A comparative Evaluation Of Selected Medicinal Plants For Possible Use As Male Anti Infertility Agents. An Animal Case Study.

Abstract: The overall purpose of this research work 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 their possible usefulness as fertility agent. The seeds of walnuts [Tetracarpidium conophorum] ,Sesame (Sesamine indicum),and Velvet bean(Mucuna pruriens) were obtained.The seeds were taken to the Department of plant Science and Biotechnology,University Of Nigeria ,Nsukka(UNN) for identification and authentification.The seeds were cut into pieces , for forty – eight hours and milled into fine powder using electric blender.Ninety sexually matured aibino rats of about twelve weeks weighing 130-180g were used for the experiment,they were in well ventilated conventional case(temperature :28-31oc),Photoperiod:12hours natural light and 12 hours dark,humidity:50-55%,where they acclimatized for two weeks with tap water and fed ad libitum before commencement of treatment.The rats were divided into eleven groups(1-11) using completely randomized design with six males 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. Rats in group 2 were administered with a drug(Ketoconazole) to induce infertility in rats and served as the control.For further evaluation for the efficacy of the seeds,the infected 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 of the treatment regime ,the rats were anaesthetized using diethyl ether.The testes and epidimides were surgically removed and weighed.Blood samles ,15ml per each rat were collected through cardiac puncture into sterile tubes for hormonal analysis.From the research ,it was shown that theconcentration of sex hormones measured in the male rats fed with medium plants (seeds) showed that 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 treatment groups 3-10(induced rats fed with 4g,8gand 12g/kg between(bw) day of the test samples). Group 11(induced rats fed with 12g/kg between day of walnut sample) also revealed a significant increase ($p< 0.05$) in the continuation of FSH in the control group 1 (normal rats) when compared with that of groups 2-11, animals fed with 4, 8 and 12g/kg between test samples. From this research therefore it was deduced that 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: medicinal plants, reproductive hormones, sperm profile, albino rats, fertility agents.

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. 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. 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. 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, sundried for 48hours and milled 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:28-31oc, 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 why 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.

Group 1: Normal rats

Group 2: Ketoconazole induced rats and commercial feed

Group 3: Induced rats fed with 4g/kg between (bw) day of the powdered test substance (velvet bean)

Group 4: Induced rats fed with 4g/kg between days of the powdered test substance (beniseed)

Group 5: Induced rats fed with 4g/kg between days of the powdered test substance (walnut)

Group 6: Induced rats fed with 8g/kg between day of the powdered test substance (Velvet bean)

Group 7: Induced rats fed with 8g/kg between day of the powdered test substance (beniseed)

Group 8: Induced rats fed with 8g/kg between day of the powdered test substance (walnut)

Group 9: Induced rats fed with 12g/kg between day of the powdered test substance (velvet bean)

Group 10: Induced rats fed with 12g/kg between day of the powdered test substance (beniseed)

Group 11: Induced rats fed with 12g/kg between day of the powdered test substance (walnut)

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 epidymides 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 epipidymides and test weight.

These epididymis 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).

(g) Determination of Testicular histology

The histology of the testes and liver of the experimental animals was determined according to the method described by Drury et al, (1967). Rats from each group were scarified and their testicles dissected out. About 4mm thick sections of each testis were fixed in 10% formal saline. The tissues were then dehydrated in ascending grades of alcohol followed by clearing in chloroform. They were thereafter embedded in paraffin, sectioned at 5 microns each using a rotary microtone. The cut section was then stained using hematoxylin and Eosin (H&E) stains, viewed under a light microscope and images captured with a motic camera.

3. RESULTS AND DISCUSSIONS

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

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 Epidiymes 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 Epididymes 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 ($p>0.05$) while mean values with different letters as superscripts are considered significant($p<0.05$).

GROUPS	Sperm Motility	Sperm Count	Sperm Viability	Sperm Head Abnormality	Sperm pH	Testes and Epididymes weight
Group 1	61.67 ± 7.64^{ab}	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^{cd}	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^{cd}	20.00 ± 5.00^{b}	44.33 ± 6.03^{abc}	63.32 ± 3.64^{ab}	7.03 ± 0.49^{a}	0.90 ± 0.17^{b}
Group 6	55.00 ± 3.23^{ab}	26.66 ± 7.63^{b}	42.00 ± 1.16^{abc}	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^{cd}	26.66 ± 2.88^{b}	43.66 ± 5.13^{abc}	63.33 ± 1.41^{ab}	7.03 ± 0.32^{a}	0.93 ± 0.12^{b}

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]

Key:

Group 1: Normal rats

Group 2: Ketoconazole induced rats and commercial feed only.

