

## Article

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## Effects of *Jatropha curcas* seed extract on reproductive functions in female Wistar rats

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### Abstract

**Background:** *Jatropha curcas* is a multipurpose plant with several industrial and medicinal importance. Its seeds contain many active compounds that are used as medicines with anti-inflammatory, and antioxidant activity. **Objective:** The objective of this study is to evaluate the effects of extract of *Jatropha curcas* seed which is patronized locally in some communities as agent of preventing childbirth on reproductive functions in female Wistar rats. **Methods:** Twenty-four animals were randomly divided into four groups of 6 rats each. Group I was administered normal saline and served as control. Groups II, III and IV were given the seed extract orally at doses of 100mg/kg, 200mg/kg and 400mg/kg body weight respectively daily for 14days orally. The animals were euthanised and blood samples collected for oestrogen, progesterone, FSH and LH assay. 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  $\pm$  SEM. Differences in mean was taken to be significant at  $P < 0.05$ . **Results:** There was significant reduction ( $p < 0.05$ ) in levels of progesterone, LH and FSH in moderate and high doses of the extract. But estrogen level was significantly ( $p < 0.05$ ) decreased in both low and moderate doses, while the high dose showed significant ( $p < 0.05$ ) increase. **Conclusion:** There was alteration in reproductive hormones due to arrest of development of ovarian follicles. This gives it the propensity of exhibiting contraceptive activity.

**Keywords:** Contraceptive, Hypothalamus, Hormones, Jatropha, Ovary, Pituitary

## Introduction

**J** *atropha curcas* (*J. curcas*) belongs to the Euphorbiaceae family and grows in tropical and sub-tropical regions like South America, Africa, India and South East Asia.<sup>1</sup> It is a multipurpose plant with several industrial and medicinal values. It was reported that flavonoid, phenolics and saponins in the extract of root of *J. curcas* plant showed antioxidant, and anti-inflammatory activities. In the same vein, the extract of *J. curcas* seed has been observed to have a high phenolic component which exhibits strong bioactivity.<sup>2-3</sup> Its benefits also extend to being a source of natural antioxidants that are used in industries.<sup>4-5</sup>

The seed extract of *J. curcas* has also been shown to have significant antioxidant activity with potent activity for protecting against oxidant and free radical injuries.<sup>6</sup> The anti-inflammatory activity of ethyl acetate, methanolic and aqueous extracts of *J. curcas* leave, stem and root has been demonstrated earlier by several researchers.<sup>7-10</sup> In a similar study, the anti-inflammatory effects of *J. curcas* extracts of leaf, stem bark, and root are attributed to their strong

iNOS inhibition.<sup>3</sup> The work of Goonasekera et al., reported antifertility activity of methanolic extract of *J. curcas* fruits administration to pregnant rats.<sup>11</sup> The fruit extracts caused foetal resorption by interrupting pregnancy occurrence at an early stage after implantation. In the same vein, Makonnen et al. also observed anti-implantation and antifertility effects of crude seed extract of *J. curcas* when administered orally to female albino pigs.<sup>12</sup> Similarly, Odusote et al. reported inhibition of pup birth by *J. curcas* oil at a dose of 2 ml/kg body weight.<sup>13</sup>

Reproduction is a biological phenomenon that requires the coordination of peripheral organs with the nervous system to ensure that the internal and external environments are optimal for successful procreation of the species.<sup>14</sup> This is accomplished by the hypothalamic-pituitary-gonadal axis that coordinates reproductive functions. The primary signal regulating female reproductive cycle from the central nervous system is gonadotropin-releasing hormone (GnRH), which modulates the activity of anterior pituitary gonadotrophs regulating follicle stimulating hormone (FSH) and luteinizing hormone (LH) release.<sup>15-17</sup> As ovarian follicles

develop, they release oestradiol, which negatively regulates further release of GnRH and FSH. As oestradiol concentrations peak, they trigger the surge release of GnRH, which leads to LH release inducing ovulation. Release of GnRH within the central nervous system helps modulate reproductive behaviors providing a node at which control of reproduction is regulated.<sup>15</sup>

