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Effects of partially purified bioactive compounds in leaves extract of *Jatropha tanjorensis* on gonadal hormones of male albino rats

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Abstract: **Gonadal hormones are responsible for the control of fertility. The understanding of their regulation is vital in solving reproductive problems such as infertility. The level at which people indiscriminately use medicinal plants due to financial and cultural factors for treating illnesses such as infertility without regards for their possible adverse effects has not been fully explored. This study was therefore conducted to determine the effects of partially purified bioactive compounds in leave extract of *Jatropha tanjorensis* on the gonadal hormones of male albino rats. The study was carried out using 20 male albino rats in 3 control groups and 2 treatment groups of 4 rats each. 25 mg/Kg body weight of the isolates Phytol and Lupeol were orally administered using intubation cannula for 14 days. The rats were anaesthetized with phenobarbital; blood samples collected and analyzed for hormonal assay at different intervals. No significant changes ($p<0.05$) in the body weights or the testis. However, the epididymal sperm count and motility was found significantly different when compared tosustanaon-control rats. The bioactive compounds lupeol and phytol present in *Jatrophat anjorensis* leaves extract are antifertility agents as seen by the significant changes in the parameters studied and the semen analysis carried out.**

***Keywords: Jatropha tanjorensis*, Lupeol, Phytol and gonadal hormones.**

1. INTRODUCTION

In many contexts, the two main classes of sex steroids are androgens and estrogens, of which the most important human derivatives are testosterone and estradiol. Other contexts will include progestogens as a third class of sex steroids distinct from androgens and estrogens [4]. Progesterone is the most important and only naturally occurring human progestogen. The understanding of the regulation of pituitary-gonadal hormones responsible for fertility is the key to solving the problem of infertility. An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of many drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies. A large and increasing number of patients use medicinal herbs or seek the advice of their physician regarding their use [1]. Many of these medicinal plants are used by males and females of reproductive age and for treating reproductive problems such as infertility [2]. Many plant extracts have been tested for their fertility or antifertility effects on human and animal models, e.g. the crude extract of *Allium sativum* (Garlic) caused a decrease in serum testosterone levels with effects being evoked at a very low [3]. *Gossypol herbaceum Linn* invoked antifertility effects in rats at 30 mg/Kg body weight with lesser dose causing infertility in humans [4]. There is a high increase in the use of medicinal plants both by females and males of reproductive age for treatment of illnesses especially infertility [2]. *J. tanjoresis* is one such plant used indiscriminately by males and females of reproductive age and for treating reproductive problems. The concern is that it is being over used without regards for its possible adverse effects. Against this backdrop, it is necessary to isolate and characterize the bioactive compounds in the leaves extract of *J. tanjorensis* as well as study their effects on gonadal hormones of male albino rats.

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Figure 1: *Jatropha tanjorensis* plant

2. MATERIALS AND METHODS

Materials

Materials used include; Stat Fax 3300 chemistry analyzer**,** Precision pipette**,** Beakers, Digital balance (model: SF-400)**,** Blender, Incubator**,** Improved Neuber counting chamber**,** Binocular Microscope (Olumpus)**,** Test tubes (anticoagulant free), General laboratory glass wares**,** Test tube rack**,** Syringe, Hexane, Methanol and needle. Enzyme Immunoassay Test (FSH), Enzyme Immunoassay Test (LH), Enzyme Immunoassay Test (Testosterone), all test kits were supplied by ChemuxBioScience, Inc South San Francisco, USA and were of analytical grade

Methods

Plant sample collection

The leaves material was collected at the college of Agriculture, Yandev, an outskirt of Gboko town, Benue State, Nigeria in May, 2018. The harvested leaves were thoroughly washed with clean water and air-dried at room temperature for two days then further dried in an oven at 40°C for 24 hours at the Veterinary laboratory Department, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria.

