International Journal of Research and Reports in Gynaecology

3(2): 21-29, 2020; Article no.IJRRGY.60393

Antifertility Propensity of *Jatropha curcas* Linn. Leaves on Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIA conceptualized and designed the study and also wrote the manuscript. Author IAA managed the analyses of the study. Authors APA and EOO managed the literature searches. Author EOA wrote the protocol while author UO performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Abdelmonem Awad M. Hegazy, Zagazig University, Egypt.
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(1) Hafiz Muhammad Arsalan, Minhaj University, Pakistan.
(2) Junali Chetia, Dibrugarh University, India.
(3) Surjyo Jyoti Biswas, Sidho-Kanho-Birsha University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60393

Original Research Article

Received 10 June 2020
Accepted 17 August 2020
Published 22 August 2020

ABSTRACT

Background: The use of *Jatropha curcas* leaves in folklore medicine has gained popularity in recent times due to its medicinal value but without regards to its adverse health effect.
Aim: This study aimed at investigating the effect of *J. curcas* leaves on the fertility of male Wistar rats.
Methodology: Healthy leaves of *J. curcas* were harvested, dried and extracted using soxhlet apparatus and ethanol as the solvent. Toxicity test was carried out using standard method. Twenty-four adult male Wistar rats were randomly divided into four groups of six each after seven days acclimatization period. Animals in group 1 were not treated, while those in groups 2, 3 and 4 were administered 100, 200 and 400 mg/kg body weight of *J. curcas* extracts respectively for twenty-eight days. Administration was done 12 hourly *via* oral route. At the end of the administration period, the rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were

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collected *via* cardiac puncture. Testes and cauda epididymis were removed and kept in sterilized watched glass. Sperm quality and concentrations reproductive hormones were determined using standard methods.

Results: Administration of *J. curcas* leaf extract for 28 days resulted in decline in the sperm count, sperm motility and seminal pH, as well as the serum levels of luteinizing hormone (LH) and testosterone. Sperm mortality and abnormality, as well as concentration of follicle stimulating hormone (FSH) were significantly ($P<0.05$) increased when animals treated with *J. curcas* leaves were compared with those in the control group.

Conclusion: It is clear from the results of this study that leaf extract of *J. curcas* reduced sperm quality and adversely affects male reproductive hormones. Thus, men interested in child-bearing should minimize its use for treatment of ailments.

Keywords: Antifertility propensity; Jatropha curcas leaves; sex hormone; sperm quality.

1. INTRODUCTION

Jatropha curcas L. (physic nut) is a species of flowering plant in the spurge family, Euphorbiaceae. It is native to the American tropics most likely Mexico and Central America [1]. It is commonly known as biodiesel fuel plant. In Nigeria, it is called 'Lapalapa' by the Yorubas, 'Cinidazugu' by the Hausas, 'Olulu-idu/uru' by the Igbos, 'Omangba' by the Iyedes in Benue State and 'Itiakpa' by the Urhobos in Delta State. It is now widely cultivated in both tropical and sub-tropical regions around the world [2, 3]. It produces flowers and fruits throughout the year. The seeds contain between 27 and 40 % oil which can be processed to produce a high-quality biodiesel fuel useable in a standard diesel engine [4].

J. curcas has been reported to have a lot of health benefits because of its wide range of medicinal uses [5]. The name *Jatropha curcas* meaning (Doctor's nutrient) was related to its numerous medicinal uses [6]. The leaves are regarded as antiparasitic, applied to scabies, rubefacient for paralysis, rheumatism and also applied to hard tumor [7]. The sap from the leaves can be used on bee or wasp sting. The leaves, when pounded can be applied on the eye of a horse to scare flies from it especially in India. The leaves contain apigenin, vitexin and ansovitexin which when combined with other factors enable them to be used against muscular pains [5]. The oil from *J. curcas* seeds is used in helping with rashes and parasitic skin diseases [8]. When the oil is mixed with benzyl benzoate, it becomes effective against scabies and dermatitis [9]. The oil from the seed can also be applied to soothe rheumatic pain. Jatropha kernel oil together with about 36% linoleic acid is a possible interest for skin care industry. The use of the oil may cause premature abortions [10]. The sap from the bark is used to dress bleeding wound and ulcer and can also be used to stop bleeding. The sap from the leaves is also used as an application for the treatment of piles. The latex is also applied topically to bee and wasp stings, boils and sores. The latex is also used to treat tooth ache, ringworm and latex is use to dress sores, ulcers and inflamed tongues [9]. Airaodion and Ogbuagu [11] have reported the abortifacient properties of *J. curcas* leaf extract in female Wistar rats. In a recent study, Airaodion and Ogbuagu [12] also demonstrated that *J. curcas* leaves possess antimalarial properties. Meanwhile, some studies have revealed that substances with antimalarial potentials have adverse effect on male fertility [13,14].

