

Assessing the Dose-Dependent Impact of Chaya Leaf (*Jatropha Tanjorensis*) Aqueous Extract on the Reproductive Health of Adult Male Wistar Rats

¹Dim Catherine Nicholate; ²Nwankwo Azubike A; ³Ezeokafor Emmanuel Nonso; ⁴Enendu Angela Chika; ⁵Nsofor Cordelia Uche; ⁶Nwankwo Samuel Ikwunne; ⁷Afubero Francis Chukwudi; ⁸Anyiam Kennedy Ekenedirichukwu*

¹Faculty of Basic Medical Sciences, Department of Human Physiology, Chukwuemeka Odumegwu Ojukwu University, Uli Campus

²Faculty of Basic Medical Sciences, Department of Human Physiology, College of Medicine and Health Science, Abia State University, Uturu..

³Faculty of Basic Medical Sciences, Department of Human Physiology, Nnamdi Azikiwe University, Nnewi Campus

⁴Faculty of Basic Medical Sciences, Department of Human Physiology, Nnamdi Azikiwe University, Nnewi Campus

⁵Faculty of Basic Clinical Medicine, Department of Pharmacology and Therapeutics, Nnamdi Azikiwe University

⁶Department of zoology, Faculty of Biociences, Nnamdi Azikiwe University, Awka

⁷Dept.of Human Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus

⁸Faculty of Basic Medical Sciences, Department of Human Anatomy, Chukwuemeka Odumegwu Ojukwu University, Uli Campus

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ABSTRACT

Jatropha tanjorensis is known for its several applications both in traditional medicine and as edible vegetable in local delicacies. It is popularly employed in Nigeria for the treatment of anemia, diabetes and Malaria. This case-control aimed to evaluate the effect of aqueous extract of dose-dependent *Jatropha tanjorensis* (chaya) leaf the reproductive parameters of male wistar rats. Total of forty n=40 male wistar rats weighing 130-150g (about 12 weeks old) were grouped into five groups of n=8 rats each, (group A to E), Group A served as negative control (just water and feed only),while Group B(Low dose of *Jatropha tanjorensis*,200mg/kg),Group C (Medium dose of *Jatropha tanjorensis* ,400mg/kg), Group D, (High dose of *Jatropha tanjorensis*, 600mg/kg), Group E (Positive control ,just folic acid drugs and feed only).Results on the relative testicular weight showed non-significant increase in groups B and C ($P > 0.05$) while groups D and E had a significant decrease ($P < 0.05$) when compared to group A. The results of sperm motility and total sperm count showed a statistically significant increase ($P < 0.05$) in active sperm cells in groups B, C, D, and E respectively when compared to A. Also, comparison made to group E showed an increase in groups B and D ($P < 0.05$) while group C had a decrease but not significant ($P > 0.05$) in group D while groups B and C were not significant. ($P > 0.05$). The result of sperm morphology showed a significant increase in the mean percentage of the normal sperm cells in groups B, C, D, and E ($P < 0.05$) compared to A. But comparison made to group E showed no significant difference in groups B, C, and D ($P > 0.05$) respectively. The abnormal sperm cell result showed a significant increase in groups B, C, D, and E ($P < 0.05$) respectively compared to A. The result of testosterone showed an

increase in the mean testosterone level in groups B, C, D, and E. Compared to placebo groups, A group showed significance difference ($P < 0.05$) in groups D and E while groups B and C had no significance differences ($P > 0.05$). The FSH result test showed a decrease in groups B, C, D, and E respectively and when compared to group A group B had no significance differences ($P > 0.05$) whereas groups C, D, and E had significance differences ($P < 0.05$) respectively

Keywords: Dose-dependent. *Jatropha tanjorensis*, Morphology, Sperm cells

BACKGROUND

Extraction and development of various medications and chemotherapeutics from medicinal plants as well as from traditionally used herbal treatments have indicated a rising dependence on the usage of medicinal plants in the industrialized nations. Many patients utilize therapeutic plants or consult their doctor about their usage; this is a growing count (Iwalewa et al., 2005).

According to reports, plant-based medicine either totally or partially relies on roughly seventy percent of the human population. With around 80% of the world's population depending on this plant-based traditional medicinal system because of its availability and affordable supply, it is indispensable in health care (Owolabi et al., 2007). Despite access to modern medication, many people in Nigeria and other underdeveloped nations of the world still mostly rely on herbal treatments to cure different ailments. Men and women of reproductive age utilize some of these medicinal herbs to treat reproductive disorders including infertility (Tor-Anyiin et al., 2016).

