

*Full Length Research Paper*

# **Histomorphology and sperm profile of animal models administered with aqueous seed extract of *Parkia biglobosa***

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This study evaluated the effects of aqueous seed extract of *Parkia biglobosa* on the micro-anatomy of the testis and sperm profile of adult Wistar rats. Twenty-one male Wistar rats weighing between 96 and 120 g were assigned into three groups A, B, and C (n=7). Group A was the control and received normal saline, while the animals in groups B and C were treated with 300 and 500 mg/kg body weight (Bwt) aqueous seed extract of *P. biglobosa* orally and daily for 30 days. The animals were sacrificed via cervical dislocation at the end of the administration, and the testes were extracted for micro anatomical and histochemical studies. The Makler counting chamber was used for semen analysis while the Hematoxylin and Eosin (H and E) stain was used to assess the microstructure of the testicular tissues. The result from semen analysis showed a significant increase ( $p < 0.05$ ) in sperm motility, vitality, and morphology in the group treated with 300 mg/kg Bwt when compared with the control. The group treated with 500 mg/kg Bwt, showed a significant decrease in sperm motility, vitality, and morphology when compared with the control. H and E studies revealed the increased thickness of the germ cell layers of the seminiferous tubules (ST), with an increased number of spermatozoa in the lumen of ST in the group treated with 300 mg/kg bwt compared to the control group. This is a biomarker for improved spermatogenic activity in testicular tissues. Ingesting of *P. biglobosa* in the conditions used in this study seems to be safe and improves sperm parameters at a low dose (300 mg/kg Bwt).

**Key words:** Semen analysis, epididymis, male fertility, *Parkia Biglobosa*, Wistar rats.

## **INTRODUCTION**

The use of herbs and spices in culinary practices has resulted in the innovation and documentation of useful medicinal plants (Tapsell et al., 2006; Lai and Roy, 2004).

Globally, there has been a renewed interest in the use of medicinal plants and natural products in healthcare (Ahmed and Hussain, 2013). In Africa, several types of

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research into the benefit of herbal extracts in the treatment of human diseases have been proven and accepted for use (Iwalewa et al., 2007). This interest in medicinal plants may be attributed to the recognized adverse effects of certain orthodox drugs, high cost, and the promulgation of an affordable traditional medicine or alternative medicine treatments in developing and underdeveloped countries (Agunbiade et al., 2012). The rural residents in some African villages depend primarily on herbs for the treatment of many ailments, while residents in urban African cities indulge in the use of both modern medicine and herbs (Palaksha and Ravishankar, 2012; Adesuyi et al., 2011). One of such commonly used herbs is the African locust beans (*Parkia biglobosa*).

*P. biglobosa* belongs to the Leguminosae family crop; it is found in the tropical regions and parts of the savannah regions of West Africa, particularly the middle belt and the southwestern regions of Nigeria (Faleye et al., 2013; Sadiku, 2010). *P. biglobosa* has leguminous pods with a tough pericarp; the seeds are used as a food condiment and as an alternative to meat due to their high protein, fat, vitamins, tannin and mineral contents (Obizoba and Atu, 1993; Enujiugha and Ayodele, 2003; Abioye et al., 2013). *P. biglobosa* pods can be picked and processed into a fermented product known as 'iru', 'dawadawa', 'ogiri' in Yoruba, Hausa, and Igbo languages, respectively. The fermented pods contain about 40% protein, 32% fat, 24% carbohydrate, essential acids, and vitamins, and they can be used as protein supplement (Diawara et al., 2000).

