

Research Article



Effect of Ethanol Extract of *Jatropha gossypifolia* (POHL) on Reproductive Parameters in Male Wistar Rats

Oyedeji K.O.¹, Akinbiyi Promise¹, Oshatimi Abayomi²

¹Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria.

²Department of Physiology, College of Health Sciences, Ladoko Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

*Corresponding author's E-mail: sinaoyedeji@yahoo.com

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ABSTRACT

This study aims at investigating the effect of Ethanol Extract of *Jatropha gossypifolia* (EEJG) on reproductive parameters in male rats. Six hundred grams of air-dried *Jatropha gossypifolia* leaves were cold macerated in 70 % ethanol and concentrated using water-bath. Eighteen Swiss male mice (20-25 g) were used for acute toxicity study. Twenty male Wistar rats (80-120 g) were divided into control (distilled water) and EEJG-treated (62.5, 125, 250 mg/kg) groups (5 per group) for hormonal assay, andrological and histopathological studies. The animals were orally treated on daily basis for 30 days. Plasma testosterone levels were assayed using Enzyme-Linked Immuno-Sorbent Assay (ELISA) and semen analysis was done microscopically. Histology of testes was also done. Data were analyzed using descriptive statistics and ANOVA at $p=0.05$. The EEJG (62.5-250 mg/kg) significantly ($p<0.05$) decreased testosterone levels relative to control. The EEJG (62.5-250 mg/kg) significantly ($p<0.05$) decreased sperm motility and sperm count relative to their respective controls. The EEJG also caused scanty germinal epithelia population in seminiferous tubules of the testes. It can therefore be concluded that *Jatropha gossypifolia* probably have deleterious effect on reproductive functions in male rats.

Keywords: *Jatropha gossypifolia*, Testosterone, Sperm motility, Sperm count, Rats.

INTRODUCTION

Jatropha gossypifolia (Pohl) belongs to the family of Euphorbiaceae, which occur preferentially in tropical and subtropical environment¹. It is commonly called Bellyache bush in English language, "Faux manioc" in French language and "Lalapapa pupa" by the Yoruba language speaking people of Nigeria.

The plant is used medicinally as an anti-diabetic, antidiarrheal, antiophidian, healing and antipyretic agent².

Pharmacologically, it is used as an anticholinesterase³, hemostatic⁴, tocolytic⁵, immunomodulatory⁶ and contraceptive agent in female rodents⁷.

Since this plant has been reported to have contraceptive property in female rodents⁷, this study therefore aims to authenticate the veracity of this claim in male rats.

MATERIALS AND METHODS

Experimental animals

Adult male rats weighing between 80-120 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, AfeBabalola University were used. They were housed under standard laboratory conditions and had free access to feed and water; they were acclimatized for two weeks to laboratory conditions before the commencement of the experiments.

All experiments were carried out in compliance with the recommendations of AfeBabalola University Ethics Committee on guiding principles on care and use of animals.

Plant material

Fresh samples of *Jatropha gossypifolia* plants were collected from the Botanical garden of the University of Ibadan, and were authenticated in the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan where a voucher specimen (No. FHI. 110178) was deposited in their Herbarium.

Preparation of ethanol extract of *Jatropha gossypifolia* (EEJG)

Large quantity (1.5 kg) of fresh specimens of the leaves of *Jatropha gossypifolia* were washed free of debris and air-dried. The dried stems and leaves were pulverized using laboratory mortar and pestle. Weighted portion (600.0 g) of the pulverized specimen was macerated with 70 % ethanol (1:2 wt./vol.) for 72 hours at room temperature. The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25 mm). The 70 % ethanol was later evaporated using water-bath to give a percentage yield of 10.96 % of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

Acute toxicity test

The method described by⁸ was used to determine the LD₅₀, which is the index of acute toxicity. Male Swiss mice (20-25 g) were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg were administered orally, one dose for each group. The treated animals were monitored for twenty-four hours for mortality and general behavior.



From the results of the above step, seven different doses were chosen and administered orally to eight groups of animals of one mouse per group respectively. The treated animals were monitored for twenty-four hours. The LD₅₀ was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

The dosages of EEJG administered in this study were obtained from the result of the acute toxicity test.

Body weight

Body weight was monitored on weekly basis throughout the duration of the experiment for each rat.