One such plant both men and women of childbearing age utilize for treating reproductive issues including infertility is *Jatropha tanjorensis*. *Jatropha tanjorensis* is a perennial herb that belongs to the family Euphorbiaceae whose common name includes: catholic vegetables, *Jatropha*, 'Hospital too far', lapalapa and Iyana ipaja in Yoruba language and has been shown to have anti-oxidant potentials as well as other health benefits (Tiwari et al., 2011). There is limited literature on the effect of *Jatropha tanjorensis* on reproductive health. This study was carried out to evaluate the effect of aqueous extract of dose-dependent *Jatropha tanjorensis* (chaya) leaf the reproductive parameters of male wistar rats.

METHODOLOGY

Ethical approval

Ethical approval was obtained from the animal ethics committee, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Abia State, Uturu.

Materials

1. 40 male Wistar rats.
2. Latex medical hand gloves
3. Standard cages
4. Vital feed rat chow (Finisher Mash for Rats, Chikun Feed, Ibadan, Oyo State, Nigeria)
5. Animal weighing balance
6. Measuring cylinder (Pyrex)
7. Paper tape and markers
8. Spectrophotometer (Shanghai Yoke Instrument Co., Ltd. China)

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9. Oral cannula
 10. Cotton wool
 11. Methylated spirit
 12. Needle and Syringes (2ml, 5ml)
 13. Spectrophotometer
 14. Normal saline
 15. Penand Paper
 16. *Jatropha tanjorensis* Leaf
 17. Gavage and syringe
 18. Ethylenediamine tetra acetic acid (EDTA) bottles
 19. Electric blender (SharkNinja, BL660, China)
 20. Drugs (Diazepam)

Plant Collection

Samples *Jatropha tanjorensis* (fresh leaves HTF) were purchased from Afor market in Uli Anambra State. The botanical identification reference with voucher number of COOU/BS/HERB/22/014 and authentication was confirmed in the herbarium of Department of Botany Chukwuemeka Odumegwu Ojukwu University, Uli Campus, and Anambra State.

Extraction of Phytochemicals

The leaves were washed thoroughly and aired-dried at room temperature. The dried leaves were ground into powdered form using electric blender. Extraction of leaf was done by cold maceration according to the method described by Hossain et al. (2013) and Evbuomwan et al. (2015). About 500 g of ground leaves were soaked into 4000 mL of ethanol and the solution was macerated for about 24 hrs with gentle shaking at 360 rpm until the soluble matter had properly dissolved. After extraction, the solution was clarified by filtration under vacuum using Whatman filter paper and the ethanol solvent evaporated completely using a rotary evaporator. The solvent free ethanol crude extract was suspended in diethyl ether to purify the extract after which it was exposed to the atmosphere for a while to ensure elimination of the solvent odour. The extracts obtained were then weighed and refrigerated till used

Experimental Design

40 male Wistar rats were randomly grouped into five groups of 8rats each

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| Group A: | Negative control (no extract, just water and feed). |
| Group B: | Low dose of <i>Jatropha tanjorensis</i> (200mg/kg) |
| Group C: | Medium dose of <i>Jatropha tanjorensis</i> (400mg/kg) |
| Group D: | High dose of <i>Jatropha tanjorensis</i> (600mg/kg) |
| Group E: | Positive control (no extract, just folic acid drugs and feed) |

All experimental protocols were observed under strict supervision, the experiment lasted for 6-weeks, and administration is done through oral gavage.

Statistical Analysis

Research objectives and hypothesis of the study was considered before analyzing data. The data generated in this study were expressed in mean **Mean** $\pm$ **SEM** where applicable. The results were statistically analyzed using the SPSS version 23 software. Means and standard errors of mean will be calculated. Statistical differences between the experimental and control groups were determined using ANOVA and values will be considered significant at $p \leq 0.05$.

Presentation of Data

Table 3.1: Effect of aqueous extract of *Jatropha tanjorensis* leaves on relative testicular weight

Groups	Relative testicular weight (g)
	Mean $\pm$ SEM
Group A (control)	0.84 $\pm$ 0.03
Group B (200 mg/kg of AJTL)	0.87 $\pm$ 0.03 ^{b*}
Group C (400 mg/kg of AJTL)	0.93 $\pm$ 0.03 ^{b,*}
Group D (600 mg/kg of AJTL)	0.94 $\pm$ 0.03 ^{a,#}
Group E (5 mg/kg of folic acid)	0.58 $\pm$ 0.92 ^a
p-value	0.041
F-ratio	13.711

Table 3.2: Effect of aqueous extract of *Jatropha tanjorensis* leaves on sperm motility and total sperm count