The hypothalamus, through its secretion of GnRH, drives anterior pituitary production of LH and FSH, which act on the ovaries to promote follicle development, ovulation, formation of the corpus luteum, and secretion of sex steroid hormones (oestrogen and progesterone). The two sex steroid hormones possess well-established actions on the female reproductive system. Both oestrogen and progesterone signaling pathways have been implicated in feedback control of gonadotropins and regulation of the female reproductive cycle.<sup>18-20</sup> GnRH is the key regulator of the reproductive system. Its pulsatile secretion determines the pattern of secretion of the gonadotropins which then regulates both the endocrine function and gamete maturation in the gonads.<sup>21</sup> The objective of this study is to evaluate the effects of extract of *Jatropha curcas* seed which is patronized locally in some communities as agent of preventing childbirth on reproductive functions in female Wistar rats.

## Methods

### *Preparation of extracts*

One hundred grams of *Jatropha curcas* seed were air dried for 48 hrs. 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 72 hours 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.

### *Confirmation of estrus*

This was done by aspirating secretion from the lumen of the vagina with a plastic pipette following introduction of 10 mL of normal saline (NaCl 0.9%). The vaginal fluid was then placed on glass slides. One drop was collected with a clean tip from each rat. The unstained vaginal smear was carefully observed under a light microscope under x40 objective lens. Three types of cells were recognized: irregular, nucleated ones were epithelial cells; irregular ones without nucleus were the cornified cells; and the little round ones were the leukocytes. The proportion among them was used for the determination of the phases of estrus cycle.<sup>22</sup> Clinically, there were manifestations of some sexual behaviours such as lordosis posture, orientation of anogenital, rapid rigorous alternate shaking of the head and ears.

### *Animal grouping*

Twenty-four (24) sexually exposed female rats of proven fertility weighing 180–200g were purchased from Ladoke Akintola University of Technology, Ogbomoso. 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°C. The animals were

acclimatized for two weeks prior to commencement of the study. 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 aqueous extract respectively daily for 14 days through oral cannula. At the end of the administration, the rats were then sacrificed under ketamine anaesthesia and blood samples were collected via cardiac puncture for estrogen, progesterone, LH and FSH assay.

#### 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  $\pm$  SEM. Differences in mean was taken to be significant at  $P < 0.05$ .

#### Results

Reproductive hormone levels in plasma in different groups was shown in Table I. There was significant ( $p < 0.05$ ) decrease in the levels of estrogen in the low and moderate doses, but a significant ( $p < 0.05$ ) increase was seen in the high dose group compared to control group. There was no significant ( $p > 0.05$ ) change in the levels of progesterone in the low dose group, but there was significant ( $p < 0.05$ ) decrease in the levels of progesterone in the moderate and high dose administration groups compared to control. There was no significant change ( $p > 0.05$ ) in the levels of LH in the low dose group, but there was significant ( $p < 0.05$ ) decrease in the moderate and high dose groups compared to control. In contrary to the results above, there was global significant ( $p < 0.05$ ) decrease in the levels of FSH in all the groups compared to the control.

**Table I:** Plasma levels of reproductive hormones in all the groups (N=24)

Parameters	Group I (n=6) (Control) 0.9% NaCl	Group II (n=6) (Low dose) 100mg/kg	Group III (n=6) (Moderate dose) 200mg/kg	Group IV (n=6) (High dose) 400mg/kg
Oestrogen (pg/ml)	131.03 $\pm$ 4.85	38.02 $\pm$ 2.53*	45.20 $\pm$ 4.58*	158.10 $\pm$ 7.06*
Progesterone (ng/ml)	41.26 $\pm$ 3.07	44.08 $\pm$ 1.06	2.50 $\pm$ 0.09*	1.10 $\pm$ 0.16*
LH (miu/mL)	19.60 $\pm$ 0.33	16.42 $\pm$ 0.43	11.02 $\pm$ 0.53*	10.80 $\pm$ 1.59*
FSH (miu/mL)	16.28 $\pm$ 0.60	12.18 $\pm$ 0.64*	12.22 $\pm$ 0.91*	9.70 $\pm$ 0.61*

Data expressed as mean $\pm$ SEM. Statistical analysis was done by ANOVA. LH: Luteinizing hormone FSH: Follicle Stimulating Hormone. \* $P < 0.05$  compared to the control. N=Total number of rats. n=number of rats in each group.

