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***Research Article***

# Acute Toxicity and Effects of Methanolic Leaves Extract of *Jatropha tanjorensis* on Gonadal Hormones of Male Albino Rats

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**Abstract**

Pituitary gonadal hormones are responsible for the control of fertility. Understanding the regulation of these hormones is vital in solving infertility problems. The levels at which humans consume these medicinal plants without regards for their possible adverse effects has not been fully explored. This study was conducted to determine the acute toxicity and effects of methanolic leaves extract of *Jatropha tanjorensis* on the gonadal hormones of male albino rats. From the study, the $LD_{50}$ of methanolic leaves extract in male albino rats was determined to be >6500 mg/kg body weight with no mortality recorded at this dose. The 650 mg/kg body weight of methanolic leaves extract was found to have a significantly ($p<0.05$) greater reducing effect on the gonadal hormones than sustanon and Amlodipine controls. No significant changes in the body weights or testis were observed. Conversely, the testosterone levels decreased significantly ($p<0.05$); similarly, a decrease was observed in FSH and LH ($p<0.05$) in treated rats. Epididymal sperm count and motility reduced significantly as compared to sustanon control. These findings reveal that the methanolic leaves extract of *Jatropha tanjorensis* are nontoxic class of substances at 650 mg/kg and is an antifertility agent.

**Keywords:** *Jatropha tanjorensis;* Medicinal plants; Acute toxicity; Gonadal hormones.

## 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 [1]. 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.

Several medicinal plants are used by males and females of reproductive age in treating illnesses and reproductive problems, such as infertility [2]. *Jatropha tanjorensis* belongs to the family Euphorbiaceae and it shows intermediacy in phenotypic characters between *J. gossypifolia* and *J. curcas* [3]. *J. tanjorensis* (Fig. 1) is known by the following common names: Yoruba, Iyana ipaja; Igbos, Ugu-Oyibo; Hausas, Bitadazugi; Tiv, Aniue chengem while it is Hospita too far in Pidgin English because of its blood boosting effects [4,5]. In Nigeria, the leaves of *J. tanjorensis* have been used locally by consuming it as vegetable and it is popular as a natural remedy against diabetes, anemia and several other uses [6].

Recent claims have it that the plant is no longer safe for use and that it could be toxic to organs in the body, although few reports on its pharmacological values and toxicological effects have been documented [7]. There is an indiscriminate use of medicinal plants due to financial and cultural factors *J. tanjoresis* is one such plant, used by males and females of reproductive age and for treating reproductive problems [2]. The concern is that it is being over used without regards for its possible adverse effects. Against this backdrop, it is necessary to study its adverse effect. Thus, the findings on methanolic leaves extract will serve as reference values for preparation of herbal drugs, bearing in mind its action on gonadal hormones and the body in general.

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Fig. 1. *Jatropha tanjorensis* plant

## 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, Methanol and needle. Enzyme Immunoassay Test (FSH), Enzyme Immunoassay Test (LH), Enzyme Immunoassay Test (Testosterone). All test kits were supplied by Chemux BioScience, Inc South San Francisco, USA and were of analytical grade

### *Plant sample collection*

The collection of the leaves was done in the month of May, 2018 from a home garden at the College of Agriculture, Yandev, Gboko-Benue State, Nigeria. The harvested leaves were identified and authenticated by Dr. Ojobo of Botany Department University of Agriculture Makurdi. Leaves were then washed and air-dried at room temperature for two days then further dried in an oven at 40°C for 24 h at the Veterinary laboratory Department, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria.

### *Preparation of extract*

The crispy leaves were ground into fine powder, 100 grams of the powder was socked in methanol in the ratio of 1:10 [8]. The mixture was agitated intermittently for 48 h. The mixture was first filtered with cheese cloth, Whatman no.1 filter paper and then evaporated to complete dryness using a thermostatically controlled water bath at 42°C using a thermostatically controlled water bath at 42°C.