Groups	Active sperm cells (%)	Non-motile sperm cells (%)	Total sperm count (%)
	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
Group A (control)	77.33 $\pm$ 1.33	22.66 $\pm$ 22.66	533.66 $\pm$ 0.33
Group B (200 mg/kg of AJTL)	86.33 $\pm$ 0.33 ^{a,#}	13.66 $\pm$ 3.33 ^{a,#}	630.33 $\pm$ 0.88 ^{a,#}
Group C (400 mg/kg of AJTL)	90.00 $\pm$ 0.00 ^{a,#}	10.00 $\pm$ 10.00 ^{a,#}	641.33 $\pm$ 0.33 ^{a,*}
Group D (600 mg/kg of AJTL)	93.33 $\pm$ 0.33 ^{a,*}	6.66 $\pm$ 3.33 ^{a,*}	704.00 $\pm$ 0.57 ^{a,#}
Group E (5 mg/kg of folic acid)	88.33 $\pm$ 1.66 ^a	11.66 $\pm$ 1.66 ^a	775.00 $\pm$ 36.85 ^a
p-value	0.021	0.021	0.005
F-ratio	37.872	37.872	29.695

Table 3.3: Effect of aqueous extract of *Jatropha tanjorensis* leaves on sperm morphology

Groups	Normal sperm cells (%)	Abnormal sperm cells (%)
	Mean $\pm$ SEM	Mean $\pm$ SEM
Group A (control)	75.00 $\pm$ 2.88	25.00 $\pm$ 2.88
Group B (200 mg/kg of AJTL)	82.00 $\pm$ 0.55 ^{a,#}	18.00 $\pm$ 0.57 ^{a,#}
Group C (400 mg/kg of AJTL)	86.66 $\pm$ 1.66 ^{a,#}	13.33 $\pm$ 1.66 ^{a,#}
Group D (600 mg/kg of AJTL)	91.33 $\pm$ 2.18 ^{a,#}	8.67 $\pm$ 2.18 ^{a,#}
Group E (5 mg/kg of folic acid)	85.00 $\pm$ 2.88 ^a	15.00 $\pm$ 2.88 ^a
p-value	0.002	0.002
F-ratio	7.477	7.477

Table 3.4: Effect of aqueous extract of *Jatropha tanjorensis* leaves on testosterone and FSH level

Groups	Testosterone (ng/ml)	FSH (mIU/ml)
	Mean $\pm$ SEM	Mean $\pm$ SEM
Group A (control)	0.31 $\pm$ 0.01	6.64 $\pm$ 1.03
Group B (200 mg/kg of AJTL)	0.51 $\pm$ 0.01 ^{b*}	5.01 $\pm$ 0.24 ^{b,*}
Group C (400 mg/kg of AJTL)	0.84 $\pm$ 0.01 ^{b,*}	4.26 $\pm$ 0.16 ^{a,*}
Group D (600 mg/kg of AJTL)	1.04 $\pm$ 0.14 ^{a,*}	2.67 $\pm$ 0.00 ^{a,#}
Group E (5 mg/kg of folic acid)	1.19 $\pm$ 0.58 ^a	3.74 $\pm$ 0.49 ^a
p-value	0.025	0.012
F-ratio	33.600	49.329

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p < 0.05$. SEM: Standard error of mean, significant (^a) and not significant (^b) when compared to A; *: significant and #: not significant when compared to E, AJTL: aqueous extract of *Jatropha tanjorensis* leaves.

Table 3.1 result showed a non-significant increase in the relative testicular weight in groups B and C ($p=0.154$, $p=0.619$) while groups D and E ($p=0.006$, $p=0.001$) had a significant decrease when compared to group A. However, comparison made to group E showed a significant increase in the relative testicular weight in groups B and C ($p=0.001$, $p=0.001$), and group D ($p=0.324$) had a non-significant increase.

Table 3.2 result showed a statistically significant increase in active sperm cells in groups B, C, D, and E ($p=0.001$, $p=0.010$, $p=0.000$) when compared to A. Also, comparison made to group E showed an increase in groups C and D ($p=0.256$, $p=0.005$) while group B had a decrease ($p=0.179$) but indicate significance in group D while groups B and C had no significance. The non-motile sperm cell indicated a statistically significant decrease in groups B, C, D, and E ($p=0.001$, $p=0.010$, $p=0.000$) when compared to A. Also, comparison made to group E showed a decrease in groups C and D ($p=0.256$, $p=0.005$) while group B had an increase ($p=0.179$) but indicate significance in group D while groups B and C had no significance. The total sperm count result showed a statistically significant increase in groups B, C, D, and E ($p=0.001$, $p=0.002$, $p=0.000$, $p=0.000$) compared to group A. Also, comparison made to group E revealed a significant decrease in groups B, C, and D ($p=0.000$, $p=0.000$, $p=0.012$).