## Discussion

Various orthodox intervention measures have been employed in women for birth control through alterations of female reproductive hormones involved in hypothalamo-pituitary-ovarian axis. Due to inadequate information, the current study explored *Jatropha curcas* seeds being consumed as local contraceptive. The study shows that administration of low and moderate doses was able to cause reduction in estrogen levels, while high dose administration revealed estrogen elevation. This could have been possibly due to the significant reduction noticed in the FSH level which consequently precipitated significant increase in the estrogen level via negative feedback loop in the hypothalamo-pituitary-gonadal axis. In spite of the increase titre of estrogen, yet there was reduction in gonadotropins as seen in table I, which could have been from reduction in gonadotropin releasing hormone (GnRH) though not assayed in the current study. On the other hand, administration of moderate and high doses caused reduction in progesterone levels, while there was no remarkable alteration in the progesterone levels of rat administered low dose. Similarly, administration of moderate and high doses also caused reduction in the levels of LH, while low dose administration did not cause any alteration in LH. It was also evident from the study that all the doses of *Jatropha curcas* seeds administered revealed a global reduction in the levels of FSH in all the groups. The reduction observed in the levels of gonadotropins in this study is in tandem with the work of Akighir et al. in 2020 on *Jatropha Tanjorensis* leaves in which there was general reduction in FSH and LH, though in male Wistar rats study.<sup>23</sup> The study found generally that the administration of moderate and high doses that revealed reduction in the assayed reproductive hormonal profiles of the rats in all the groups except the estrogen level of the high dose could have been due to a modulator role

of the seeds causing negative feedback effect on the hypothalamo-pituitary-ovarian axis. In contrary, the increased estrogen levels seen in the group administered high dose perhaps, was due to hyperstimulation of the ovarian tissues which is a reversal effect of the low and moderate doses. From another point of view, the administration of moderate and high doses caused reduction in the levels of all the reproductive hormones investigated except the level of estrogen in the high dose *Jatropha* administration. This indicates that moderate dose of *Jatropha* was able to cause reduction in estrogen and progesterone levels while high dose *Jatropha* also caused reduction in progesterone but in contrary caused elevation in the plasma level of estrogen. The reductions observed in the primary sex hormones (estrogen and progesterone) could have also been responsible for the reduction seen in FSH and LH by a negative feedback loop on the pituitary or through a direct physiological effect of the moderate and high doses of *Jatropha* on the pituitary, thus causing less secretion of FSH and LH from the pituitary gland. Invariably, the two pathways above will progressively lead to arrest of follicular development. The overall appearance of the efficacy of these pathways can be said to be similar to mechanism of action of combined oral contraceptives which are agents that suppress ovulation by inhibition of GnRH from the hypothalamus, and inhibition of FSH and LH. The phytochemical components of *Jatropha* especially flavonoids could be said to have anti-estrogenic property if used at low and moderate doses which was responsible for precipitating the general reduction (except at high dose) observed in the estrogen levels, and by extension all other female reproductive hormones. This is similar to the research of Yakubu et al. in 2008 where Johnston leave extract in female Wistar rats demonstrated reduction in all reproductive hormones.<sup>24</sup>

## Conclusion

*Jatropha curcas* seed is a multipurpose plant with medicinal values associated with propensity in modulating female reproductive hormones which leads to arrest of development of ovarian follicles through its effects on the pituitary, ovaries and possibly the hypothalamus.

**Conflict of Interest:** The authors have no conflict of interest to disclose.

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