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Fertility Potentials of Methanol Extract of *Sesamum Indicum* seeds

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Abstract
The effects of methanol extract of *Sesamum indicum* seeds (obtained from Kogi state) on male hormonal profile and sperm quality in adult male albino rats was evaluated. Thirty six adult male rats (170 – 200 grams) were randomly divided into four groups replicated thrice with each replicate having three rats. Group A (normal control) and groups B, C and D (test groups) were administered 0, 125, 250 and 500 mg/kg of the extract respectively. Sperm parameters, male hormonal profile and histology of the organs evaluated after 28 days showed that a significant ($p<0.05$) increase in the concentrations of sperm motility, viability and count across all groups treated with methanol(MEOH) extract of *Sesamum indicum* seeds(SIS) when compared with control group. The percentage normal sperm concentration in the MEOH SIS treated groups decreased significantly ($p<0.05$) while total sperm abnormality concentrations increased significantly across all groups when compared with the control group. The percentage of detached head increased significantly ($p<0.05$) across all groups treated with MEOH extract while percentage of bent-mid piece and twisted tail only increased significantly ($p<0.05$) only in the group treated with the 500mg/kg of the extracts when compared with control group. Serum concentration of follicle stimulating hormone (FSH), Leutenizing hormone (LH) and testosterone significantly ($p<0.05$) increased in group treated with 250mg/kg of MEOH SIS extract also, a significant ($p<0.05$) increased was also observed in the concentrations of LH administered 125 mg/kg of the extract when compared with the control group. The histology of the testes of all groups showed intact seminiferous tubules and interstitium with orderly germ cell maturation variable around the tubule. The findings of this study suggest that methanol extract of *Sesamum indicum* seeds may possess fertility potentials since it stimulate and improve male hormonal concentration, sperm quality and maintain the architecture of the testes of the adult male albino rats. However, the high dose of 500mg/kg of the extract may be toxic at the administered duration.

Keywords: Sesamum indicum seeds, Male hormones, sperm parameters, sperm abnormality,

Introduction

Roughly 8% of men seek out for medical help for problems associated with fertility (Kumar and Singh, 2015). So many etiologies causes male infertility, so different approaches are required to resolve this problem (Ashamu *et al*., 2010). Many studies have been done to find out new effective treatments, and important advances and progress have been attained in both diagnosis and treatment of male infertility (Esteves *et al*., 2011). According to the underlying cause, non-surgical or surgical treatments, including hormone therapy with testosterone, human chorionic gonadotropin (hCG), clomiphene citrate and bromocriptine(Baker, 2001) or *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI), may be used to treat an infertile man (Friedl *et al*., 2002; Vernaeve *et al*., 2003) regrettably, some of these treatments are often too costly, especially to underprivileged and developing countries. Thus, some of these patients have frequently referred traditional practitioners who are reliant on the use of promising botanicals to manage or prevent this trial (Oyedemi *et al*., 2017). Different techniques of traditional Chinese medicine (TCM) including herbal remedies and various other traditional methods are few of the several traditional methods which are different from classic approaches that have been administered in animal models or in human for male infertility treatment (

Cheong *et al*., 2010; Lin and Huang, 2007).

Sesame plant especially the seed, oil and leaves are consumed locally as a staple food by subsistence farmers in South-West and Middle Belt areas of Nigeria (Akpan –Iwo *et al.,* 2006) and Shittu (2006) reported that it may be the reason for the high productiveness among the adult male population in these areas. It has been reported that phytochemicals in sesame leaves may have effect on improving fertility potentials and improve or increase epididymal spermatocytes reserve in adult male Sprague Dawley (Shittu *et al*., 2007). So due the presence of these phytochemicals and antioxidants and also some unconfirmed information from some people in kogi state who claims of considerable success in infertility issues with the use of this plant, our research interest was raised on the need to explore this plan. Also the potentials of this plant was probably neglected in Kogi State due to how common it is. However, some authors reported the effect of sesame leaves on male fertility but it has not been wholly explored considering the chemical compositions in this seed. Hence, this study aimed at evaluating the effect of methanol extract of *Sesamum indicum* seeds (obtained from Kogi state) on male hormonal concentration and sperm quality in adult male albino rats.