### *Experimental animals*

Twenty four (24) male albino rats weighing 180-200 g were purchased from the animal farm of the College of Health Sciences, Benue State University, Makurdi and were acclimatized in the animal house College of Veterinary Medicine, University of Agriculture, Makurdi for 2 weeks. Animals were allowed free access to standard feeds (Pfizer feed PLC, Lagos, Nigeria) and water *ad libitum* [9].

### *Acute oral toxicity study*

To determine acute toxicity, the up-and-down method described by [10,11] was used. Single oral dose of the plant extract was administered to the animals. An initial dose of 2000 mg/kg body weight of animal was administered to one animal and monitored for the first four hours for any behavioral changes, toxicity and mortality. After 24 h, another animal was given 3500 mg/kg body weight and observed. 5000 mg/kg and 65000 mg/kg body weight were given to two additional animals and were observed continuously for 14 days respectively.

### *Animal grouping*

The study was carried out using 20 male albino rats in 3 control groups and 2 treatment groups of 4 rats each. Picric acid was used to distinctly label each animal for easy identification. The treatment groups received 650 mg and 325 mg/kg body weight of the crude extract of *J. tanjorensis* orally using intubation cannula for 14 days.

### *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 immunoasorbent assay (ELISA) [12-14].

### *Sperm Count*

The analysis was carried as described by [15]. Light microscope (Olumpus Binocular

 

Microscope) was used, first x10 and x40 objective lens. The number of sperms was counted using a hemacytometer and sperm count expressed as a number of sperm/mL [15].

**Results**

In table 1, the results of acute toxicity of *Jatropha tanjorensis* are presented. Acute toxicity study revealed non-toxic effect at these test doses: 2000 mg/kg, 3500 mg/kg, 5000 mg/kg, and 6500 mg/kg of the plant extract. There was no mortality, fast respiratory rate, convulsion, dullness or other clinical symptoms observed within the treatment period. Therefore the $LD_{50}$ of this plant is considered to be above 6500 mg/kg body weight.

In table 2, the results of the effects of *J. tanjorensis* methanolic leaves extract is presented. Results show that 650 mg/kg body of the plant extract decreased the levels of FSH, LH and TST (2.90±0.33, 2.47±0.19 & 12.98±0.52) more than the sustanon control (7.52±0.02, 6.48±0.57 & 20.88±1.04) and Amlodipine control (3.40± 0.99, 2.87±0.17 & 13.3±0.63) respectively. 325 mg/kg dose of the extract had varied effects in reduction of the gonadal hormones as compared to the control groups.

Table 1. Acute toxicity study of *Jatropha tanjorensis* methanolic leaves extract

| Number | Body weight | Body weight | Survival status |
|---|---|---|---|
| 1. | 92 | 2000 | 0 |
| 2. | 90 | 3500 | 0 |
| 3. | 93 | 5000 | 0 |
| 4. | 94 | 6500 | 0 |

Toxicity sign: no-toxicity sign observed for the 1st four hours to 14 days
LD50 > 6500 mg/kg body weight

Table 2. Effects on the levels of TST, FSH and LH of sustanon-induced rats treated with Crude of *Jatropha tanjorensis* leaves extract

| 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±1.04$^{*b}$ |
| SUS + AMLODIPINE | 3.40±0.99$^{*}$ | 2.87±0.17$^{*}$ | 13.3±0.63$^{*a}$ |
| SUS + 650 mg/kg C.E | 2.90±0.33$^{*}$ | 2.47±0.19$^{*}$ | 12.98±0.52$^{*a}$ |
| SUS + 325 mg/kg C.E | 2.20±0.16$^{*}$ | 2.78±0.25$^{*}$ | 15.08±0.52$^{*ab}$ |

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: SUS= Sustanon C.E= Crude Extract TST=Testosterone FSH=Follicle Stimulating Hormone LH= Luteinizing Hormone

The results of testicular weights of sustanon induced rats treated with methanolic leaves extract of *J. tanjorensis* and those of the control groups are presented in table 3. The results show that the testicular weights of the rats treated with the two doses (650 mg/kg & 325 mg/kg) did not vary greatly. Therefore, the methanolic leaves extract of *J. tanjorensis* have no adverse effects on the testicular weights of sustanon induced albino rats.