The testes are paired male reproductive organs located in the scrotum; each testis has a whitish fibrous capsule known as the tunica albuginea. Septal extensions from the tunica albuginea divide the parenchyma of the testis into approximately 250 lobules (Inderbir, 2011). Each lobule contains about 1 to 4 seminiferous tubules embedded in a stroma of loose interstitial connective tissue which contains blood and lymphatic vessels and macrophages (Chaurasia, 2010). Each seminiferous tubule is about 150 to 250 cm in diameter, 30 to 70 cm long, and has a complex stratified epithelium (Singh and Chakravarty, 2000). The seminiferous tubule is the site where spermatozoa are produced. The interstitial connective tissues of the testis take part in main activities of the testis viz., mechanical support to the seminiferous tubules and blood vessels, production of testosterone by interstitial cells (Leydig cells), participation in the sustentacular cell barrier, and regulation of the sustentacular cell (Sertoli cell) functions. Due to the rich nature of the *P. biglobosa* and its widespread use in Nigeria, there have been reports that it can enhance reproduction by contributing to the quality of sperm in males (Soetan et al., 2011). This speculative report of the effectiveness of *P. biglobosa* on sperm profile may have also added to the increasing number of its patronage perceived in Nigeria. Therefore, this study seeks to institute a scientific basis of the traditional use of *P.*

*biglobosa* by assessing its impact on sperm profile in Wistar rats.

## MATERIALS AND METHODS

### Plant material and extraction

Fresh seeds of *P. biglobosa* were bought from a modern fruits shop in Benue State, Nigeria. The seeds were washed properly and air-dried for two weeks after which they were boiled and pounded using mortar and pestle to remove the shaft. With the shaft removed, the seeds were washed and fermented for two days, then sun-dried for one month and blended into powdery form.

The powdery *P. biglobosa* was distributed in 1500 ml of distilled water in a plastic rubber container. The mixture was vigorously stirred intermittently with a stick and then allowed to stand for 24 h before it was filtered with a Whatman filter paper tinned funnel into a conical flask. The filtrate was evaporated at 45°C with water bath for three weeks to obtain the crude solid extract which was stored in the refrigerator before use.

### Experimental animals

Twenty-one adult Wistar rats were used for the study. The rats weighed between 96 and 120 g in body weight and were kept in the Animal House of the Anatomy Department, Cross River University of Science and Technology (CRUTECH) Okuku, of Cross River State. The animals were kept under standard laboratory conditions in rubber cages with a 12 h daylight cycle. They had unrestricted access to feed and water and were acclimatized to laboratory conditions for one week before the commencement of the experiment.

### Experimental design and procedure

The twenty-one Wistar rats were assigned into three groups (n=7). The rats in group 1 were the control and received normal saline. Animals in group 2 served as the low dose group and were treated with 300 mg/kg Bwt of the seed extract of *P. biglobosa*. The animals in group 3 served as the high dose group and were treated with 500 mg/kg Bwt of the extract.

Before commencement of the experiment, ethical approval was obtained from the Ethics and Research Committee of the Faculty of Basic Medical Sciences of the Cross River University of Technology, Okuku, Nigeria, in line with the guideline and principles of the use of experimental animals.

### Administration of extract

*P. biglobosa* extract weighing 157 mg was dissolved in 317 ml of distilled water. The high dose group (group 3) was administered 500 mg of the extract, the low dose group (group 2) was treated with 300 mg of the extract and the control group (group 1) was given normal saline.

### Termination of the experiment

Before and after *P. biglobosa* was administered to determine the morphological observations, all animals were weighed using a sensitive weighing balance (Kerro-BI, 2001). Animals in all the groups were sacrificed by cervical dislocation. Their testes were removed and weighed. The testicular weight was recorded and

**Table 1.** Results from sperm analysis of all experimental animals.

Sperm parameter	Control	Low dose (300 mg/kg/Bwt)	High dose (500 mg/kg/Bwt)
Motility (%)	63.00±1.225	60.80±0.4899	56.40±2.619*
Vitality (%)	73.00±1.225	75.00±0.5477	72.00±1.225
Morphology (%)	56.40±2.619	72.00±1.225***	42.80±0.9695**

Values are presented in Mean ± Standard Error of Mean (SEM). \* = Significantly lower than control and low dose at  $p < 0.05$ .

\*\*Significantly lower than control and low dose at  $p < 0.01$ . \*\*\*Significantly higher than control and high dose at  $p < 0.001$ .

processed for testicular glycogen distribution and testicular histology. The caudal epididymis was removed from the testis and processed for the epididymal sperm profile.