Materials and Methods

Collection and identification of plant

Sesamum indicum seeds were purchased from an Inye farmer at Engenma ward 3 in Ankpa Local Government Area of kogi State. The plant was identified by Mr Ibeh Ndukwe of the Department of Forestry and Enviromental Mangement Micheal Okpara University of Agriculture Umudike. A voucher specimen was kept at the herbarium unit in the Department of Physiology and Pharmacology, College of Veterinary Medicine.

Preparation of Crude Extract

The *Seamum indicum* seeds was thoroughly washed and air-dried at room temperature. The dried seeds were pulverized by the use of a miller. The pulverized sample of *Sesamum indicum* seeds was introduced into the extraction chamber of a soxhlet extractor and extraction was carried out using methanol with temperature maintained at 60^0C. At the end of the extraction, the extract was concentrated to dryness with rotary evaporator which was maintained at 40^0C and stored in the refrigerator until required.

Animals

Thirty six male adult albino rats weighing between 170-250g was randomly selected and used for the study. The animals were housed in well ventilated stainless-steel cages under standard laboratory condition and were fed with standard pellets (Finisher mash, Chikun Feeds, Crown Flour Mill LTD, Lagos State, Nigeria, with crude protein of 17.00% and metabolizable energy of 3000Kcal) and water *ad libitum.* They were allowed to acclimatize for two weeks before commencement of the experiment. The guidelines and the experimental protocols were approved by the ethical committee, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria. The experimental rats were maintained using the guide for the care and use of laboratory animals (NRC, 2011). The experiment was laid done in a complete randomized design of four treatments with three replicate each, and each replicate having three rats. Group B, C and D (test groups) were given 125, 250 and 500 mg/kg BW of the extract respectively while group A which served as control group was given 5 ml/kg of water for 28 days.

Acute Oral Toxicity Study

This method as described by Lorke (1983) was used.

Experimental Design

Thirty six rats were randomly assigned to four groups (A – D) of nine animals each and they were treated as follows: group A (normal control) received distilled water (5 ml/kg) only, groups B – D received 125, 250 and 500 mg/kg of methanol extract of *Sesamum indicum* seeds respectively. The treatments were administered once per day orally via gavage for 28 days. Twenty-four (24) hours after the last treatment on day 28, Blood was collected by ocular puncture with the use of capillary tube into anti-coagulant free bottles. The blood sample was centrifuged and serum was collected to determine the male hormonal profile. Sperm cells were harvested from the epididymis reserve. The rats were anaesthetized with chloroform (inhalation), and their epididymis extracted. The caudal portion of each epididymis was incised and a smear was made on the preheated glass slides for sperm analysis evaluation and thereafter, the rats were anaesthetized with chloroform fume in a desiccator, laparotomized and testes of all groups were excised immediately and fixed in 10% formalin for histopathological examination.

Determination of Sperm parameters

Sperm motility, sperm cell viability and sperm morphology: They were evaluated by the method described by Zemjani (1970). **Sperm count:** The count of the sperm cells in the semen samples were evaluated using the haemocytometer, a method described by Clegg *et al*. (2001).

Determination of male hormonal profile: Enzyme-linked immunosorbent assay (ELISA) method was used to determine the testosterone, Leutenizing hormones (LH) and follicle stimulating hormones (FSH) concentrations as described by Uotila *et al*. (1981).

Histological Examination: The testes was excised and transferred to a sterile universal container containing 10% neutral formalin. They were processed and embedded in paraffin wax to provide a hard support for sectioning. Every third section was mounted in glass slide and stained with Haematoxylin and Eosin and photomicrographed.

Statistical Analysis: Data collected were analysed with one way analysis of variance (ANOVA). Significant means were separate with post-hoc least significant difference (LSD) multiple comparisons. Significance was accepted at $p \leq 0.05$. Results were expressed as

Mean ± SD. Data analysis was done using Statistical Package for Social Scientists (SPSS) version 20.

