EFFECT OF JATROPHA CURCAS SEED AQUEOUS EXTRACT ON QUALITY OF SEMEN AND REPRODUCTIVE HORMONES IN MALE WISTAR RATS

T.O. Ayinde[1], H.O. Abubakar[1], L.S. Ojulari[1], M.E. Balogun[2], M.T. Liadi[3], L.A. Olayaki[1], M.A. Belewu[4]

[1]Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria,[2]Department of Physiology, Faculty of Health Sciences, Al-Hikmah University, Ilorin, Nigeria,[3]Department of Animal Production, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria, [4]Department of Crop Production, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria

Abstract: Background: Jatropha curcas is gaining popularity in recent times due to its medicinal value but with less regards to its adverse health effect especially as it concerns male infertility. The current study was aimed at investigating the effect of Jatropha curcas seed extract on quality of semen and reproductive hormones in male Wistar rats. **Methodology**: Twenty-four male Wistar rats were randomly divided into four groups of six rats each. Group I served as control and was given 0.9% normal saline, while groups II, III and IV were administered 100, 200 and 400mg/kg of the extracts respectively for 60 days. The rats were then euthanised and blood samples collected via cardiac puncture for testosterone, LH and FSH assay; and semen collected for seminal fluid analysis. **Results**: There was significant ($p<0.05$) decrease in sperm count, significant ($p<0.05$) increase in total sperm abnormality, and significant ($p<0.05$) increase in sperm motility only at high dose when compared with the control. There was also significant ($p<0.05$) reduction in testosterone and FSH, while LH showed significant ($p<0.05$) increase. **Conclusion**: Jatropha curcas seed extract severed sperm quality with affectation of male reproductive hormones. Thus, male desirous of having babies should exercise caution in its patronage.

Keywords: Jatropha, Spermatozoa, Hormone, ATPase

Corresponding author: Ayinde Taofeek Olanrewaju, Department of Physiology, University of Ilorin, Ilorin, Nigeria ,E-mail: toayinde@gmail.com, Phone no: +2348036221914

Introduction

Jatropha is an ubiquitous in the spurge family, Euphorbiaceae. It contains approximately 170 species of succulent plants, shrubs and trees (some are deciduous, like Jatropha curcas). Most of these are native to the Americas, with 66 species found in the Old World.[1] The Plant produces separate male and female flowers, and like many members of the family Euphorbiaceae, Jatropha contains compounds that are highly toxic. Jatropha curcas has been reported to have a lot of health benefits because of its wide range of medicinal uses. In Nigeria, it is called 'Lapalapa' by the Yorubas, 'Cinidazugu' by the Hausas, 'Olulu-idu/uru' by the Igbos.[2] The name Jatropha curcas meaning (Doctor's nutrient) is related to its numerous medicinal uses. [3] Jatropha curcas shows a wide range of pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, analgesic and abortifacient effects. Jatropha curcas has been a widely used source of medicine for decades in many cultures.

Male infertility is found in 50% of infertile couples.[4] Reasons for infertility in couples have been found to be 55% male-related and 35% to be female related, while 10% constitutes infertility of unknown origin.[5] Some of the etiologies of declining male fertility can be related to falling androgen levels, decreased sexual activity, alterations in sperm quality, especially, concentration, motility, morphology, and DNA integrity.[6]

Semen is an organic fluid that contains spermatozoa. It is produced by the male gonads and other accessory sex organs, and can fertilize female ova. Besides spermatozoa, semen contains several other components such as proteolytic enzymes as well as fructose which is the major energy source of spermatozoa, and provide a medium through which they can move.[7] Thus, along with clinical examinations, male infertility is usually confirmed by seminal fluid analysis and hormonal profile. The aim of current study

was to determine the effect of Jatropha curcas seed extract on sperm quality and reproductive hormones in male Wistar rats.

Methodology

Preparation of extracts

One hundred grams of Jatropha curcas seed were air dried for 48hrs. It was then pulverized using a Blender/Miller III, (model MS - 223, China), Forty grams of the powder then extracted in 1 L of distilled water for 72hours at room temperature with constant shaking on a shaker (Stuart Scientific Orbital Shaker, UK). The extract was then filtered with Whatman No. 1 filter paper. The resulting filtrate was concentrated on steam bath to give 22.47 g of the residue. Calculated amounts of the residue 100mg/kg, 200mg/kg and 400mg/kg were then reconstituted in 100 ml of normal saline for the administration regimen.