### Semen analysis

The semen was collected from the caudal part of the epididymis by scrapping the lumen into a lumen tube and pre-warmed in a water bath at 50 to 60°C. A drop of this sample was placed on a slide in the chamber and covered with a cover glass. The sperm motility, vitality, and morphology (Table 1) were assayed using the Makler counting chamber and the sperm parameters were observed using an Olympus microscope. Semen analysis was carried out to ascertain the effect of the extract on sperm quality and male fertility.

### Tissue processing

The testes were extracted and kept in a container with 10% neutral buffer formalin for 72 h to realize effective fixation, and then placed in ascending grades of ethanol for dehydration. First, they were treated with two changes of 70% ethanol each lasting for 1 h 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 15 min. Then, they were soaked in molten paraffin wax at 58°C till the next day, before the tissues were embedded in wax to form blocks. These tissue blocks were trimmed and sectioned at 3 to 5  $\mu$ m thickness using a microtome. The sections were floated in warm water (28°C) and then taken up on albumenized glass slide, air-dried, and stained with hematoxylin and eosin (H and E) (Harris et al., 2011).

### Hematoxylin and Eosin staining

The tissue sections were stained as outlined by Feldman and Wolfe (2014) and Harris et al. (2011). The tissues were infiltrated and embedded in paraffin wax. Then, they were dewaxed/deparaffinized using xylene, followed by clearing. They were hydrated to facilitate staining with H and E.

The tissue sections were stained in hematoxylin for approximately 30 min. The stained slide was rinsed in tap water for 5 min until the section turned blue. The sections were differentiated in 70% ethanol containing 1% HCl for about 5 s, to remove excess stain. The tissue sections were rinsed for 5 min in tap water, then stained in eosin solution for about 10 min. Sections were further washed for 5 min in tap water. Finally, the stained sections were dehydrated, cleared and cover-slipped for light microscopy (Feldman and Wolfe, 2014; Harris et al., 2011).

### Statistical analysis

Statistical analysis was carried out using Statistical Package for

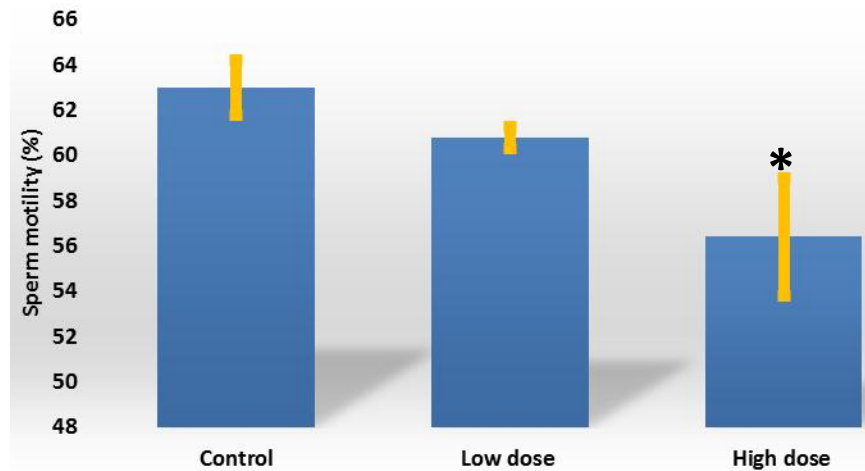
Social Sciences (SPSS) version 16 Chicago Inc, one-way ANOVA, and multiple data comparison test. Results were presented as mean and standard error of mean (Mean + SEM). Paired sample T-test was used to test for significance across groups and it is contemplated to be statistically significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