Table 3.3 result a significant increase in the mean percentage of the normal sperm cells in groups B, C, D, and E ($p=0.050$, $p=0.004$, $p=0.000$, $p=0.010$) compared to A. Also, comparison made to group E had showed no significant difference in groups B, C, and D ($p=0.361$, $p=0.606$, $p=0.071$) with groups C and D had an increase and group B showed a decrease. The abnormal sperm cell result showed a significant increase in groups B, C, D, and E ($p=0.050$, $p=0.004$, $p=0.000$, $p=0.010$) compared to A. However, comparison made to group E showed no significant difference in groups B, C, and D ($p=0.361$, $p=0.606$, $p=0.071$) with groups C and D had a decrease and group B showed had an increase.

Table 3.4 result showed an increase in the mean testosterone level in groups B, C, D, and E ($p=0.599$, $p=0.194$, $p=0.001$, $p=0.000$) when compared to group A; but showed significance in groups D and E while groups B and C had no significance. However, comparison to group E showed a significant decrease in groups B and C while group D had a significant increase. The FSH result showed a decrease in groups B, C, D, and E ($p=0.088$, $p=0.001$, $p=0.002$, $p=0.000$) when compared to group A, but group B had no significance and groups C, D, and E had significance. Further, comparison to group E had showed a non-statistically significant decrease in-group D ($p=0.148$), while groups B and C ($p=0.000$, $p=0.009$) had a statistically significant increase.

Spermatogenesis is a key factor in the male reproductive system, and it involves several physiologically complex processes that involve multiplication of spermatogonia, meiosis of spermatocytes, and differentiation

of spermatids into the male gamete, which is capable of motility and fertilizing an egg (Bergmann, 2006). The study findings of the aqueous extract of *J. tanjorensis* leaves on relative testicular weight reported a significant decrease at 600 mg/kg of the extract but showed no significant difference at low and moderate doses of *J. tanjorensis*. The physiology linked to the decrease in testicular weight could result from apoptosis or necrosis of testicular tissue.

The study reported that administration of an aqueous extract of *Jatropha tanjorensis* leaves resulted in a significant increase in sperm motility, as indicated in treatment groups B, C, and D. The physiology linked to the significant increase in sperm motility following *Jatropha tanjorensis* leaf extract in the experimental rats is linked to the presence of flavonoids, which help promote the process of spermatogenesis acting through the Akt pathway. The study by Yousef *et al.* (2006) revealed a significant increase in sperm motility following *Jatropha tanjorensis* leaves against chromium VI induced testicular damage in rats, and they agree with the study findings.

The study also revealed that administration of an aqueous extract of *Jatropha tanjorensis* leaves demonstrated significantly higher normal sperm cells and lower abnormal sperm cells, as indicated in groups B, C, and D. The physiology behind the significance is the presence of bromelain, a high class of flavonoids that help boost the spermatogenesis process. The study agrees with the report of Yousef *et al.* (2006) but contradicts the findings of Akighir *et al.* (2020).

The study revealed that an aqueous extract of *Jatropha tanjorensis* leaves showed significantly higher levels of testosterone in groups B, C, and D compared to the control group. The physiology linked to the significantly higher levels following *Jatropha tanjorensis* leaf extract is bromelain, a flavonoid, which has the potency to initiate regulation of gonadotrophs in the hypothalamic axis.

The study findings showed that administration of the aqueous extract of *Jatropha tanjorensis* leaves in experimental rats revealed a significant decrease in FSH levels compared to the control. The physiology linked to the significant decline in the FSH level is the presence of phytol and lupetol, which resulted in the inhibition of anterior pituitary hormone synthesis. Akighir *et al.* (2020) and Ayinde *et al.* (2022) reported a significant decline in the FSH level following the extract of *Jatropha tanjorensis* leaves, which agrees with the study findings.

Conclusion

The study revealed improved sperm parameters, as shown by increased levels of active sperm motility, total sperm count, sperm morphology, and hormonal levels (testosterone and FSH), aqueous leaf extract of *J. tanjorensis* be used to improve male fertility, following the active phytoconstituents it possesses. Also, folic acid treatment could serve as a good supplement in sperm quality, and hormonal activities (testosterone and FSH), which can serve as an alternative in people with male infertility issues.

Recommendation

Future research should focus on exploring the underlying mechanisms of *J. tanjorensis* potential reproductive effects against other forms of toxicity, using more sensitive biomarkers and advanced laboratory procedures to detect possible changes as well as dose-response relationships.

Contribution to Knowledge

The study revealed that the aqueous leaf extract of *J. tanjorensis* had impaired liver function, reduced immune function, and could help boost hematological indices in anaemia, as indicated by a higher levels in hematinic parameters. Also, the study showed that *J. tanjorensis* leaf extract has great potential in treating male infertility of any aetiology since it has high flavonoids. However, it was shown to improve sperm quality and hormonal levels. However, folic acid administration could be used as an alternative to manage haematological indices, renal function, and male reproductive function. Thus, its consumption should be at a minimal level to avoid hepatic dysfunction, as indicated in this study.

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