Infertility is defined as the inability to achieve pregnancy after 12 months of unprotected intercourse [15]. Male infertility is found in 50% of infertile couples [16]. According to Speroff and Fritz [17], 55% of the reasons for infertility are found to be male-related and 35% to be female-related, while 10% constitutes infertility of unknown origin [17]. Some of the etiologies of declining male fertility can be related to falling androgen levels, decreased sexual activity, alterations in sperm quality, especially, motility, morphology, and DNA integrity [18]. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus elicits the release of gonadotropins i.e. follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland [19]. LH is a glycoprotein that regulates testosterone synthesis by the extratubular Leydig cells. The other gonadotropic hormone, FSH controls spermiocytogenesis and spermiogenesis by affecting both the germinal epithelium and Sertoli cells [20]. The levels of these hormones are under negative feedback control by the gonads [21]. Testosterone is responsible for normal growth, development of

male sex organs, and maintenance of secondary sex characteristics. A high intratesticular level of Testosterone is an absolute prerequisite for sperm production, and function. Testosterone improves sperm motility and epididymis function [22]. Failure of pituitary gland to secrete FSH and LH will result in disruption of testicular function leading to infertility [23].

Semen is an organic fluid that contains spermatozoa. It is secreted by the gonads (sexual glands) and other accessory sex organs of male, and can fertilize female ova. In humans, semen contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose which is the major energy source of spermatozoa, and provide a medium through which they can move or "swim" [24]. Male infertility can be assessed through semen analysis and hormonal profile [25].

Male impotence also called erectile dysfunction (ED) is a common medical condition that affects the sexual life of millions of men worldwide [26, 27]. Erectile dysfunction is defined as the inability of a man to achieve and maintain an erection sufficient for naturally satisfactory intercourse. Sexual dysfunction is a serious medical and social symptom that occurs in 10-52% of men and 25-63% of women [28]. It is the repeated inability to achieve normal sexual intercourse male impotence (or) erectile dysfunction is a significant problem that may contribute to infertility [29]. Erectile dysfunction is adversely affected by diabetes mellitus, antihypertensive, antipsychotic, antidepressant therapeutic and antimalarial drugs [30]. Previous studies have reported that substances with antimalarial potentials have adverse effect on male fertility [13,14]. This study therefore sought to investigate the effect of *J. curcas* leaves on the fertility of male Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh and healthy leaves of *J. curcas* free from disease were harvested from Odo-Ona area of Ibadan, Nigeria and were identified by a botanist. They were washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electric blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion et al. [31, 32]. Amount of 25 g of the powder was packed into the thimble of the soxhlet extractor. 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35 ^{o}C with a yield of 2.77 g which represents a percentage yield of 11.08%. The extract was preserved in the refrigerator at 4 ^{o}C for further analysis.

2.2 Oral Acute Toxicity Studies

Oral acute toxicity study was carried out according to the method described by Airaodion et al. [33]. Twenty-five rats were divided into five groups of five rats per group. Group A was given distilled water (10 mL/kg) while groups B, C, D and E were separately given 500, 1000, 1500, and 2000 mg/kg body weight of *J. curcas* extract respectively. Treatments were administered orally by gastric intubation. The animals were observed for 24 hours post treatment for signs of toxicity and then 48 hours for possible death.