Preparation of Extract

The preparation of the plant was done as described by [5].The crispy leaves were ground into fine powder and preserved in moisture-free, airtight laboratory containers for further use. The powered plant material (100 g) was macerated with methanol (1000 ml) in ratio of 1:10 and was agitated intermittently for 48 hours, filtered into a clean glass jar. Filtration of the mixture was done first with cheese cloth, filter paper, and then filtrates were evaporated to complete dryness using a thermostatically controlled water bath at 42 ^{0}C

Experimental animals

Twenty (20) albino rats (male only) weighing averagely 200 -250 g were bought from the animal farm of the college of health science, Benue State University, Makurdi and were acclimatized in the animal house college of veterinary medicine, University of Agriculture, Makurdi for 2 weeks while allowing them free access to standard feeds (Pfizer feed PLC, Lagos, Nigeria), and water ad libitum [6].

Animal grouping

The study was carried out using 20 male albino rats in 3 control groups and two treatment groups of 4 rats each. Picric acid was used to distinctly label each animal for easy identification. The treatment received 25 mg/kg body weight of the isolated bioactive compounds Phytol and Lupeol orally using intubation cannula for 14 days.

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Collection and preparation of sera samples

The rats were anesthetized with phenobarbital and cardiac puncture performed at different intervals to obtain blood for male fertility hormone profile. The blood samples were stored in plain tubes (i.e. without anticoagulant) for the hormonal assay.

Determination of the parameters

The sera samples were separated and then assayed for Testosterone, Follicle Stimulating Hormone and Luteinizing Hormone using enzyme-linked immune-absorbent assay (ELISA) as described by [7], [10]

Sperm Count

Sperms from the right and the left cauda epididymis were released by cutting into 10 mL of normal saline in small petri-dish and then minced by using manual glass homogenizer; it was incubated using a temperature controlled water bath at 40 ºC. Binocular Microscope Olumpus was used; the number of sperms was counted using a hemocytometer. Sperm count was expressed as a number of sperm per milliliter [9].

3. RESULTS

Table1 presents the results of the effects of isolated bioactive compounds in *J. tanjorensis* leaves extraction the levels of TST, FSH and LH of sustanon-induced rats. The results show that non treated rats which were induced with sustanon had very high levels of TST (20.88±0.04) as opposed to lupeol which had the highest lowering effect on TST (12.72±0.79), and varied with Amlodipine (13.3±0.63) by just 0.58. Phytol treated group also recorded great reduction in the level of TST with a slightly lesser effect than lupeol (13.67±0.74). Compared to the lowering activity of Amlodipine (2.87±0.17) phytol is a more efficacious. Thus lupeol is very effective in lowering TST and FSH level in albino rats but not more than the standard drug Amlodipine while phytol is very effective in lowering LH levels in albino rats and even better than the standard drug Amlodipine.

Table1: Effectson the Levels of TST, FSH and LH of Sustanon- Induced Rats Treated with Isolated Bioactive Compounds in*Jatrophajanjorensis*Leaves Extract.

G Group	FSH (ng/mL)	LH(ng/mL)	TST(ng/mL)
NORMAL CONTROL	5.96±0.81^{ab}	7.45±0.66^{ab}	0.45±0.13^{ab}
SUS CONTROL	7.52±0.02^{*b}	6.48±0.57^{*b}	20.88±0.04^{*b}
SUS + AMLODIPINE	3.40±0.99*	2.87±0.17*	13.3±0.63^{*a}
SUS + 25MG/KG PHYTOL	3.22±0.13*	2.38±0.29*	13.67±0.74^{*a}
SUS+25MG/KG LUPEOL	3.00±0.24*	3.08±0.13*	12.72±0.79^{*a}

Each value is a mean of 4 replicates determinations ± SEM, values with different superscripts across the columns are significantly different at $p<0.05$

Key: 1=Normal control 2=Sustanon control 3=Amlodipine control 4=phytol 5= lupeol

The result in table 2 showed the effects on testicular weight of the isolated bioactive compounds in leaf extract of *Jatropha tanjorensis.* The results show that there was no significant difference in all the treatment groups Phytol2.47±0.13lupeol2.20±0.0 when compared to thesustanon (1.20±0.08) and Amlodipine (1.20±0.08) controlrats respectively.