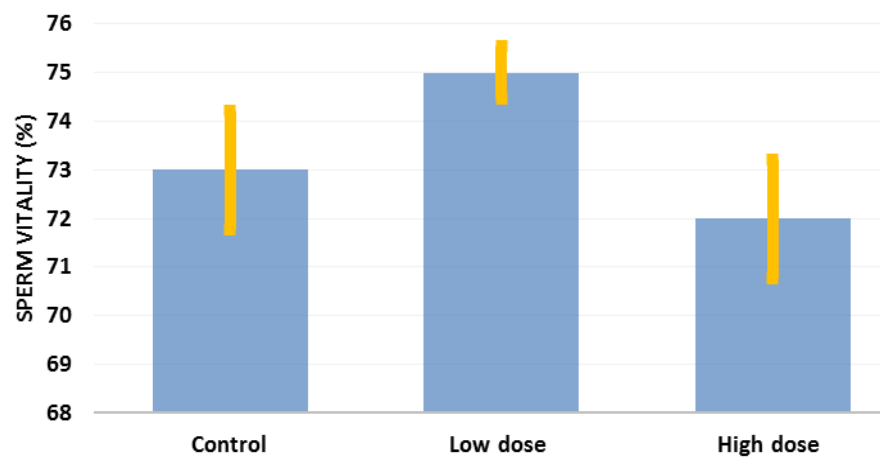
### Microscopic examination of the testes

Semen analysis and testicular biopsy are routine laboratory tests conducted to assess male fertility. It has been established that semen motility, vitality, and morphology (Figures 1, 2 and 3), are causative elements of male infertility (Brugh and LipShultz, 2004; Mishra et al., 2012), in addition to other factors such as malnutrition, genetic abnormality, critical illness, amongst others (Haslett et al., 2002; Biswas et al., 2010). Another study reported that only a few populations of men seek proper medical treatment to firmly find solution to their infertility issues because majority of the men are either too shy or ashamed to visit a clinic for proper medical consultations, and-as-such patronize herbal/plant formulations for self-medication (Vital and Health Statistics, 2002).

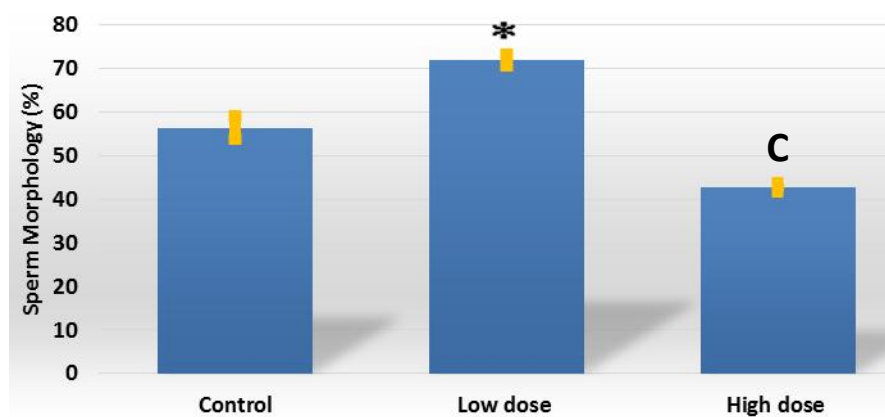
Most medicines contain plant extracts as their active ingredients and are culturally accepted because they have slight side effects. This has made herbal formulations to gain more acceptance in a traditional African home than orthodox medicines. Plants have provided mankind with a large variety of potent drugs to alleviate or eradicate infections and suffering from diseases. Despite advancements in synthetic drugs, some of the plant-derived drugs still retain their importance and significance (Adetutu et al., 2011; Karou et al., 2011). Despite the advancement in modern medicine, there are still a large number of ailments or infections (diseases) for which suitable drugs are yet to be found. This has brought an urgent need to develop safer drugs from the environment for the treatment of inflammatory disorders such as diabetes, liver diseases, gastrointestinal disorder, and infertility (Wake, 2012). The multidisciplinary approach used in finding solutions to some diseases has promulgated the research on herbal plants or medicine, and there seems to be a great development in the pharmacological appraisal of various



**Figure 1.** Effect of *parkia biglobosa* seed extract on sperm motility in Wistar rats. Values are expressed in Mean $\pm$ SEM; n=5, \*p<0.05 vs control.