Experimental design

Twenty male (80-120 g) rats were randomly divided into four groups, with each consisting of five animals. The four groups were subjected to the following oral treatments once a day for thirty (30) days:

Group I: received 0.5 ml/100 g of distilled water as control group

Group II: received 62.5 mg/kg of EEJG

Group III: received 125 mg/kg of EEJG

Group IV: received 250 mg/kg of EEJG

Collection of blood samples

Twenty four hours (day 31) after the last dosing of the groups, blood samples were collected from all the animals through the medial cantus for the determination of plasma testosterone levels. All the animals were later sacrificed by cervical dislocation and the testes were removed along with the epididymides for semen analysis.

Hormonal assay

Plasma samples were assayed for testosterone using the enzyme-linked immunosorbent assay (ELISA) technique using the Fortress kit.

Semen collection

The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen.

Semen analysis

Progressive sperm motility: This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27 °C) and two drops of warm 2.9 % sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using ×400 magnifications. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labeled as motile, sluggish, or immotile. The percentage of motile

sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100)⁹.

Sperm viability (Life/dead ratio): This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope using ×400 magnifications. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperms were counted and the percentage was calculated¹⁰.

Sperm morphology: This was done by adding two drops of warm Walls and Ewas stain (Eosin/Nigrosin stain can also be used) to the semen on a pre-warmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using ×400 magnifications¹⁰. Five fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the five fields; the number of abnormal spermatozoa were expressed as a percentage of the total number of spermatozoa.

Sperm count: This was done by removing the caudal epididymis from the right testis and blotted with filter paper. The caudal epididymis was immersed in 5 ml formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5 ml formol-saline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the Improved Neubauerhemocytometer under the microscope.

Testicular histology

After removing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70 % alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and were dehydrated by passing through different grades of alcohol: 70 % alcohol for 2 hours, 95 % alcohol for 2 hours, 100 % alcohol for 2 hours, 100 % alcohol for 2 hours and finally 100 % alcohol for 2 hours. The tissues were then cleared to remove the alcohol, the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten paraffin wax for 2 hours in an oven at 57 °C, thereafter the tissues were embedded. Serial sections were cut using rotary microtome at 5 microns (5 µm). The satisfactory ribbons were picked up from a water bath (50 °C - 55 °C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1 %



acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at $\times 40$, $\times 100$ and $\times 400$ magnifications.

Statistical analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparisons between the control and the treated groups were done using one-way analysis of variance (ANOVA) with Duncan’s Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

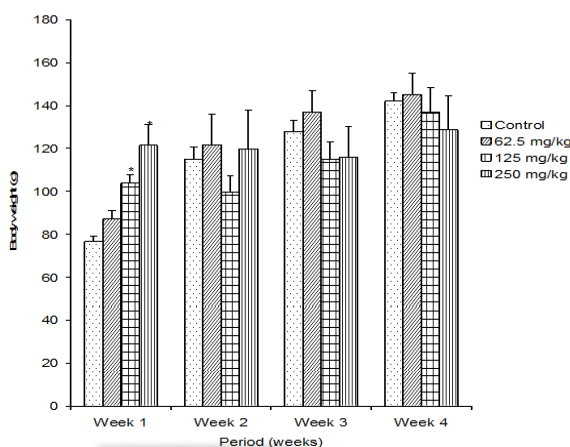
RESULTS

The LD₅₀ of the crude extract was found to be 6500 mg/kg *per os*.

The effect of 30 days treatment of rats with EEJG on body weight is shown in Figure 1. Treatment of rats with 125 mg/kg and 250 mg/kg of EEJG on the first week produced a significant ($p < 0.05$) increase in body weight relative to the control, while 62.5 mg/kg of EEJG produced no significant ($p > 0.05$) change in body weight. Treatment of rats with all the treatment doses (62.5 mg/kg, 125 mg/kg and 250 mg/kg) of EEJG produced no significant ($p > 0.05$) changes in body weights in the second, third and fourth week of treatment.

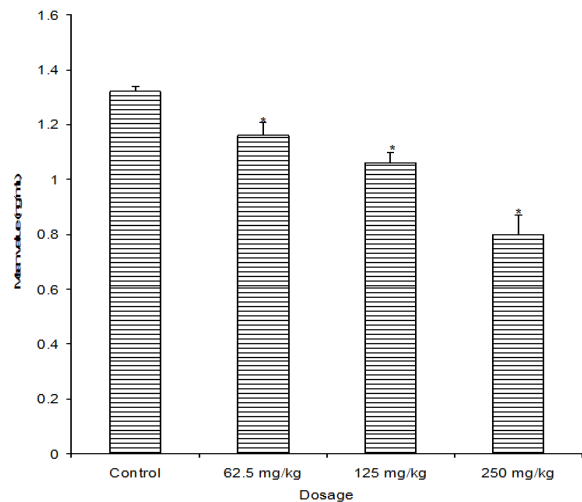
The effects of 30 days treatment of rats with EEJG (62.5 mg/kg, 125 mg/kg, 250 mg/kg) on testosterone levels is shown in Figure 2.

