°C, themixture was filtered, after which cheese cloth was use for filtration, followed by filter paper (whatman No. 1).

2.2. Experimental Animals

Twenty one (21) adult male albino rats were purchased from the animal house of the department of Human Anatomy and Forensic Anthropology, Cross River University of Technology, Okuku campus and were used for this study. The animals were randomly divided into three (3) group, and were allowed one week of acclimatization. The animals were housed in Perspex cage under controlled light schedule (12 hours light and 12 hours dark cycle) and were fed with standard growers' vital feed and water *ad libitum* before the start of administration. They were weighed prior to the experiment.

2.3. Experimental Design and Procedure

Twenty one (21) adult male rats were grouped in three (3) groups of control, low dose and high dose according to their weight respectively.

Group A (CONTROL) animals received normal saline orally at a dose of 300 mg/kgBw.

Group B (LOW DOSE) received aqueous extract of *Parkia biglobosa seed* orally at a dose of 300 mg/kgBw.

Group C (HIGH DOSE) animals received aqueous extract of *Parkia biglobosa seed* orally at a dose of 500 mg/kgBw.

2.4. Termination of the Experiment

At the end of the four weeks period, animals in all the groups were sacrificed a day after the end of the administration under cervical dislocation. The prostate of each animal were removed and washed with normal saline.

3. Histological Analysis

Tissue blocks were sectioned at 5µ with a rotary microtome then was dewaxed in xylene for two minutes per two changes. Xylene is being cleared in 95% alcohol for one minute per two changes and 70% alcohol for another minute. The sections were hydrated in running tap water until sections turned blue. They were then counterstained with 1% alcohol eosin for one minute, followed by a rapid dehydration through ascending grades of alcohol, cleared in xylene and mounted with DPX mutant. Stained sections viewed under a light microscope and photomicrograph.

4. Statistical Analysis

Statistical analysis was carried with 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. Descriptive statistics of the experimental data and presented as Mean standard error of the Mean (Mean + SEM). Paired sample T-test were taken as statistically significant at $P<0.05$.

5. Results

Table 1. *Morphological observation of body weight.*

BODY WEIGHTS		
GROUPS	**INITIAL**	**FINAL**
Control	101.8±2.273	145.0±3.225[a]
Low dose	112.5±1.677	185.2±4.790[a]
High dose	120.5±1.891	156.6±4.226[a]

Values are presented as Mean±SEM.

The morphological study shows an observable significant

($p<0.05$) increase in the final mean body weight when compared with the initial body weight. The final body weight of the control animals (145.0±2.273) was significantly ($p<0.05$) higher than its initial body weight (101.8±2.273). Also, it was observed that the mean final body weights of the low dose group (185.2±4.790) and high dose treated animals (156.6±4.226) were significantly ($p<0.05$) increased than their initial body weights (112.5±1.677) and (120.5±1.891). respectively.

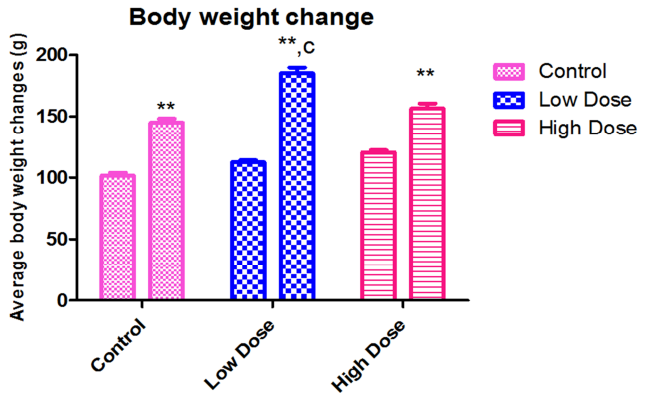

Figure 1. *Showing effect of Parkia biglobosa extract on average weight changes in Wistar rats. Values are expressed in Mean ± SEM n=5, ***=p<0.01 vs control, c = p<0.01 vs High dose.*

Table 2. *Effect of aqueous extract of Parkia biglobosa (seed) on the relative weight of the prostate.*

		MEAN	±SEM	P-VALUE	F-VALUE
Relative Organ weight (g)	Control				
	Low dose	1.49	±0.07	0.046	
	High dose	1.41	±0.03	0.132	3.318

Values are in mean ± SEM (n-3). **p<0.05 vs normal control is significant.

From the table above, the group administered with low dose shows significant increase in the relative weight of the prostate, while the high dose shows an insignificant increase in the relative organ weight.