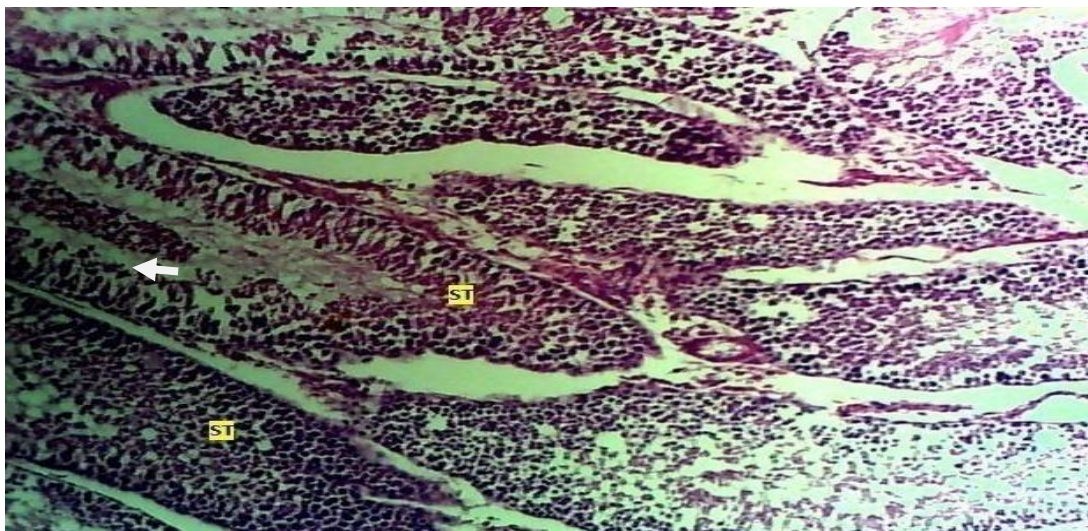


**Figure 2.** Effect of *parkia biglobosa* seed extract on sperm vitality in Wistar rats. Values are expressed in Mean $\pm$ SEM; n=5, there was no significant difference across groups.

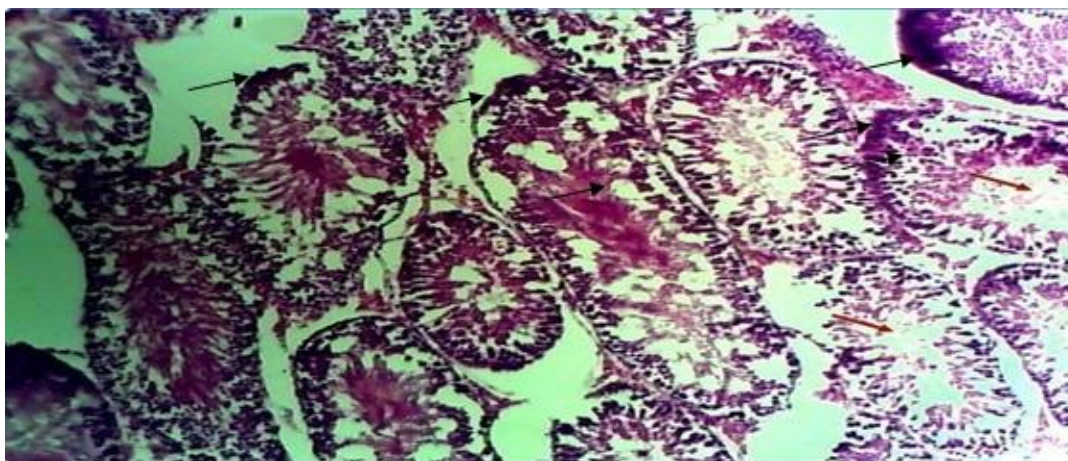


**Figure 3.** Effects of *Parkia biglobosa* extract on sperm morphology in Wistar rats. Values are expressed in Mean  $\pm$ SEM n=5, \*P<0.001 vs control, <sup>C</sup>p<0.01 vs high dose.