Results and Discussion

Results

Acute Oral Toxicity Study Results

The acute toxicity study did not show any toxicity sign and symptom at 5000mg/kg. Each of the animals were observed for behavourial signs of toxicity (changes in skin, hair, eyes, mucous membranes, and respiratory, circulatory, autonomic and central nervous systems, motor activity, convulsion, tremors, salivation, diarrhoea, lethargy, or sleep) for 24hrs. After 24 hours post administration of the methanol extracts and observation at both high and low doses, no death was recorded in all the extract groups and the distilled water group. Furthermore, the animals did not display any drug-related changes in behavior, breathing, skin effects, water consumption and impairment in food intake even after the mice were allowed for another 7 days in case of any delay toxicity. Therefore, the extract was deemed to be safe at doses of 5000 mg/kg, and the median lethal dose (LD50) was considered to be > 5000 mg/kg.

Effect of methanol extract of Sesamum indicum (SIS) seeds on sperm motility, sperm viability and sperm concentration of wistar albino rats

The results of table 1 as indicated below showed a significant ($p<0.05$) increase in the concentrations of sperm motility, viability and count across all groups treated with methanol extract of *Sesamum indicum* seeds when compared with control. The improvement of the sperm quality declined with the increasing dose of the extract.

Effect of methanol extracts of Sesamum indicum seeds (SIS) on percentage of normal sperm cells (PNS) and total sperm abnormality (TSA) of wistar albino rats

The PNS concentration in the MEOH SIS treated groups decreased significantly ($p<0.05$) while TSA concentrations increased significantly across all groups when compared with the control group as indicated in Table 2.

Effect of methanol extracts of SIS on sperm abnormalities indices of wistar albino rats

Table 3 showed that the percentage of detached head increased significantly ($p<0.05$) in the groups treated with high, medium and low doses of MEOH extract when compared with control group. However, percentage of bent-mid piece and twisted tail increased significantly ($p<0.05$) only in the group treated with the high dose (500mg/kg) of the extracts.

Effects of methanol extract of Sesamum indicum (SIS) seeds on the male hormonal profile of wistar albino rats

The results of male hormonal profile as shown in table 4 indicated that serum concentration of FSH and testosterone significantly ($p<0.05$) increased in group treated with 250mg/kg of MEOH SIS extract also, a significant ($p<0.05$) increased was also observed in the concentrations of LH administered 125 mg/kg and 250 mg/kg when compared with the control group

Histology Results

The histology of the testes of all groups showed intact seminiferous tubules and interstitium with orderly germ cell maturation variable around the tubule. However, The control groups shows average seminiferous tubule density of 76 per LPF (low per field) and the mature spermatid density is variable in tubules averaging 380 per tubule while the group B, C and D showed 76, 77 and 75 seminiferous tubule density per LPF and 360, 370 and 350 mature spermatid density respectively. The histology of the test group were almost similar with the control group

Discussion

In acute toxicity study, no deaths were recorded after oral administration of low doses(10, 100, 1000mg/kg) and high doses(1600, 2000, 5000mg/kg) methanol extract of *Sesamum indicum* seeds to the mice after were observed for 24 hours and were allowed to stay for 7 days in case of any delay toxicity. There were no observed physical signs of toxicity at any stage of the experiment; no mortality was equally recorded on administration of all the doses. At a dose of 5000mg/kg body weight, methanol extract of *sesamum indicum* seeds were observed to be safe for consumption in mice. Therefore, the LD50 of the extract could be equal to or greater than 5000 mg/kg. This study support the claim by Yusuf *et al.,* 2017 who reported high safety margin (above 5000mg/kg bw) of the ethanol extract of *Sesamum Indicum* seeds. According to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, substances with LD50 > 5000 mg/kg are categorised category 5 (Organization for Economic Development, 2008) and are considered very safe. Hence, extract of *sesamum indicum* seed extract maybe considered relatively safe on acute exposure