Animal grouping

Twenty-four (24) female rats weighing 180–200g were purchased from Ladoke Akintola University of Technology, Ogbomoso. The animals were acclamatised for two weeks prior to commencement of the study. They were housed and maintained in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. They were kept in standard plastic cages containing wood chips (sawdust) bedding, with good ventilation, free access to standard rat pellet feeds and water *ad libitum*. The animals were subjected to artificial day and night cycle of 12 hours by 12 hours light, under optimum temperature of 25-30^{o}C. The experimental protocols which involved invasive and non-invasive procedures were approved by the Animal Research Ethical Committee of University of Ilorin, and were conducted in accordance with internationally accepted principle for laboratory animal use and care.

Experimental procedure

They were randomly divided into four groups of 6 rats each. Group I served as control and was given 0.5ml/kg 0.9% NaCl. Groups II, III, and IV were administered 100mg/kg, 200mg/kg and 400mg/kg body weight of Jatropha curcas seed aqeous extract respectively daily for 60 days through oral cannula. At the end of the administration, the rats were then sacrificed under ketamine anaesthesia. An abdominal midline incision was made and extended to the chest to expose the heart. The apex of the heart was punctured with a needle and syringe. Blood sample was collected and poured into appropriately labeled heparinized sample bottle.. The samples were centrifuged at 3000 rpm for 15 minutes to separate the plasma, which was then separated and stored appropriately till required for analysis of testosterone, FSH and LH. The quantitative determination of total testosterone concentration in plasma was done using Microplate Enzyme- linked Immunoassay (EIA) using Monobind assay kit (Lake Forest, USA) according to the manufacturer's instructions. Semen sample was obtained from the caudal region of the epididymis of each rat by carefully milking down the vas deferens. The sample was milked directly into a petrish dish already filled with diluent prepared from non-fatty milk powder (11%, w/v) and distilled water heated to 95°C for 10 min. After cooling to room temperature, penicillin (64.2mg) and streptomycin (100mg) were added at 37°C. Following this, the semen and diluent were gently mixed together with the tip of a pipette (sucking and release) being careful to avoid trauma to the spermatozoa. About 15µL of the diluted semen was then pipetted on to a glass microscope slide and cover slip placed on top. It was then observed under light microscope at ×40 magnification. Sperm quality was determined through assessment of the following parameters: sperm concentration, motility, and morphology.[8,9]

Sperm concentration was analyzed using the haemocytometer method.[10] The diluted semen sample was put into the counting chamber, and the number of spermatozoa was counted using a haemocytometer with improved doubles Neubauer ruling under a light microscope. The number of spermatozoa in five squares was

counted. The mean was multiplied by 10^6 in order to obtain the sperm count. The sperm concentration was expressed as $\times 10^6$ ml^{-1}.

Sperm motility was analyzed and averaged by counting the motile and non-motile spermatozoa under a light microscope and expressed as the percent motility.[10] The motility was determined by eye-estimation of the proportion of spermatozoa moving progressively straight forward at higher magnification (x40) and expressed as percentage. With this assessment, the spermatozoa were classified into three categories according to their motility: progressive, in situ and immobile. The spermatozoa with progressive motility are those with lineal forward movements; in situ motility refers to those with circular or local movements, and immobile sperm are spermatozoa without movements.[11] The motility was observed using the ×40 microscope objective lens. The number obtained in each category was expressed as a percentage.

Sperm total abnormality was assessed by adding two drops of warm eosin–nigrosin stain[10] to the semen (10µl) on a pre-warmed slide. A uniform smear was then made and air-dried. The stained slide was immediately examined under an oil immersion lens. For each rat, 200 spermatozoa were examined randomly for abnormalities in the head and/or the tail in different fields, and the percentage of total abnormalities was determined.[12]

Table 1: Treatment administered to rats in the control and experimental groups

Groups of rats	Normal Saline solution	Jatropha (mg/kg)
I	0.9%	0
II	0	100
III	0	200
IV	0	400

Statistical analysis

Statistical analysis was done using statistical package for social sciences (SPSS). Differences in means was obtained using ANOVA test. All values reported in the study were expressed as MEAN ± SEM. Differences in mean was taken to be significant at $P<0.05$.

ResultsTable 2: Effect of J. curcas seed aqueous extract on sperm qualities and reproductive hormones in male Wistar rats

parameter	Group I (Control) 0.9% NaCl	Group II (Low dose) 100mg/kg	Group III (Moderate dose) 200mg/kg	Group IV (High dose) 400mg/kg
Sperm count ($x10^6$)/ml	81.34 ±0.71	53.78 ±0.89*	44.32 ±0.31*	41.58±0.53*
Sperm Motility %	65.07±5.1	63.15±7.1	67.09±3.5	74.03±2.6*
Total abnormality %	28.20±0.27	46.04±0.19*	51.16±0.28*	58.24±0.11*
Testosterone mmol/L	4.07±0.14	1.53±0.05*	3.09±0.19*	3.21±0.12*