2.3 Experimental Design and Animal Treatment

Twenty-four adult male Wistar rats with body weight between 180 and 200 g were used for this study. They were acclimatized for 7 days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into four groups of six rats each. Animals in group 1 were administered distilled water while those in groups 2, 3 and 4 were administered 100, 200 and 400 mg/kg body weight of *J. curcas* extracts for twenty-eight days [31]. Administration was done 12 hourly via oral route. At the end of

the administration, the rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected via cardiac puncture.

2.4 Determination of Sperm Quality

The cauda epididymis were separated from both of the testes and tinged with 2 mL of normal saline then teased the cauda epididymis of each rat. The suspension was mixed through a metallic net to avoid any other tissue contamination. Sperms counts were done with the aid of hemocytometer according to the method of Eliasson [34]. Motility of spermatozoa was determined according to the methods of Tijee and Oentoeng [35]. Sperm abnormality was determined according to the method of Airaodion et al. [14] while seminal pH was measured using a pH meter as described by Airaodion et al. [14].

2.5 Determination of Male Reproductive Hormones

The serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were measured by using enzyme-linked immunosorbent assay (ELISA) according to the methods described in Manafa et al. [36].

2.6 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean ± standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Tukey's (HSD) post hoc multiple comparison tests. Differences between means were considered to be significant at $p<0.05$.

3. RESULTS

3.1 Acute Toxicity Studies

Ethanolic leaf extract of *J. curcas* was safe in rats at the tested oral doses (500–2000 mg/kg). There was no mortality within the study period. However, there were behavioral changes such as depression, reduced motor activity and ataxia.

3.2 Male Fertility Parameters

The effect of *J. curcas* leaf extract on sperm qualities and male reproductive hormones are presented in Tables 1 and 2 respectively.

4. DISCUSSION

Herbs have been used for centuries in the treatment of different ailments [37, 38, 39]. Some of them have unexploited effects which can result in serious unplanned consequences. These consequences could be teratogenic, and as such babies born of such pregnant mothers can be malformed with ignorance of its cause [40]. This study therefore sought to investigate the effect of ethanolic leaf extract of *J. curcas* on sperm qualities and male reproductive hormones.

Table 1. Effect of *J. curcas* leaf extract on sperm qualities of Animals after 28 days of administration

Sperm qualities	Control	100 mg/kg	200 mg/kg	400 mg/kg
Sperm Count (x10^4 mL)	276.34 ± 15.83^{b}	284.06 ± 13.92^{a}	252.73 ± 14.83^{c}	248.11 ± 13.78^{d}
Sperm Motility (%)	82.21 ± 4.32^{a}	70.22 ± 9.34^{b}	67.33 ± 5.67^{bc}	60.53 ± 7.83^{c}
Sperm Mortality (%)	17.79 ± 2.98^{c}	29.78 ± 6.36^{b}	32.67 ± 3.52^{ab}	39.47 ± 6.03^{a}
Sperm Abnormality (%)	27.67 ± 0.83^{b}	32.72 ± 2.62^{ab}	36.63 ± 1.92^{a}	39.28 ± 2.93^{a}
Sperm pH	7.59 ± 0.85^{a}	7.38 ± 1.00^{b}	7.14 ± 0.35^{c}	6.89 ± 0.73^{d}

Values are presented as Mean±SD, where n = 6. Values with different superscripts along the same row are significantly different at p<0.05

Table 2. Effect of *J. curcas* leaf extract on Male sex Hormones of Animals after 28 days of administration

Sex hormones	Control	100 mg/kg	200 mg/kg	400 mg/kg
Luteinizing Hormone (IU/L)	7.59 ± 0.84^{a}	5.84 ± 1.02^{b}	5.39 ± 0.63^{b}	5.12 ± 0.77^{b}
Follicle Stimulating Hormone (IU/L)	8.63 ± 1.11^{b}	11.08 ± 0.98^{a}	11.92 ± 1.07^{a}	12.34 ± 1.20^{a}
Testosterone (IU/L)	31.67 ± 2.22^{a}	29.63 ± 1.89^{ab}	25.19 ± 1.60^{bc}	20.03 ± 1.54^{c}

Values are presented as Mean±SD, where n = 6. Values with different superscripts along the same row are significantly different at p<0.05

The result of the acute toxicity test of this study showed that *J. curcas* leaves is not toxic to health as no mortality was recorded after 48 hours of administration. The change in behavioural conduct of animals observed might be an indication that consumption of *J. curcas* leaves in high amount could lead to agitation or depression [12].