The sperm cell count, motility, live/dead sperm cell ratio, morphology, the seminal volume, sperm pH, consistency and colour are usually referred to as andrological parameters and they are used to determine the fertility of a male subject (Saba *et al.*, 2009). This present study recorded a significant ($p<0.05$) increase in sperm motility and sperm viability percentage across all the groups treated with different doses of methanol extract of *Sesame indicum* seeds, also sperm count was significantly higher in the groups administered 125 and 250mg/kg bw of methanol extract as shown in table 1. The extract might have prevented peroxidative changes in the sperm cells and in the membranes of the testis, thus causing enhanced sperm motility and decreasing spermatozoa abnormalities (Farombi *et al.,* 2007). Oluyemi *et al*., 2007 documented that plants rich in antioxidants are capable of increasing sperm counts, sperm motility and enhance sperm morphology thus, the presence of vitamins C, E, phenols and flavonoids (antioxidants) in sesame seeds may justify its ability to

scavenge free radicals and protect sperm against lipid peroxidation (Amini *et al*., 2013). The results of Ibrahim *et al*, 2019 on sperm viability is in line with the result of our present study.

Teratozoospermia is characterized by the presence of spermatozoa with abnormal morphology over 85 % in sperm (De Braekeleer *et al*., (2015). Morphologic evaluation of spermatozoa is a part and parcel of semen analysis work-up for infertility (French *et al.,* 2010). When critical percentages (i.e.≤ 10%) of sperm cell abnormalities are present in the semen, the male subject is usually considered infertile (Saba *et al.,* 2009). A significant decrease was observed in the percentage of normal sperm cells across all groups treated with methanol extract and also a significant increase in the percentage of total abnormal spermatozoa across all groups treated with methanol extract as shown in table 2. This result was consolidated by the significant increase observed in three out of the six morphometric parameters analyzed i.e a significant increase in %detached head across all groups of extracts, %bent mid pieces and twisted tail were significantly higher in the rats that received 500mg/kg of methanol extracts as indicated in table 3, this could be attributed to prolonged administration of high dose of sesame seed extract which caused damaging effects on the seminiferous tubules which is reflected as high percentage of secondary sperm cell abnormalities and this may eventually lead to tetrazoospermia following chronic administration of this extract. This result is almost in tandem with the outcome of study of Khani *et al*. (2013) who reported that sesame seeds did not improve the sperm morphology of infertile men. On the contrary, the present study did not agree with the works of Ibrahim *et al*., (2019) who reported a significant increase in the normal sperm morphology on male albino rats treated with *Sesamum indicum* seeds. The difference may be because of variation in seed specie, method and period of harvest, difference in solvent used for extraction, dose administered and period of administration.

Follicle stimulating hormone (FSH) affects the proliferation, maturation and function of the supporting sertoli cells that produce regulatory signals and nutrients for the maintenance of developing germ cells (Oduwole *et al*., 2018). Deficiency of FSH or its receptor decrease spermatogenesis while increased FSH increases spermatogenesis, the sertoli cell number and testis size above normal in rats (Soffientini *et al*., 2017). The significant increase in the FSH concentration of the groups administered 250 mg/kg methanol extract as reported in table 4 could be indicative that the extract increases spermatogenesis. The increase in spermatogenesis could be through expression of a large number of sertoli cell genes (Sadate-Ngatchou *et al*., 2004) since FSH most prominently stimulates many genes involved in spermatogenesis like Kruppel-like factor 4 (Klf4) and also stimulate sertoli cells (Godmann *et al*., 2008). The result of this current study agrees with the work of Alaauldeen, (2017) who reported a significant increase in FSH concentration in acrylamide-intoxicated rats treated with sesame seeds oil. Studies have shown that FSH deprivation not only lowers sperm count but also affects the quality of the remaining sperm (Nieschlag *et al.,* 1999). So the increase in FSH concentration explained the increase in sperm count observed in this present study.