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Table 2: Effects on Testicular Weight of Sustanon-Induced Rats Treated with Crude and Isolates of *Jatropha tanjorensis* Leaves Extract.

GROUP	WEIGHT
NORMAL CONTROL	1.67 ± 0.05^{ab}
SUS CONTROL	$1.20\pm0.08^{*}$
SUS + AMLODIPINE	$1.20\pm0.08^{*}$
SUS + 25Mg/Kg PHYTOL	2.47 ± 0.13^{ab}
SUS+25Mg/Kg LUPEOL	2.20 ± 0.0^{ab}

Each value is a mean of 4 replicates determinations ± SEM, values with different superscripts across the columns are significantly different at $p<0.05$

Key: 1=Normal control 2=Sustanon control 3=Amlodipine control 4=phytol 5=lupeol

Table 3: Presents the results of the effects on the levels of spermpercent motility of rats induced with sustanon and treated with isolated bioactive compounds in leave extract of *Jatropha tanjorensis.* The results showed that the bioactive had significant difference on semen analysis after treatment for 14 days. There was a decline in the sperm concentration and motility when compared to the sustanon61.67±2.36and Amlodipine46.33±2.62control rats. Phytol had highest effect 38.32±2.36 while lupeol 41.67±6.24

Table 3: **Percentage Sperm Motility of Rats Induced with Sustanon and Treated with Isolated Bioactive Compounds in *Jatropha tanjorensis* Leaves Extract**

GROUP	***% MOTILITY***
NORMAL CONTROL	66.67 ± 2.36^{b}
SUS CONTROL	61.67 ± 2.36^{b}
SUS + AMLODIPINE	$46.33\pm2.62^{*a}$
SUS + 25Mg/Kg PHYTOL	$38.32\pm2.36^{*a}$
SUS+25Mg/Kg LUPEOL	$41.67\pm6.24^{*a}$

Each value is a mean of 4 replicates determinations ± SEM, values with different superscripts across the columns are significantly different ($p<0.05$)

Key: 1=Normal control 2=Sustanon control 3=Amlodipine control 4= phytol5= lupeol

4. DISCUSSION

The oral administration of the isolated bioactive compounds to rats using intubation cannula for 14 days resulted to a significant decrease ($p<0.05$) in the levelsof testosterone with concomitant decrease in follicle stimulating hormone and luteinizing hormone. The oral administrations of these isolated bioactive compounds (phytol and lupeol) in leaves extract of *J. tanjorensis* at 25 mg/kg body weight decreased the levels of gonadal hormones. The findings in gonadal hormones (Testosterone, Follicle stimulating hormones and Luteinizing hormones) decrease in the level recorded in the treated groups may be the likely cause of spermatogenic arrest and failure of spermatogenesis in the histology of rats' testes. The findings collaborates the ascension that LH through specific receptors controls the production and secretion of testosterone and testosterone is critical for the completion of meiosis and entry into progress through spermatogenesis in rats [10], [11]. This also agrees with the earlier works that, the crude extract of *Allium sativum* (Garlic) caused a decrease in serum testosterone levels with effects being evoked at a very low dose [12], [13].

The testosterone at pre-induction was within the range of 1-2 ng/ml as indicated in the graph, while in the post-induction, testosterone levels increased significantly to the range of 23-25.5. It was observed the testosterone levels in treated rats dropped remarkably with lupeol having greater hormone reducing effect as was with the crude extract which was about 13.5. This agrees with the findings of [14] on Caricpryl-99 seed alkaloid extract on the serum of testosterone, which showed that the extract decreased the hormone levels greatly in non-induced rats.