Treatment of rats with all the treatment doses of EEJG (62.5 mg/kg, 125 mg/kg, 250 mg/kg) produced significant ($p < 0.05$) reductions in testosterone levels relative to control.



*Significant different as compared with control group at $p < 0.05$

Figure 1: Body weight changes in control rats and rats treated with EEJG for 30 days

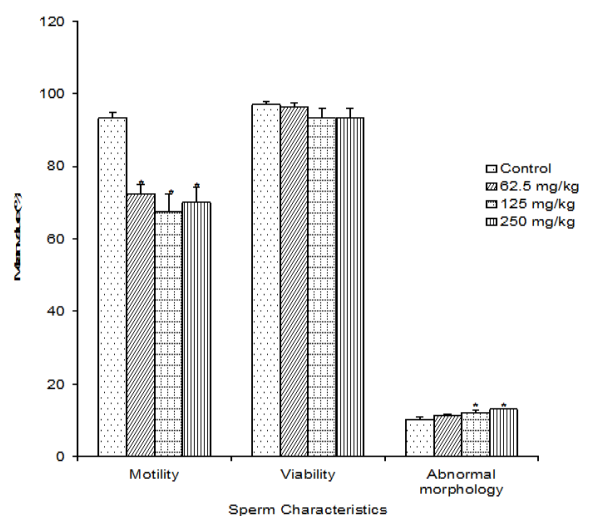


*Significant different as compared with control group at $p < 0.05$

Figure 2: Effect of treatment of rats for 30 days with EEJG on testosterone plasma level

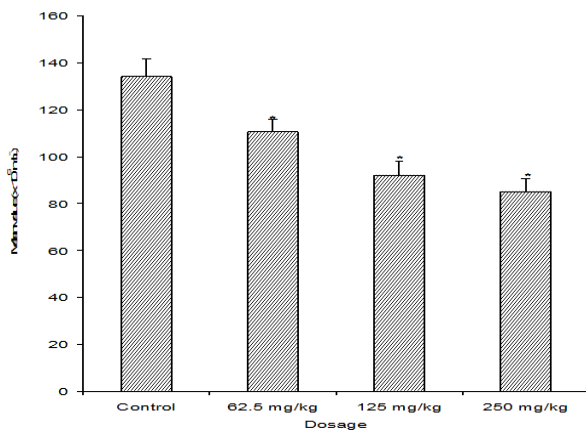
The effect of 30 days treatment of rats with EEJG (62.5 mg/kg, 125 mg/kg, 250 mg/kg) on sperm characteristics are shown in Figures 3 and 4.

Treatment of rats with all the treatment doses of EEJG (62.5 mg/kg, 125 mg/kg, 250 mg/kg) caused significant ($p < 0.05$) reductions in progressive sperm motility relative to the control. Treatment of rats with all the treatment doses of EEJG (62.5 mg/kg, 125 mg/kg, 250 mg/kg) produced no significant changes ($p > 0.05$) in the percentage of viable sperms (live/dead ratio) relative to the control. Treatment of rats with 125 mg/kg and 250 mg/kg of EEJG caused significant ($p < 0.05$) increases in the percentage of abnormal sperm cells relative to the control. Treatment of rats with all the treatment doses of EEJG (62.5 mg/kg, 125 mg/kg and 250 mg/kg) produced significant ($p < 0.05$) reductions in sperm counts relative to the control.



*Significant different as compared with control group at $p < 0.05$

Figure 3: Spermogram showing the effect of 30 days treatment of rats with EEJG on sperm characteristics



*Significant different as compared with control group at $p < 0.05$

Figure 4: Spermogram showing the effect of 30 days treatment of rats with EEJG on sperm count

Figures 5 and 6 respectively show the transverse sections through the testes of control rat and rat treated with 250 mg/kg of EEJG for 30 days.

Treatments of rats with EEJG caused scanty germinal epithelia population of the seminiferous tubules with mild to severe interstitial edema. However, the control rats presented with normal interstitial cells with normal germinal epithelia.

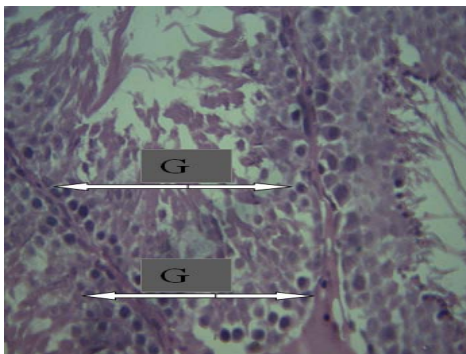


Figure 5: Effect of 0.5 ml distilled water (control) on rat testis after treatment of rat for 30 days $\times 400$.

