A comparative Evaluation Of Selected Medicinal Plants on Male fertility indices (reproductive hormones and sperm profile) of albino wistar rats. An Animal Case study

Abstract: The study aims to investigate the effects of the medicinal plants{seeds} on the reproductive hormones and sperm profile of male albino rats to ascertain their possible usefulness as fertility agent. Walnuts [Tetracarpidium conophorum] ,Sesame (Sesamine indicum),and Velvet bean(Mucuna pruriens) seeds were obtained and taken to the Department of plant Science and Biotechnology, University Of Nigeria , Nsukka(UNN). The seeds were milled into fine powder. One hundred and ten sexually matured aibino rats of about twelve weeks weighing 130-180g were divided into eleven groups (1-11) using completely randomized design. There were two different control groups and rats in group 1 served as the Control 1 and were fed with normal commercial feed. Rats in group 2 were administered with a drug (Ketoconazole) to induce infertility. The Infertility Induced groups were treated with low dose (groups 3-5), medium dose (groups 6-8) and high dose (9-11) for the period of nine (9) weeks. At the end and testes and Epididymides were surgically removed and weighed. Blood sample analysis revealed that the concentration of sex hormones measured in the male rats fed with medium plants (seeds) showed that the testosterone concentration significanty increased ($p<0.05$) in animal control group 1(normal rats) Therefore, these medicinal plants walnut seeds showed significant increase in their testosterone concentration, luteinizing hormone (LH) and follicle stimulating hormone (FSH) which significantly enhanced the production of reproductive hormones which enriched the fertility status of these animals.

Keywords: albino rats, medicinal plants, reproductive hormones, sperm profile, testis histology

1. INTRODUCTION

Medicinal plants are plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs (WHO 2008). The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can lead to the development of novel and safe medicinal agents (Golla et al., 2011). Historically, all medicinal preparations were derived from plants whether in the simple form of raw plants materials or in the refined form of crude extracts, mixtures (Krishnaraju et al., 2005). The rich knowledge base of countries like India and China in medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs (Mukherjee & Wahile, 2006). Nigeria is also among countries blessed with a wide range of such medicinal plants which have not been thoroughly evaluated for their bioactive/ therapeutic agents. Primary among this list of properties is their antioxidants, antibacterial and antifungal activities (Ajaiyeoba and Fadara , 2006). However, some of such botanicals are Tetracarpidium conophorum, Sesame indicum L and Mucuna pruriens. These plants have antimicrobial activity which may cure some sexually transmitted infections that could be responsible for male fertility (Ajaiyeoba and Fadara 2006). Researchers have also shown that these plants possess some essential antioxidants and Vitamins capable of fighting

off reactive oxygen species, thereby assisting seminal plasma exhibiting a strong capacity to maintain a relatively neutral and protective environment for sperm function (Orth et al; 1993; Foresta et al, 2004). There are reports that abnormalities in sex hormone biosynthesis may impair spermatogenesis. The failure of the pituitary to maintain proportionate levels of FSH,LH and PRL may lead to disruption of testicular function, leading to infertility (Dashistani and Dayem, 2007).The seeds have also been found to contain major essential fatty acids (Omega-3DHA and Omega-6) which include oleil, linolenic and linolenic acids (Zwarts et al; 1999; savage, 2001; Ozkan and koyuncu, 2005, Ahmed et al, 2008,). Essential fatty acids act as hormone regulators and its purported that sperm contain high concentrations of Omeg-3's.

The main purpose of the research work therefore is to investigate the effects of the medicinal plants (seeds) on the reproductive hormones and sperm profile of male albino rats in order to ascertain possible usefulness as a fertility agent.

2. MATERIALS AND METHODS

The seeds of walnut (Tetracarpidium conophorum), Sesame (Sesamium indicum), and Velvet bean (Mucuna pruriens) were procured and sent to the department of plant science and Biotechnology, University of Nigeria (UNN) for identification and authentification. **The seeds were cut into pieces and dried for fort-eight hours** and ground into fine powder using electric blender.

Experimental Animals and Administration of Seeds. Ninety sexually matured male albino rats of about twelve weeks weighing between 130-180g were used for this experiment. They were kept in a well ventilated conventional cage (temperatures: 28-31°c, photoperiod: 12hours of natural light and 12hours of darkness; humidity: 50-55%) and acclimatized for two weeks with tap water before commencement of treatments. The rats were divided into eleven groups (1-11) using completely randomized design with six male and four female rats in each group. There were two different control groups in this research. Rats in group (1) served as the control 1 and were fed with normal commercial feed only and rats in group (2) served as control ii and were administered with a drug (ketoconazole) reported to induce infertility to rats, including dog and primate (Vawda and Davies, 1986; Donald et al , 1990). To evaluate further the potentials of the seeds, the infected groups were treated with low dose (groups 3-5), medium dose (groups 6-8) and high dose (groups 9-11) using dietary inclusion for the period of nine weeks.

Preparation of Serum and Semen for fertility Examination.