**Plate 1.** Photomicrograph of the testis from the control group (A), Stain shows well defined seminiferous tubule (ST) showing different stages of spermatogenesis. Flagella of immotile sperms can be seen in lumen of ST (arrow). H and E, X40.



**Plate 2.** Photomicrograph of the testis of low dose group treated with 300 mg/kg bwt aqueous seed extract of *Parkia biglobosa* showing increased thickness of germ cell layer, increased population of spermatozoa with flagella seen in lumen of ST (arrow) H and E, X40.

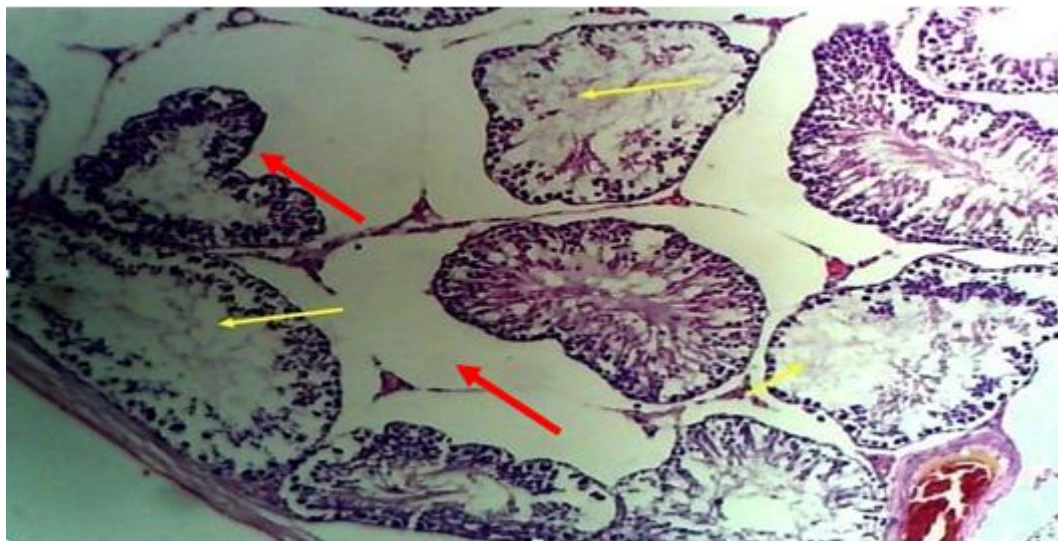
plants used in traditional systems of medicine of recent (Wuthi, 2010).

The effect of digitalization has brought about situations where men sit for long hours in offices using computers or are exposed to radiation from these machines which may impact reproductive health (Eisenberg et al., 2014; Gaskins et al., 2015). The importance of sound reproductive health among people is the capacity to have a satisfying and safer sex life, the ability to reproduce and the liberty to decide if, and when often to do so (Hajizadeh et al., 2017). Plants used as fertility boosters contain bioactive substances which are very effective in improving the immune system, raising and renewing the

body's liveliness, and treatment of different health issues (Ebong, 2015; Karou et al., 2011).

### Semen analysis

Results from this study showed there was a significant increase ( $p < 0.01$ ) in percentage sperm motility in the experimental animals that were treated with 300 mg/kg Bwt (Plate 2), while the animals treated with 500 mg/kg Bwt (Plate 3), aqueous seed extract of *P. biglobosa* showed reduced motility compared to the control (Plate 1). This suggests that a low dose of *P. biglobosa* may



**Plate 3.** Photomicrograph of high dose group treated with 500 mg/kg bwt aqueous seed extract of *Parkia biglobosa*, showing alteration of some seminiferous tubules, decreased population of spermatozoa with flagella seen in the lumen of ST, mildly reduced germ cell layers and dilated interstitial spaces (ARROW) H and E, X40.

increase sperm motility and augment reproductive health. This result supports the findings of Auta and Hassan (2016) that the mice exposed to different dose concentrations of aqueous wood ash extract of *P. biglobosa* showed significantly increased sperm motility. This increase observed was attributed to the rich iron content in the extract.