Photomicrograph showing normal viable germinal epithelia (GE) with no visible lesion seen.

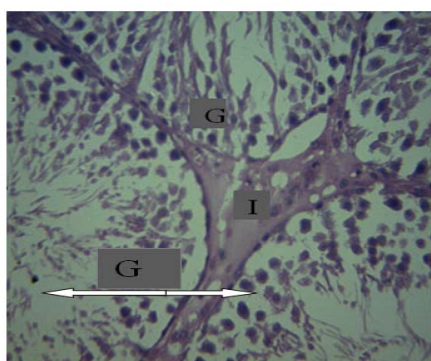


Figure 6: Effect of 250 mg/kg of EEJG on rat testis after treatment of rat for 30 days $\times 400$.

Photomicrograph showing scanty germinal epithelia (GE) with moderate interstitial edema (IO).

DISCUSSION

This plant extract exhibits low toxicity and wide safety margins which is reflected by its high LD_{50} , since it has been reported that any compound or drug with an oral LD_{50} estimate greater than 1000 mg/kg could be considered of low toxicity and safe¹¹. Similar result was reported by¹² in *Physalisalkekengi* extract treated rats.

This study has shown that the treatment of rats with the extract produced no significant changes in body weights of rats in the second, third and fourth weeks of treatment. This could be due to the absence of androgenic property in this plant, since it has been reported that androgens possess anabolic activities¹³. It could also be due to absence of anorectic and lipolytic properties in this plant¹⁴. Similar result was reported by¹⁵ in *Vernoniaamygdalina* extract treated rats.

The extract caused significant decrease in testosterone levels. This decrease in the testosterone levels could indicate that the extract inhibit the mechanism intervening in the process of hormone synthesis in the Leydig cells. Similar report was given by¹⁶ in rats treated with *Aeglemermelos* extract.

The extract caused significant decrease in sperm motility. This suggest that the extract was able to permeate the blood-testis barrier with a resultant alteration in the micro environment of the seminiferous tubules, since it has been reported that the decrease in sperm motility caused by chemical agents was due to their ability to permeate the blood-testis barrier¹⁷ and thus creating a different microenvironment in the inner part of the wall of the seminiferous tubules from the outer part¹⁸. Similar report was given by¹⁹ in rats treated with *Sarcotemmaacidum* extract.

There was an insignificant decrease in sperm viability as well as a significant increase in the percentage of morphologically abnormal sperm cells induced after treatment of rats with the extract. This could be due to the ability of the extract to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis^{20,21}. Similar result was reported by²² in isolated tetracyclic steroid treated rats.

Sperm count is considered to be an important parameter with which to access the effect of chemicals on spermatogenesis²³. Spermatogenesis is influenced by the hypothalamic adenohipophysial-Leydig cell system relating gonadotropin releasing hormone, luteinizing hormone and androgen. This implies that the decrease in sperm count caused by the extract in the treated rats might be as a result of decrease in plasma level of testosterone, because this hormone has been reported to

be important in the initiation and maintenance of spermatogenesis²⁴. Similar report was given by²⁵ in *Terminalia chebula* extract treated rats.

Photomicrographs of the extract treated rats have revealed that the extract suppressed sperm production as evidenced by the scanty germinal epithelia population. This scanty germinal epithelia population may be due to insufficient amount of testosterone, since it has been reported that spermatogenesis is activated by testosterone which is synthesized by Leydig cells and act on Sertoli cells and peritubular cells²⁶. Similar result was reported by²⁷ in *Colebrookia oppositifolia* extract treated rats.

The results also revealed that the extract caused mild to severe interstitial edema. It has been reported that there were five pathophysiological causes of edema which can be due to (i) increased hydrostatic pressure (ii) reduced oncotic pressure (iii) lymphatic obstruction (iv) sodium retention or (v) inflammation²⁸. Hence, the edema induced by this extract might be caused by any of the aforementioned causes. Similar result was reported by²⁹ in deltamethrin treated rats.

CONCLUSION

In conclusion, this study has shown that the crude extract of *Jatropha gossypifolia* has spermatotoxic or antispermatogenic effect in male rats. However, the effect of this extract on human reproductive functions are unknown. Nevertheless, considering these findings in animal model, it is recommended that moderation should be exercised in the consumption of *Jatropha gossypifolia* by those taking it for contraceptive purpose⁷.

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