In this study, it could be clearly demonstrated that administration of *J. curcas* leaf extract perturbed sperm qualities after 28 days of administration as presented in Table 1. Administration of 100 mg/kg was observed to significantly ($p<0.05$) increase sperm count of animals when compared with those in the control animals. This might be an indication that at this dosage, *J. curcas* leaves stimulated steroid hormone biosynthesis as well as spermatogenesis. However, increasing the dosage had adverse effect on the sperm count of animals. This was evidence when the sperm count of animals treated with 200 and 400 mg/kg body weight of *J. curcas* were compared with those in the control group as well as the group treated with 100 mg/kg body weight of *J. curcas* leaf extract. The significant ($p<0.05$) decline observed in the sperm count of animals treated with 200 and 400 mg/kg body weight of *J. curcas* leaf extract when compared with those in animals in the control group showed that at these doses, *J. curcas* leaf extract is toxic to the sperm. This might be suggestive that the extract at these doses inhibited steroid hormone biosynthesis, which led to impairment of spermatogenesis [41]. This has been reported to affect the seminal quality of animals [42].

Results of this present study indicated a significant ($p<0.05$) decrease in the sperm motility of animals treated with leaf extract of *J. curcas* when compared with those in animals in the control group after 28 days of administration. The declined sperm motility observed in this present study might be suggestive that *J. curcas* leaf extract possesses the propensity to inhibit the activity of ATPase in all tissue of the animals [43]. This causes suppression of energy metabolism. If ATPase activity is inhibited, it could suppress the motility rate of sperm, as ATP is the main energy source of sperm and it is directly related to sperm motility. The decrease observed in the sperm motility of animals in this study is dose-dependent. The result of this study is similar to that of Ubah et al. [44] who investigated the semen characteristics of Wistar rats treated with methanolic extract of *Jatropha gossypifolia*.

Administration of ethanolic extract of *J. curcas* leaves to animals for 28 days led to a dose-dependent increase in sperm abnormality when compare with animals in the control group. This elevation was significant ($p<0.05$) when animals treated with 200 and 400 mg/kg were compared with those in the control group (Table 1). This might be an indication that at these doses, extract of *J. curcas* leaves resulted in damage of Sertoli cells [14]. For normal testicular function Sertoli cells play vital role in maintaining conducive environment for spermatogenesis. Damage in Sertoli cell may affect the maturation process of spermatozoa, which might result in increased abnormality of sperms observed in this study sequel to *J. curcas* administration.

Extract of *J. curcas* leaves was observed to significantly ($p<005$) reduced the seminal pH of animals when compared with those in the control group. A decrease in pH makes the medium of seminal plasma to become acidic which in turn makes sperms highly fragile, thus leading to higher rate of mortality. There is an inverse correlation between seminal pH and sperm mortality. A decrease in seminal pH (increase in acidity) leads to an increase in sperm mortality. The significant dose-dependent increase observed in the sperm mortality of animals treated with leaf extract of *J. curcas* might be attributed to the significant dose-dependent decrease in seminal pH. Low pH of epididymal fluid of bovine has been reported to result in increased rate of mortality of spermatozoa [45].

There are several possible mechanisms for the antigonadal actions of plant extracts. They may exert a direct inhibitory action on the testis; they may affect the pituitary, causing changes in gonadotrophins concentrations and thus subsequent spermatogenic impairment; or they may change the concentration of neurotransmitter [46]. Antiandrogens can disrupt male differentiation by several mechanisms, including antagonism of receptor binding, or by inhibition of the production, transport, or metabolism of androgens [47].

The effect of leaf extract of *J. curcas* on male reproductive hormones of animals after 28 days of administration is presented in Table 2. From this result, *J. curcas* was observed to significantly ($p<0.05$) reduced serum concentrations of luteinizing hormone (LH) when compared with those in animals in the control group. This could be sequel to the disruption of spermatogenic process leading to a decrease in the sperm count as well as infertility observed in this study. This

disruption may have occurred by direct effects of the extract on cells and tissue, or it might also occur because of imbalanced hormonal levels [48]. The extract might have direct effect on testis tissue or by entering into the pituitary gland which could cause decrease in the level of LH in the serum. The major role of LH in male is to stimulate and enhance the production of testosterone by the Leydig cells [49].