Animals treated with 300 mg/kg Bwt had better sperm vitality than animals treated with 500 mg/kg Bwt aqueous seed extract of *P. biglobosa* compared to the control. The results of sperm vitality from this study agree with Chinyerum and Thomas (2020), who stated that animals treated with green tea extract showed a substantial increase in sperm vitality. Sperm vitality refers to the proportion of living and healthy sperm present in the semen (Guzick et al., 2001).

Sperms morphology serves as an important and profound indicator of chemical toxicity on the cells of the reproductive system (Isidori et al., 2006). They can be used to evaluate spermatogenic impairment, fertility, and heritable genetic variations, which offer a direct measure of the quality of sperm production in chemically treated experimental animals (Gautam et al., 2010; Devi et al., 2011).

The present study showed a significant ( $p < 0.05$ ) decrease in percentage sperm morphology in the groups treated with 500 mg/kg Bwt aqueous seed extract of *P. biglobosa* when compared with the control, the group treated with 300 mg/kg Bwt *P. biglobosa*. This result agrees with the study of Adeoye et al. (2021), who reported a similar significant decrease in sperm parameters, that is, in the count, motility, and even normal sperm in their study. The findings from semen

parameters observed in this study also corroborate the study conducted by Hussain et al. (2018), who demonstrated that a polyherbal formulation could produce a synergistic efficacy leading to an improvement in the major semen parameters in oligospermic males. The semen analysis results suggest that our herbal formulation of *P. biglobosa* aqueous seed extract improved semen parameters in the experimental animals at doses of 300 mg/kg Bwt; but it was cytotoxic at 500 mg/kg Bwt causing a decrease of semen parameters.

### Histological observation

Hematoxylin and Eosin (H and E) results revealed increased thickness of germ cell layers, and increased population of spermatozoa in the low dose group (Plate 2). These observations have been previously documented to improve male sexual activity and male reproductive functions (Cao et al., 2012; Aversa and Fabbri, 2001; Gauthaman et al., 2002). The observed increased thickness of germ cell layers may be due to increased cell division sparked by the plant extract. This is in line with the studies conducted by Emmanuel et al. (2013), which reported that the presence of alkaloids activities of the *P. biglobosa* plants is an important pharmacological property that could be responsible for the observed effect on spermatogenesis.

The group which received the highest dose (Plate 3) showed alteration of some seminiferous tubules with mildly reduced germ cell layers, decreased population of spermatozoa and dilated interstitial tissues. This result corroborates the study conducted by Obeten et al. (2019),



who reported a dose-dependent adverse effect on almost all the testicular parameters from a plant extract studied. Observations from our study suggest that at 500 mg/kg body weight, the extract does not support spermatogenesis. This observation is in agreement with other studies (Wake, 2012; Palaksha and Ravishankar, 2012).

The histological observations from our study support the findings we saw in the seminal analysis. There was an improvement in sperm parameters (motility, vitality, and morphology) in the low dose group treated with 300 mg/kg Bwt of *P. biglobosa* compared to the high dose group treated with 500 mg/kg Bwt of *P. biglobosa* which showed a decrease of sperm parameters (motility, vitality, and morphology). Thus, the aim of the study has been addressed.

## Conclusion

The study recounted that the aqueous seed extract of *P. Biglobosa* comprises some pharmacological properties that could improve sperm motility, vitality, and morphology; thus providing a scientific basis for the folk belief that it may boost or improve male fertility. The results from this study are similar to those reported by Hussain et al. (2018), who compared pre-intervention with post-treatment semen analysis following a polyherbal treatment. Their results showed a 256% increase in sperm concentration in males. However, the present study showed that at higher concentration care should be taken in consuming the plant as this is seen to be unfavorable to sperm parameters. Also, the decrease in sperm parameters observed at higher dose (500 mg/kg Bwt) needs further investigation.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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