In table 4, the results of sperm motility in rats induced with sustanon and treated with methanonic leaves extract is presented. The results show that, the rats treated with 650 mg/kg had reduced motility (37.00±0.2.16), as compared to the normal control (66.67±2.36), sustanon control (61.67±2.36) and Amlodipine control (46.33±2.62) respectively. The 325 mg/kg treated rats also showed reduced motility in the sperm motility (46.67±2.36), though with lesser degree. Thus, the methanolic leaves extract of *J. tanjorensis* reduces the motility of sperm in sustanon induced albino rats.

Table 3. Effects on testicular weight of sustanon induced rats treated with methanolic leaves extract of *Jatropha tanjorensis*

| GROUP | WEIGHT |
|---|---|
| NORMAL CONTROL | 1.67±0.05$^{ab}$ |
| SUS CONTROL | 1.20±0.08$^{*}$ |
| SUS + AMLODIPINE | 1.20±0.08$^{*}$ |
| SUS + 650 mg/kg C.E | 1.50±0.08$^{b}$ |
| SUS + 325 mg/kg C.E | 1.48±0.09$^{b}$ |

 

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: SUS=Sustanon C.E=Crude Extract TST=Testosterone FSH=Follicle Stimulating Hormone LH= Luteinizing Hormone

Table 4. The percentage sperm motility of rats induced with sustanon, and treated with methanolic extracts of *Jatropha tanjorensis*

| GROUP | % MOTILITY |
|---|---|
| NORMAL CONTROL | $66.67\pm2.36^{b}$ |
| SUS CONTROL | $61.67\pm2.36^{b}$ |
| SUS + AMLODIPINE | $46.33\pm2.62^{*a}$ |
| SUS + 650 mg/kg C.E | $37.00\pm2.16^{*a}$ |
| SUS + 325 mg/kg C.E | $46.67\pm2.36^{*a}$ |

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: SUS= Sustanon C.E= Crude Extract TST=Testosterone FSH=Follicle Stimulating Hormone LH= Luteinizing Hormone

## Discussion

Acute toxicity study is determined to be > 6500 mg/kg because neither death nor any other sign of toxicity (mortality, fast respiratory rate, convulsion and dullness) was observed during the period of the experiment. Although, earlier studies have proven that $LD_{50}$ of the aqueous extract of *J. tanjorensis* is above 6500 mg/kg [16], confirming the safety of its oral administration.

The oral administration of leaves extract of *J. tanjorensis* at 650 mg/Kg and 325 mg/kg body weight decreased the levels of gonadal hormones significantly ($P<0.05$) as compared to the control groups. These findings on the gonadal hormones (Luteinizing follicle stimulating and testosterone hormone) in rats of the treatment groups may be the cause of spermatogenic arrest and failure of spermiogenesis in the histology of rat testes [17]. This also agrees with the works on crude extract of *Allium sativum* (Garlic) on testosterone levels in rats which revealed that, there was a decrease in serum testosterone levels with effects being evoked at a very low dose [18,19]. The findings in this study collaborate with the assertion that LH through specific receptors controls the production and secretion of testosterone and testosterone is critical for the completion spermatogenesis in rats [17,20]. Studies revealed that aqueous leaves extracts of *J. tanjorensis* has ameliorative effects on anemia and osmotic fragility induced by protein-energy malnutrition in male wistar rats which could be associated with the presence of flavonoids and allied substances [5]. We submit that these effects of the methanolic leaves extract of *J. tanjorensis* on gonadal hormones may be orchestrated by flavonoids or other bioactive compounds.

## Conclusions

The methanolic leaves extract of *Jatropha tanjorensis* is non-toxic at >650 mg/Kg body weight. The methanolic leaves extract of *Jatropha tanjorensis* also causes decrease in gonadal hormones, semen concentration and motility. Thus it should be used with caution. It is recommended therefore, that further studies be conducted on the methanolic leaves extract to ascertain the bioactive compounds responsible for gonadal hormones, semen concentration and motility reduction.

## Conflicts of interest

The authors declare no conflict of interest.

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