Lutenizing hormone (LH) mediate their actions on spermatogenesis through its cognate receptors, luteinizing hormone/choriogonadotropin receptor (LHCGR in humans), a plasma membrane associated G-protein coupled receptor expressed on leydig cells (McLachlan *et al*., 2002), where it stimulates testosterone production (O'Shaughnessy, 2014). The result of the LH concentrations of the present study shows a significant ($p<0.05$) increase in the groups treated with the low and medium doses of methanol extracts of *Sesamum indicum* seeds. This result is in accordance with the work of Mahabadi *et al*, (2015) who reported that LH concentration increased significantly in regimen group of adult rat fed with sesame supplemented diet. This indicate that that sesame seeds may also increase testosterone and could induce onset of puberty since LH stimulates leydig cell production of testosterone and regulate the epididymal epithelial morphology and epididymal steroidogenesis (Nosek *et al*., 2016).

Testosterone is responsible for maintaining blood testis barrier, supporting the completion of meiosis and adhesion of elongated spermatids to sertoli cells to release sperm. Maturation of spermatid is essentially testosterone dependent, a step that cannot be completed in spite of high FSH (Shittu *et al*., 2008). A significant increase in testosterone level was observed in the groups treated with 250mg/kg methanol extract of *Sesamum indicum* seeds while non-significant reduction was observed in the higher dose. This result agrees with the work of Shittu *et al.* (2009) who reported an increase in testosterone concentration in normo-glycaemic adult male sprague dawley rats fed with *Sesamum radiatum.* The low testosterone concentration observed at higher doses of the extract may not be as a result of the destruction of the leydig cells but a reflection of the complex hormonal interplay at the level of the hypothalamic-pituitary-testicular axis owing to the fact that at such high doses, the extract could possibly lead to more production of estradiol which competes with dihydrotestosterone for aromatization, thereby leading to a decline in the level of testosterone (Mahabadi *et al.,* 2015).

Conclusion

The findings of this study suggest that the methanol extract of *Sesamum indicum* seed may possess spermatogenic activity since it stimulate and improve male hormonal concentration, sperm quality and maintain the architecture of the testes of the adult male albino rats, however the high dose of 500ng/kg may be toxic at the duration administered.

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Table 1: Effect of methanol and hexane extract of *Sesamum indicum* (SI) seeds on sperm motility, sperm viability and sperm concentration of wistar albino rats

Treatment	Sperm motility (%)	Sperm viability (%)	Sperm Count($\times 10^6$/C.Ep)
Control	69.13 ±1.23	78.20 ± 2.46	116.58 ± 4.02
MEOH SI, 125 mg/kg	79.93 ± 2.83*	89.25 ± 2.69*	141.12 ± 7.83*
MEOH SI,250 mg/kg	78.63 ± 2.41*	87.89 ± 0.93*	133.70 ± 5.64*
MEOH SI, 500 mg/kg	77.27 ± 1.13*	85.80 ± 0.48*	129.62 ± 0.61

Values with (*) are significantly (p<0.05) different when compared with the control

Table 2: Effect of methanol extracts of *Sesamum indicum* seeds (SIS) on percentage of normal sperm cells (PNS) and total sperm abnormality (TSA) of wistar albino rats

Treatment	PNS (%)	TSA (%)
CONTROL	96.16 ± 0.36	3.84 ± 0.36
MEOH SI, 125 mg/kg	94.11 ± 0.18*	5.89 ± 0.18*
MEOH SI, 250 mg/kg	93.34 ± 0.89*	6.66 ± 0.89*
MEOH SI, 500 mg/kg	92.04 ± 0.58*	7.96 ± 0.58*

Values with (*) are significantly (p<0.05) different when compared with the control. PNS=Percentage normal spermatozoa, TSA= Total Sperm abnormality

Table 3: Effect of methanol extracts of SIS on sperm abnormalities indices of wistar albino rats