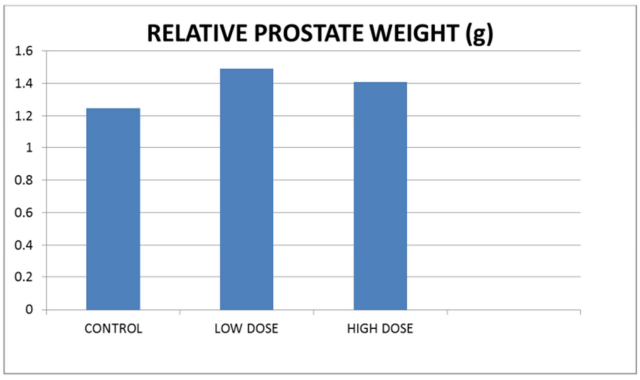

Figure 2. *Bar chart showing the relative prostate weight of experimental animals.*

6. Histological Analysis

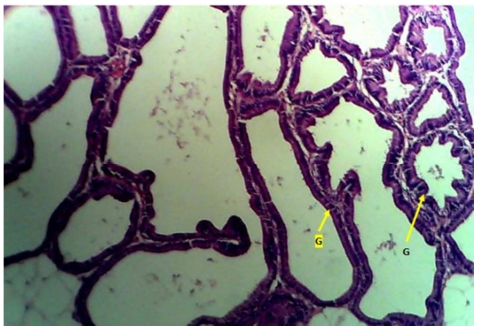

Figure 3. *Photomicrograph of the prostate CONTROL showing the glandular epithelium (G) Appearing normal. (H&E X40).*

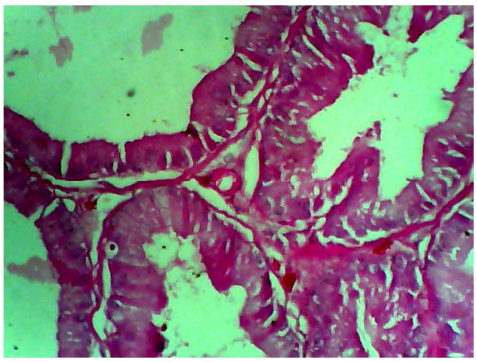

Figure 4. *Photomicrograph of the prostate CONTROLPAS- positive epithelium and cytoplasm (PAS X40).*

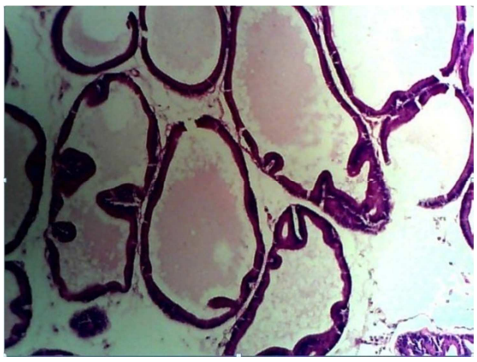

Figure 5. *Photomicrograph of the prostate LOW DOSE showing cystically dilated glands (H & E X40).*

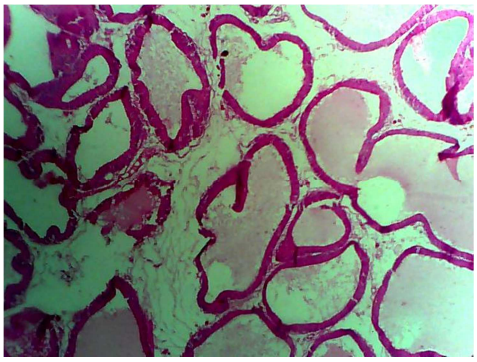

Figure 6. *Photomicrograph of the prostate LOW DOSE showing PAS-positive epithelium a d cytoplasm (PAS X40).*

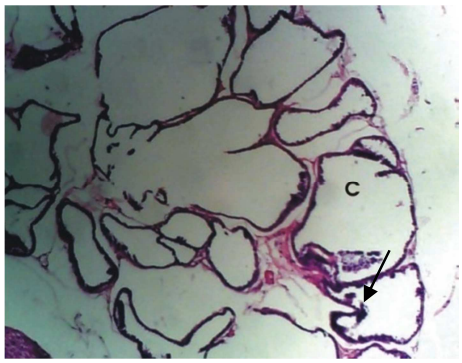

Figure 7. *Photomicrograph of the prostate HIGH DOSE DOSE showing focal area of cystic changes (C) (H&E X40).*

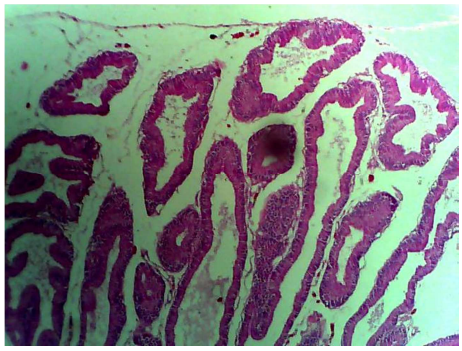

Figure 8. *Photomicrograph of the prostate HIGH DOSE showing weakly PAS-positive epithelial nuclei with a weakto negative PAS positive cytoplasm (PAS X10).*