At the end of the treatment regime, the rats were anaesthetized using diethyl ether. The testes and Epididymides were surgically removed and weighed. Blood samples, 15ml per each rat were collected through cardiac puncture into sterile tubes for hormonal analysis.

Hormone Assay

The blood samples collected were centrifuged at 2500rpm for 5minutes using a centrifuge at 10-20oc to obtain the serum samples which were analyzed for testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol hormone level using enzyme linked immunoassay (ELISA) technique (Ekaluo et al; 2010)

Semen Analysis

(a) Determination of Epididymides and weight.

These Epididymides and testes were dissected out and excess blood damped into cotton wool and placed in a clean weighing balance to record the weight.

(b) Evaluation of sperm motility

Semen samples from the different treatment groups were dropped on a glass slide and viewed under the microscope. A minimum of five microscopic fields were assessed to evaluate sperm motility and were analyzed for progressive motile sperm (PMS), non-progressive motile sperm (NPMS) and non-motile sperm (NMS) distinguished by the movement of the sperm cells (World Health Organization, 1992)

(c) Estimation of mean sperm count

The mean sperm count was carried out according to the method of (Ekaluo et al; 2009)

(d) Estimation of sperm viability

Sperm viability was estimated using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples were mixed with equal volume of eosin-nigrosin stain and air dried smears were prepared on glass slides for each samples as described by Bjorn dahl et al, (2003).

(e) Estimation of Semen PH

The PH of semen was measured using a specially treated calibrated paper blot that changes colour according to the PH of the semen that it is exposed to (Comhaire and Vermeulen, 1995)

(f) Sperm head abnormalities

A fraction of each of the sperm suspension was examined by placing the solution (10:1) for 30 minutes on a glass slide. The slide was examined for percentage abnormalities in every 200 spermatozoa on each slide and fine air dried smear prepared on glass slide for each sample according to (Ekaluo et al; 2005).

3. RESULTS AND DISCUSSIONS

Table 1 shows the results of the impacts of medicinal plants on the sperm quality of the albino rats. The result revealed no significant increase ($p>0.5$) in the sperm motility between the animal groups 1,6,7,9 and 11 and these groups showed a significant increase ($p<0.5$) when compared with that of other experimental groups. The sperm motility was significantly lower ($p<0.05$) in the animal groups 2,3,4,5,8 and 10.The result in respect of sperm count showed a significant increase ($p<0.05$) in the group 1 compared across all the induced animal groups 2-11. However, there was no observed significant difference ($p>0.05$) between the group 2 and the animal groups treated with the medicinal plant samples. Sperm viability was observed to be significantly high($p<0.05$) in the groups

1,5,6,8,10,and 11 compared to other treated groups. Also, there were no significant difference (p>0.05) in the sperm viability between the animals in groups 2,3,and these groups showed significant decrease(p<0.05) in th e sperm PH of the rats in all the groups. Meanwhile, Testes and Epididymides weight of the group 6 was significantiy higher(p<0.05) than that of groups 1,4,5,and 8.However,the results revealed no significant increase (p>0.05) of the Testes and Epididymides weight between the groups 2,3,7,9,10 and11.

All values are expressed as Mean +Standard Deviation (SD).Mean values with the same letters as superscripts along the column are considered non-significant difference (p>0.05) while mean values with different letters as superscripts are considered significant(p<0.05).

Table1: Effects of the medicinal plants (Tetracarpidium conophorum,Sesame indicum L,and Mucuna pruriens) seed on the sperm profile of the albino rats

GROUPS	Sperm Motility (mean±SD)	Sperm Count (mean±SD)	Sperm Viability (mean±SD)	Sperm Head Abnormality (mean±SD)	Sperm pH (mean±SD)	Testes and Epididymides weight (g/kg) (mean±SD)
Group 1	61.67±7.64^{a} b	53.33±2.89 a	58.33±3.51^{a}	38.33±7.63^{b}	7.50±0.49^{a}	0.93±0.2^{b}
Group 2	26.67±5.77^{d}	26.67±7.64 b	34.00±8.72^{c}	71.67±6.36^{a}	6.63±0.72^{a}	0.97±0.06^{ab}
Group 3	33.33±5.77^{c} d	26.67±7.64 b	29.67±7.50^{c}	60.00±1.50^{ab}	6.90±0.42^{a}	1.13±0.58^{ab}
Group 4	31.67±6.07^{d}	21.66±7.60 b	41.33±5.51^{bc}	60.00±2.42^{ab}	6.77±0.40^{a}	0.73±0.12^{b}
Group 5	33.33±2.58^{c} d	20.00±5.00 b	44.33±6.03^{ab} c	63.32±3.64^{ab}	7.03±0.49^{a}	0.90±0.17^{b}
Group 6	55.00±3.23^{a} b	26.66±7.63 b	42.00±1.16^{ab} c	56.67±2.89^{ab}	7.00±1.20^{a}	1.53±0.23^{a}
Group 7	65.00±5.00^{a}	28.33±2.89 b	27.00±2.65^{c}	61.66±4.64^{ab}	7.07±0.57^{a}	1.00±0.35^{ab}
Group 8	45.00±1.00^{c} d	26.66±2.88	43.66±5.13^{ab}	63.33±1.41^{ab}	7.03±0.32^{a}	0.93±0.12^{b}

		b	c			
Group 9	48.33±1.41[b] c	28.33±2.80 b	36.67±2.58[bc]	66.66±2.88[ab]	7.00±0.72[a]	1.10±0.10[ab]
Group 10	35.00±8.03[c] d	35.00±1.54 b	42.60±4.93[ab] c	61.24±2.08[ab]	7.10±0.61[a]	1.07±0.31[ab]
Group 11	58.33±1.93[a] b	30.00±5.00 b	49.32±5.13[ab]	58.33±2.16[ab]	7.33±1.10[a]	1.07±0.32[ab]