Parameters	Control	MEOH SIS, 125mg/kg	MEOH SIS, 250mg/kg	MEOH SIS, 500mg/kg
%Detached head	0.89 ± 0.12	1.57 ± 0.18*	2.08 ± 0.06*	2.30 ± 0.18*
%Bent mid piece	0.54 ± 0.02	0.64 ± 0.11	0.54 ± 0.23	0.95 ± 0.07*
% Twisted tail	1.43 ± 0.29	2.01 ± 0.09	2.28 ± 0.41	2.51 ± 0.39*
% Short tail	0.05 ± 0.03	0.17 ± 0.07	0.20 ± 0.08	0.33 ± 0.03
%Distal cytoplasmic droplets	0.45 ± 0.08	0.45 ± 0.05	0.45 ± 0.22	0.45 ± 0.01
%Proximal cytoplasmic droplets	0.48 ± 0.14	0.67 ± 0.14	0.82 ± 0.09	0.85 ± 0.08

Values with (*) are significantly (p<0.05) different when compared with the control. PNS=Percentage normal spermatozoa, TSA= Total Sperm abnormality

Table 4: Effects of methanol and hexane extract of *Sesamum indicum* (SIS) seeds on the hormonal profile of male wistar albino rats

Treatment	FSH (µIU/mL)	LH(µIU/mL)	TESTOSTERONE (ng/mL)
Control	2.25 ± 0.29	2.72 ± 0.87	5.35 ± 0.95
MEOH SI, 125 mg/kg	3.25 ± 0.29	10.72 ± 0.87*	6.20 ± 1.44
MEOH SI, 250 mg/kg	6.75 ± 0.58*	13.22 ± 0.58*	11.60 ± 0.64*
MEOH SI, 500 mg/kg	2.70 ± 0.02	3.67 ± 0.32	5.54 ± 0.29

Values with (*) are significantly (p<0.05) different when compared with the control. FSH = follicle stimulating hormone, LH = luteinising hormone

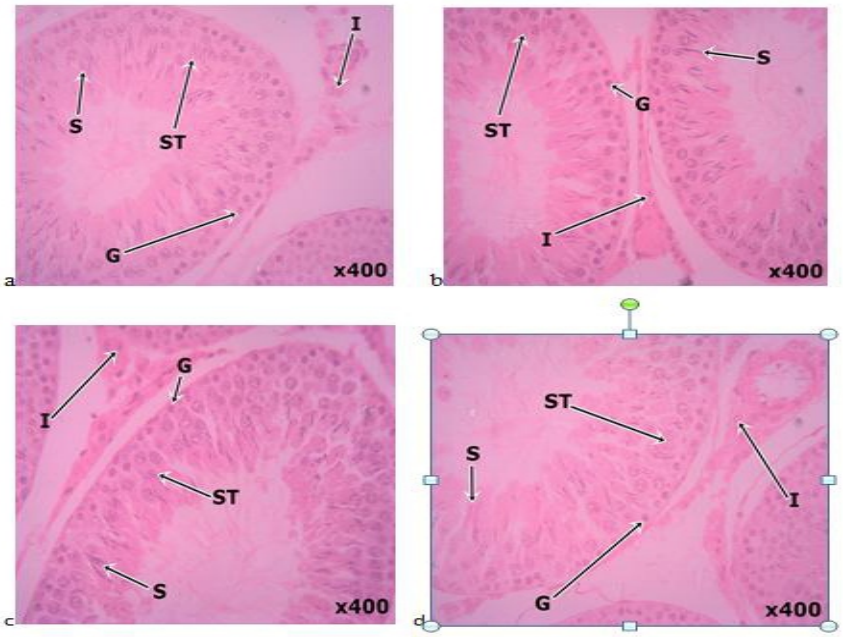

Figure 1: The photomicrograph (H & E, x400) of the testes of rats exposed to methanol extract of Sesamum indicum seeds for 28 days

Legend: a = normal control; b = extract, 125 mg/kg; c = extract, 250 mg/kg; D = extract, 500 mg/kg. G – Germinative cells. I=Interstitial cell of Leydig. ST=Sertoli cell. S=mature Sperm