Follicle stimulating hormone (FSH) showed levels in all the groups at post-treatment and was within the range of (7.3-8.3 ng/ml), except the normal control group which was not induced (5.8ng/ml). At post-treatment, mean hormone values of the negative control and treated groups was observed to be within the range (2.5-3.5 ng/ml), with negative being the least reduced. While, the 325 mg/kg plant extract recorded the highest reduction. The hormones concord with the findings of [14], however, disagrees with the submission (Modares and Heidari, 2015). FSH regulates the development, growth, pubertal maturation and reproductive process of the human body. In both male and female FSH stimulates the maturation of germ cells. In male induces sertolic cell to secrete androgen-binding protein (ABP), regulated by inhibin's negative feedback mechanism on anterior pituitary. Specifically, activation of sertolic cell by FSH sustains spermatogenesis and stimulates inhibin-B secretion [16]. It is therefore suggested that the low sperm count recorded in this study may have been orchestrated through this mechanism.

Luteinizing hormone at post-treatment did not show great difference when compared with pre-induction levels .At post-treatment however; it was observed that the LH levels were greatly reduced in the treated groups, and negative control. Phytol orchestrated the highest reducing effects (2.7 ng/ml), while lupeol was the least (3.5 ng/ml). Reduced levels of the treated groups ranged between 2.7- 3.5 ng/ ml at post-treatment in the treated rats. [14] also recorded reducing effects (0.33±0.07) in the LH levels at 150 mg/kg body weight in non-induced rats, which was significantly different as compared to the control. Contrary to these findings [15] observed that Allium Sativum at 800 mg/kg body weight increased LH levels on heat stress female mice (3.2 Iμ/ml). These variations in the levels of the hormones may be as the result of varying bioactive compounds in the various plants used by the researchers. Also, sex is another determining factor in organisms' reaction to drugs or substances as in the case of [15]. LH is produced by gonadotropic cells in the anterior pituitary gland. In females, an acute rise of LH ("LH surge") triggers ovulation and development of the corpus luteum [17]. In males, LHin synergy with FSH, stimulate Leydig cell production of testosterone [18]. Suggestion is therefore made that, the reduction created by the treatment substances on the levels of these two hormones may be the reason for the reduction in the testosterone levels recorded in induced rats

The testicular weights of albino rats induced and treated with sustanon did not significantly differ at post treatment. Contrary to these findings in the plant solvent extracts and isolated bioactive compound treated groups, [19] observed a significant decrease ($P<0.001$) in the testicular weight of rats treated with Bisphenol® as compared to the control group. These findings however agree with the findings in this study with reference to amlodipine control group. Amlodipine and bisphenol are both antihypertensive drugs [19]. The findings of [20] also agree with the findings in this study that amlodipine significantly decreased ($P<0.05$) the testicular weights of albino rats. However this contradicts with the findings in this study on the extracts and isolated bioactive compounds.

The percentage motility of rats induced with sustanon, treated with crude extract and bioactive compounds showed significant difference. Phytolrecorded the highest reducing activity (38.32±2.36), followed by Lupeol (41.67±6.24). There was a significant decrease ($P<0.05$) in the levels of hormones when compared to the sustanon-induced rats (61.67±2.36) and the non-treated group. The reduction in the percentage motility of sperm and its total count may chiefly be as a result of the bioactive compounds present in the methanolic leaves extract of *J. tanjorensis* affecting the leydig cells which are responsible for production of testosterone, and testosterone is responsible for the completion of spermatogenetic process [21].

5. CONCLUSION

In the study, the bioactive compounds in leaves extract of *J. tanjorensis* at 25 mg/Kg body weight were not safe because of the reducing effects they had on the gonadal hormones. It is therefore; clear that the bioactive compounds lupeol and phytol in *J. tanjorensis* leaves extract are antifertility agents as seen by the significant changes in the parameters studied. Lupeol and phytol have shown to have reduced levels of gonadal hormones in addition to reducing the sperm concentration and motility.

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