LH (mIU/ml	36.28±0.10	29.19±0.34*	24.46±0.18*	21.03±0.54*
FSH (mIU/ml	1.98±0.16	1.29±0.64*	0.97±0.11*	0.42±0.20*

There was significant ($p<0.05$) reduction in sperm count in all the groups, while there was significant ($p<0.05$) increase in total sperm abnormality in all the the groups compared with control. There was significant ($p<0.05$) difference in sperm motility of high dose administration, but there was no significant ($p>0.05$) difference in sperm motility of low and moderate doses. There was significant ($p<0.05$) reduction in plasma levels of testosterone and FSH in all the groups, while the plasma levels of LH showed significant ($p<0.05$) increase in all the groups compared with control.

Discussion

In this study, it was demonstrated that administration of J. curcas seed caused reduction in total sperm count in all the groups compared with control. This is in tandem with another study on leaf extract of Jatropha tanjorensis which also demonstrated decrease sperm count.[13] The pattern of reduction was seemingly dose dependent with severe affectation of high dose J. curcas administration. The sperm motility at high dose was however increased remarkably while the low and moderate doses are favourably comparable with the control. This is in contrary to the work of Ubah et al. in 2016 that concluded on a total reduction in sperm motility, though Jatropha gossypifolia was used and not Jatropha curcas.[14] The increased motility observed in high dose administration could have been possibly due to increase in energy storage of sperm cells of rats in this group. And that J. curcas leaf extract possesses the propensity to stimulate the activity of ATPase in all sperm cells. This causes elevation of energy metabolism. If ATPase activity is stimulated, it could increase the motility rate of sperm, as ATP is the main energy source of sperm and it is directly related to sperm motility. However, the particular motility involved was not observed in this study, but likely to be far from being progressive judging from outcome of other semen parameters explored in the study. The study also shows increase in total sperm abnormality in all the groups when compared with control. The highest dose of Jatropha administration demonstrated the most severe structural abnormality while the low dose structural defect was mild, yet remarkable. The study also revealed a global reduction in plasma testosterone compared with control. This reduction is also seemingly dose dependent, with the lowest dose of Jatropha seed extract having the lowest testosterone levels. In the same vein, the plasma FSH level was also generally reduced compared with control. The reduction seen here was also dose dependent, however, the highest dose of Jatropha administration witnessed most severe reduction. In contrary, the plasma level of LH revealed an overall increase that was also dose dependent with the lowest dose of Jatropha administration having the highest increase of LH when compared with the control. Although, decrease in plasma levels of testosterone may not always translate to deficiency at the level of seminiferous tubules, but the observation of the current study shows that the diminished plasma level of testosterone was a clear demonstration of absolute depletion at the seminiferous tubules considering the general reduction observed. The low level seen could have been responsible for the increase LH levels seen in this study perhaps via negative feedback in the hypothalamo-pituitary-testicular axis when compared with the control. It is also of interest to note that higher administration of Jatropha precipitated lowest plasma level of LH and highest levels of testosterone in this study. And because testosterone is required for

spermatogenesis, this low level could have been responsible for the low sperm count revealed in this study, which is also seemingly dose dependent. In a similar manner, the global plasma FSH level is consistent with the low sperm count seen in this study. This possibly could have been due to alteration of spermatogenic activity of seminiferous tubules which are the primary testicular structures involved in sperm production. The sperm cells also suffered some levels of abnormalities from the administration of Jatropha especially the highest dose. This could be closely related to the phorbol ester constituent in Jatropha seed which had been established in several studies to be very toxic[15] due to its ability to induce mobilization of several cytokines including IL-1, IL-2 and IL-6 which could have caused affectation of Sertoli cells which are required for maintaining conducive environment for spermatogenesis.[16,17] In a reversed manner, this is in concordance with studies that have revealed that usually, lower numbers of abnormally shaped sperm are associated with better quality of sperm count of the semen.[18] The study also indicates that reduction in plasma testosterone does not always translate to absolute poor quality of all parameters of semen as erroneously upheld, especially by some drug prescribers who overzealously administer testosterone to men inspite of good sperm motility. Thus prospectively, the inclusion of extracts of this seeds having impacted negatively on sperm quality and plasma reproductive hormones could be explored as male contraceptive in another broader study. This invariably may be a replacer, being cost effective and non-invasive compared to the popular vasectomy.

Conclusion

The results of this study demonstrated that Jatropha curcas seed exract could decrease sperm quality and affects male reproductive hormones. Thus, male individuals desireous of babies should be very cautious while consuming it as nutritional delicacy. However, further study is ongoing to explore the efficacy of Jatropha curcas seed extract as male contraceptive.

No conflict of interest

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