7. Discussion

The use of plant extracts as fertility enhancer in animals is now in the increase because of the shifting of attention from synthetic drugs to natural plant products [15]. Plants that were once considered of no value are now use to produce important drug to the society with little or no side effect [16]. This accounts for the abuse of the plant as evident in the prolonged administration without appropriate dose evaluation and hence undermining the greater potential for adverse effect. 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, there is excessive use of these herbal drugs without considering the dose required to treat certain ailment, thus there is the need for screening methods to be developed to ascertain the safety and efficacy of these herbal plants [17]. In the morphological study, the body weight of the normal and test groups were statistically significantly ($P<0.001$) increased. The experimental animals in the low and high doses groups that received 0.3 ml and 0.5 ml of the extract per kilogram body weight showed significant increase in the final weight ($P<0.001$) and ($P<0.01$) 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 low dose groups, indicating the toxic effect of *Parkia biglobosa*at high a dose. Some studies reported that weight loss is a simple and sensitive index of toxicity after exposure to toxic substance [18-20].

The decreased weight also could be due to decreased food intake in the rats due to the high carotenoid content in the high dose which is one of the components of *Parkia biglobosa*

Organ weight is one of the most sensitive drug toxicity indicators and it changes often precede morphological changes [21]. Organ data is necessary because there are use to determine whether treatment group animal organ weight are in the range of background data or not, and there also provide an important reference to pathologist for gross anatomy and microscopic examinations [22].

From the result of the study above, there was significant increase in the relative weight of the prostate (Figure 2) when comparing the low dose to the high dose, this significant increase, may be an indication that the extract did not cause inflammation at the cellular levels when administered at low dose. This is in line with work done by Mohd. Nazrulislam*et al.,* 2009 who carried out research on the effect of an indigenous contraceptive herbal formulation on gonadotrophs of the pituitary gland of the rats.

The histological findings of the study reveal that the control groups of the prostate showed normal histology with their concretions, connective tissues, were also found with the slim border lined glandular epithelium grossly present. The low dose revealed cystically dilated gland and intraluminal budding of cell. While the high dose reveals focal area of cystic changes which might be as a result of the administration of the extract at high dose over the duration of 30 days, may have adverse effect on the integrity of the prostate gland. These focal areas of cystic changes are also prognosis for benign prostatic hyperplasia. [23].

Histochemical analysis of PAS (CONTROL) Control group shows PAS-positive epithelial an also showed the cytoarchitecture of the glandular acini with its mucosal surfaces lined by simple columnar epithelium and surrounded by a stroma composed of smooth muscles, fibro-collagen and blood vessels. The LOW DOSE experimental group animals revealed a normal glandular architecture of the prostate gland. The HIGH DOSE reveals weakly PAS-positive epithelial nuclei with a weak to negative PAS- positive cytoplasm, studies reveal that this could cause atrophic tubules, reactive hyperplasia involving cellular in- filtration.

8. Conclusion

The finding of this research study revealed that the extract of *Parkia biglobosa* is dose dependent and may cause adverse effect to the prostate gland. Thus, intake of aqueous extract of *Parkia biglobosa* at highlevels and long duration in males may cause benign prostatic hyperplasia and could affect male fertility.

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Credit Authors Statement

Kebe E. Obeten: Conceptualization, Methodology, Investigation, Writing–review and editing.

Ettah E. Nkanu: Software, Formal analysis, Data curator.

Gabriel Udo-Affiah: Resources, Visualization, Validation.

Wonah E. Justice: Writing–original draft, Supervision, Project administration.

Appendix

Appendix A. Parkia Biglobosa

African locust bean (*Parkia biglobosa*) also known as dawadawa is an important tree species which generates non-timber forest products. The pulp of the fruit is rich in sucrose and the seeds are rich in carbohydrates, proteins and lipids thus constituting an important source of energy. *Parkia biglobosa* is use to treat various ailments, such as hypertension, wound and malaria. The locust beans have over time served as food and nutrition plants during food shortage and drought periods especially in the Sahelian, Sudanian and Savanna and transitional zones of West Africa.

Appendix B. Material and Method

Fresh dawadawa seed was gotten from a market in Makurdi, Benue State Nigeria. Makurdi is located in the middle belt along the Benue River and hold the base for the Nigeria air force's MIG 21 and SEPECAT jaguar's aircraft squadrons in 2007, Makurdihas an estimate population of 500,797.

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