(Tetracarpidium conophorum,Sesame indicum L,and Mucuna pruriens) seeds.

The results of the concentration of sex hormones measured in the male rats fed with medicinal plants (seeds) shown in the table 2. From the result, the testosterone concentration showed significant increase ($p<0.05$) in animal control group 1 (normal rats) while the concentration of the hormone in group 2 (ketoconazole induced animals with no treatment) significantly decreased ($p<0.05$) when compared across the treated groups 3-10 (Induced rats fed with 4g, 8g and 12g/kg between day of the test samples). Group 11 (Induced rats fed with 12g/kg between day of walnut sample) also revealed a significant increase ($p<0.05$) when compared with the rest of the treatment groups 3-10. The result of the present study also revealed a significant increase ($p<0.05$) in the concentration of FSH in the control group 1 (normal rats) when compared with that of groups 2-11 (animals fed with 4,8 and 12kg/g test samples). However, there was also an observed significant increase ($p<0.05$) in the concentration of Luteinizing hormone (LH) in the group 1 compared to that of groups 2-10 but the result revealed no significant increase ($p>0.05$) when compared group 1 (normal rats) and that of group 11 (animals fed with 12kg/g walnut sample). Group 5 (animals fed with 4kg/g walnut sample) and 6 (animals fed with 8kg/g velvet bean sample) showed significant decrease ($p<0.05$) when compared across other treatment groups. Moreover, the results of the estradiol in table 2 indicate a significant decrease ($p<0.05$) in group 1 (normal rats) compared to that group 2 (ketoconazole induced animals with no treatment) and that of the treatment groups 3-10 (animals fed with 4, 8 and 12kg/g test samples) but no significant difference ($p>0.05$)was observed when compared with that of group 11 (animals fed with 12kg/g walnut sample).

Table 2: The concentration of sex hormones measured in the male rats fed with medicinal plants

GROUPS	Testosterone	FSH (µl/ml)	LH (µl/ml)	Estradiol (µl/ml)

	(µl/ml) (mean±SD)	(mean±SD)	(mean±SD)	(mean±SD)
Group 1	1.63±0.15^{a}	2.57±0.86^{a}	2.60±0.31^{a}	32.90±3.24^{c}
Group 2	0.33±0.06^{d}	1.37±0.12^{b}	1.90±0.10^{bc}	50.96±4.94^{a}
Group 3	0.40±0.20^{cd}	1.60±0.17^{b}	1.93±0.12^{bc}	42.77±11.01^{ab}
Group 4	0.43±0.25^{cd}	1.63±0.15^{b}	1.83±0.06^{bc}	43.80±8.58^{ab}
Group 5	0.53±0.23^{cd}	1.80±0.10^{b}	1.07±0.06^{c}	37.46±3.04^{b}
Group 6	0.50±0.26^{cd}	1.40±0.10^{b}	1.07±0.05^{c}	44.20±8.80^{ab}
Group 7	0.50±0.35^{cd}	1.53±0.06^{b}	1.10±0.10^{bc}	43.47±7.05^{ab}
Group 8	0.63±0.32^{cd}	1.43±0.06^{b}	1.10±0.01^{bc}	39.13±7.98^{b}
Group 9	0.47±0.11^{cd}	1.53±0.05^{b}	1.53±0.11^{bc}	50.23.6.44^{ab}
Group 10	0.67±0.06^{bc}	1.37±0.06^{b}	1.43±0.15^{bc}	48.70±8.55^{ab}
Group 11	0.77±0.05^{b}	1.63±0.28^{b}	1.47±0.05^{ab}	38.77±4.54^{bc}

4. CONCLUSION

The concentration of sex hormones measured in the male rat fed with medicinal plants (seeds) showed that the testosterone concentration significantly increased ($p<0.05$). It was also deduced that these medicinal plants including Walnut seeds significantly increased their Luteinizing homone(LH) and Follicle Stimulating Hormone (FSH). This eventually enhanced the production of the reproductive hormones which enriched the fertility status of these animals.

Disclaimer:

This paper is based on preliminary dataset. Readers are requested to consider this paper as preliminary research article, as authors wanted to publish the initial data as early as possible. Authors are aware that detailed statistical analysis is required to get a scientifically established conclusion. Readers are requested to use the conclusion of this paper judiciously as statistical analysis is absent. Authors also recommend detailed statistical analysis for similar future studies.

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