Group 3: Induced rats fed with 4g/kg bw day of the powdered test substance (Velvet bean)

Group 4: Induced rats fed with 4g/kg bw day of the powdered test substance (Beniseed)

Group 5: Induced rats fed with 4g/kg bw day of the powdered test substance (Walnut)

Group 6: Induced rats fed with 8g/kg bw day of the powered test substance (velvet bean).

Group 7: Induced rats fed with 8g/kg bw day of the powered test substance (Beniseed)

Group 8: Induced rats fed with 8g/kg bw day of the powered test substance (Walnut)

Group 9: Induced rats fed with 12g/kg bw day of the powdered test substance (Velvet bean)

Group 10: Induced rats fed with 12g/kg bw day of the powdered test substance (Beniseed)

Group 11: Induced rats fed with 12g/kg bw day of the powered test substance (walnut)

Table 2: The concentration of sex hormones measured in the male rats fed with medicinal plants (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 bw 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 bw walnut sample). Group 5 (animals fed with 4kg/g bw walnut sample) and 6 (animals fed

with 8kg/g bw 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 bw test samples) but no significant difference ($p>0.05$)was observed when compared with that of group 11 (animals fed with 12kg/g bw walnut sample).

GROUPS	Testosterone	FSH	LH	Estradiol
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}

KEY:

Group 1: Normal rats

Group 2: Ketoconazole induced rats and commercial feed only

Group 3: Induced rats fed with 4g/kg bw day of the powdered test substance (Velvet bean)

Group 4: Induced rats fed with 4g/kg bw day of the powdered test substance (Beniseed)

Group 5: Induced rats fed with 4g/kg bw day of the powdered test substance (walnut)

Group 6: Induced rats fed with 8g/kg bw day of the powdered test substance (Velvet bean)

Group 7: Induced rats fed with 8g/kg bw day of the powdered test substance (Beniseed)

Group 8: Induced rats fed with 8g/kg bw day of the powdered test substance (Walnut)

Group 9: Induced rats fed with 12g/kg bw day of the powdered test substance (Velvet bean)

Group 10: Induced rats fed with 12g/kg bw day of the powdered test substance (Beniseed)

Group 11: Induced rats fed with 12 g/kg bw day of the powdered test substance (Walnut)

4. CONCLUSION

From research results it was shown that the concentration of sex hormones measured in the male rat fed with medicinal plants(seeds) showed that the Testosterone concentration showed significant increase ($p<0.05$). It was also deduced that these medicinal plants including Walnut seeds showed significant increase in their Luteinizing homone(LH) and Follicle Stimulating Hormone (FSH) which significantly enhanced the production of the reproductive hormones which enriched the fertility status of these animals.

REFERENCES

AJaiyeoba, E. O. and Fadare,D.A.(2006) Antimicrobial potential of extracts and fractions of the African Walnut Tetracarpidium conophorium. **African Journal of Biotechnology**,5 (22):2322-2325.

Bjorndahl, L., Sodourlund, I., Kvist, U.(2003).Evaluation of the one –step eosin-nigrosin staining technique for human sperm vitality assessment.Human Reproduction.18:813-816.

Comhair , F., and Vermeulem,(1995). Human semen analysis .Human Reproductive Updates. 1(4):343 362.

Dashistani , H .I and Dayem, M .A.(2007). Hyperprolactinemia and hyper gonadotro-pins in infertile men with severe oligospermia and azospermia**. International Journal of Endocrinology**,3:3-8.

Ekaluo,U.B., Ikpeme,E.V., Udensi.O., Markson,A.A., Madunagu,B.E., Omosun,G. and Umana,E. (2010)Effect of aqueous ieaf extract of neem(Azadijirachta indica) on the hormonal milieu of male rats. **International Journal of Current Research.**

234 Ekaluo ,U.B., Udokpoh, A.E.,Udofia . U.U. Ajang , R.O.(2005).Comparative Toxicity of five Commonly
235 Used Analysis ,on Rat Sperm Count and Morphology. **Global Journal of Pure and Applied**
236 **Sciences.** 2(1):81-82.

237 Orth, A .B., Royse, D.J.,and Tien,M.(1993).Ubiquity of lignin –degrading peroxidases among various
238 wood-degrading fungi.Applied Environmental Microbiology,59 :4017-4023.

239 Vawda ,A.I. and Davies, A .G.(1986). An Investigation into the effects of Ketoconazole on testicular
240 function in Wistar rats. Acta Endocrinology. 111(2): 246 -251.

241 World Health Organization .(1992).WHO laboratory manual for the Examination of human Semen
242 and Sperm.Cervical Mucus Interaction Cambridge University Press.

243 Zwarts,L., Savage ,G.P. and Mc Neil, B.L.(1999) Fatty Acid Content of New Zealand-Grown
244 Walnuts(juglans regia L**), International Journal of Food Sciences and Nutrition**.,50: 189