Administration of ethanolic extract of *J. curcas* leaves to animals for 28 days resulted in to significant increase in the serum concentration of follicle stimulate hormone (FSH) when compared with those in animals in the control group. This might possibly be due to suppression of feedback inhibition of anterior pituitary [48]. The suppression of feedback inhibition may secondarily increase the secretion of FSH. The results observed in this study revealed that the extract could have a direct effect on the pituitary, which led to increase in circulating FSH levels in the blood. FSH has important effects on Sertoli cells. Inhibin and other factors secreted by Sertoli cells could elevate circulating FSH levels by feedback on the pituitary. This corresponds to the findings of Airaodion et al. [49] who reported a significant increase in serum FSH level when animals were treated with extract of *Garcinia kola* seed. The mechanism of action of *J. curcas* leaves on male sex hormones is unclear but might be similar to that of *Garcinia kola* seed since they are both antimalarial agents.

Subhan et al. [50] has proved that increase in FSH levels may reflect decreased testicular activity resulting in an alteration of the normal feedback mechanism between the testes and the hypothalamic pituitary axis, through an impairment of Sertoli cells, and decreased inhibin secretion. Mann et al. [51] found that tubular damage is always accompanied with an increase in serum FSH. Increase in the serum FSH level observed in this study could be an indication that administration of *J. curcas* leaves might have led to tubular damage in the animals. Yanam et al. [52] discovered in infertile males with abnormal histopathology (Sertoli cell only syndrome, hypo spermatogenesis, and spermatid arrest), the mean FSH levels were significantly elevated compared to the control group.

According to the result of this present study, ethanolic extract of *J. curcas* leaves decreased the levels of serum testosterone when compared with levels in the control group. This decline was only significant when animals treated with 200 and 400 mg/kg body weight of *J. curcas* leaves were compared with those in animals in the control group. Decreased levels of LH and the damage of Leydig cells might account for reduced testosterone production as well as decreased levels of serum testosterone released from testicles observed in this study. Decreased levels of serum testosterone can stimulate the release of gonadotropin-releasing hormone (GnRH) through a negative feedback mechanism. Decreased levels of testosterone may also lead to reduced secretion of seminal fluids from seminal vesicles [53].

Testosterone is a requirement for the differentiation of sex organs and production of sperms [54]. *J. curcas* leaf extract might have decreased serum LH and testosterone levels by increasing steroid catabolism and elimination or directly inhibits steroid hormone production, or both [55]. Maintenance of testosterone levels is very critical for spermatogenesis and fertility [56]. Thus, reduced serum testosterone levels arising from administration of *J. curcas* leaf extract might cause a reduction in spermatogenesis and fertility in animals and possibly man. Spermatogenesis in the testes is also regulated by the hypothalamic-pituitary-testicular axis. Gonadotropin (GTH) cells secrete LH and FSH in response to GnRH. GnRH release could also be regulated by testosterone through a negative feedback loop. LH stimulates testosterone production in Leydig cells and FSH stimulates androgen-binding protein (ABP) production in Sertoli cells. ABP binds to testosterone and promotes meiosis of the spermatocytes [53].

Generally, antimalarial remedies have been reported to have antifertility effects. Some of these remedies reported include *Vernonia amygdalina* leaves [13], *Carica papaya* leaves [14], *Garcinia kola* seed [49], chloroquine [57], *Azadirachta indica* [58], *Alstonia boonei* [59] and dihydroartemisinin [60]. Airaodion and Ogbuagu [12] have recently reported that ethanolic extract of *J. curcas* leaves possess antimalarial properties. Thus, there is a possible relationship between its antimalarial properties and antifertility activities.

5. CONCLUSION

It is clear from the results of this study that the leaf extract of *J. curcas* could reduce sperm quality and adversely affects male reproductive hormones. Thus, men interested in child-bearing might minimize its use for treatment of ailments.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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