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Full length Research Article

Consumption of Aqueous Leaf Extract of *Jatropha tanjorensis* Improves Fertility Potential and Gestational Outcome in Virgin Female Wistar Rats

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Summary: In spite of the overwhelming patronage of the plant *Jatropha tanjorensis* (J.T) popularly called "*Hospital too far*" by pregnant and women of reproductive age for its supposed reproductive benefits, the scientific evidence are hardly known. This study thus sought to examine the effect of consumption of aqueous leaf of J.T extract on female fertility potential and gestational outcome using forty (40) virgin female Wistar rats weighing 120-150g. The extract LD50 was >5000 mg/kg. The female rats were divided into unmated and mated groups (n=10 rats per subgroups). Group A (unmated group) consisted of control which received 0.2mL of normal saline (vehicle) and J.T-treated which received 500mg/kg (orally) once daily for 28 days. For Group B (mated group), the treated dams received 500 mg/kg (orally) once daily prior to mating (2 weeks), during mating (1 week) and throughout gestation, while the control received 0.5ml of physiological saline orally during the same period. All animals had free access to food and water. For the unmated group, after 28 days of treatment samples were collected for hormonal assay. J.T increased follicle stimulating hormone (FSH) and estrogen. For Group B, the extract was assessed for it reproductive and gestational performance, as well as pregnancy outcome. Nine (9) J.T-treated rats against seven (7) of control got pregnant. J.T increased mean weight gain, food and water intake. The Birth weight, body length and tail length of pups whose mothers were treated with the aqueous leaf extract of J.T increased significantly. Intake of J.T was effective in boosting female reproductive health, pregnancy health and outcome.

Keywords*: Virgin coconut oil; sodium benzoate; acetylcholinesterase; catalase; superoxide dismutase; oxidative stress*

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INTRODUCTION

Female fertility is largely used to specify the definite reproductive act of females. Its uneasiness or dysfunction brings about undesirable effects on mammalian yield (Tamboura *et al*., 2006).

In the past few decades the rate of infertility has increased tremendously (Oehninger, 2001; Venkatesh, 2009). Cinar *et al*., (2015) reported a decline in fertilization and implantation rate, while Ngoula *et al*., (2007) reported a fall in procreative performance. Environmental toxicant, lifestyle (Mateo, 2009), stress, ageing and drugs usage (Hafez and Hafez, 2005) are a major risk factor considering hormonal disruption (Sarkar *et al*., 2003) associated with decrease fertility (Ait *et al*., 2009).

Pregnancy has been described as a stress test for life (Williams, 2003). It involves diverse physiological changes in mammals. These changes and fear of losing the pregnancy leads to expectant mothers opting for other forms of medication. John and Shantakumari, (2015) reported that such mothers choice of care is to seek herbal medicines. Additionally, growth and development of the newborn is significantly determined during the pregnancy period which is an essential phase. Nutrition is really significant during this period as it supports the fetus growth and development. Expectant mothers especially in rural areas believes that medicinal plants are rich in nutritional values and are therefore used as an intervention to better their health as well as that of the unborn fetus, as well as reduce birth effects.

The plant Jatropha tanjorensis (J.T) popularly known as 'Hospital too far', (Oyewole *et al*., 2012) have been found valuable in treatment of various ailments such as lowering blood cholesterol level and treatment of cardiovascular diseases due to hyperlipidemia (Oyewole and Akingbala, 2011). The leaves of J.T was reported rich in antioxidant properties (Atansuyi *et al*., 2012). Ehimwenma and Osagie, (2007) observed that J.T leaf contains varying bioactive compounds as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins.

There is no scientific prove on the effects of J.T in female reproductive health. Though, the plant has been used to treat female infertility and to ease delivery (Nindaratnasari, 2017). This study therefore aimed at examining the effects of aqueous leaf of J.T extract on reproductive performance and pregnancy assessment of female rats.

MATERIALS AND METHODS

Extract Preparation: The leaves of Jatropha tanjorensis (J.T) was procured from Marian market at Calabar in Cross River State, Nigeria. Plants preparation was by the method of (Agarwal *et al*., 2007). The leaves were carefully washed

with fresh water and air-dried at room temperature for two (2) days, before further drying in an oven (40°C) for 24 hours. The leaves crisp was then grounded into powdered form and conserved in moisture-free, airtight laboratory containers until usage. The powdered form of the plant (100 g) was softened using water (1000 ml) in ratio of 1:10 and agitation was done occasionally for 48 hours before filtering into a clean glass jar. Mixture filtration was done by using filter cloth, filter paper, and thermostatically controlled water bath at 42 0C.

Determination of LD50 and extract dosage: The LD50 was done according to Lorke, (1983) method whereby 12 female albino wistar rats which comprised of two phases where used and observed for any adverse effect at 24 hours.

Experimental animals: Fifty (50) albino rats (40 virgin females and 10 males) weighing averagely 120 -150g were purchased from the animal farm, University Calabar, Nigeria and was permitted one week acclimatization in the animal house, having access to fed and water. They were housed in cages which floor was covered with wood dust (bedding material), with room highly ventilated in the animal house in Physiology Department, University of Calabar, and allowed fed and water ad libitum.

Experimental design: After acclimatization, the rats were divided into unmated and mated groups (n=10 rats per subgroups). Group A was the unmated, it consisted of control which received 0.2mL of normal saline (vehicle) and treated which received 500mg/kg of Jatropha tanjorensis once daily. Group B constituted the mated group, it consisted of control which received 0.2mL of normal saline (vehicle) and treated which received 500mg/kg of Jatropha tanjorensis. For the unmated group (Group A) treatment was for 28 days before sacrifice, and its serum collected for examination of hormonal profile. For the mated group (Group B) oral dosage was carried out once daily for two weeks (14 days) before cohabitation. This also help ruled out pre-existing pregnancy. The virgin female rats were after then cohabitated with male rats (untreated) of the same strain in a ratio (1:2) for one week. The female rats were treated once daily during this period. The existence of virginal plug was deemed gestation Day 0, and all female rats with virginal plug were housed singly in metabolic cages and oral treatment continued throughout gestation. After two weeks of observation, the number of non-pregnant rats was recorded and excluded from the study.

Collection of blood samples and Serum Preparation: Rats in the unmated group after 28 days of treatment were euthanized using 60 mg kg-1 of ketamine-hydrochloride and blood samples collected via cardiac puncture and emptied into plain sample containers and kept to stand for 2 hours, thereafter a bucket centrifuge (B-Bran Scientific and Instrument Company, England) was used as centrifuge at 1,000 rpm for 5 minutes. After centrifugation the serum samples were stored (-20 °C) until each hormones were assayed.

Hormonal assay: Estrogen, progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in serum were determined using ELISA kits following manufacturer's protocol.

Assessment reproductive parameters: The assessed reproductive parameters include:
(1) Females cohabited defined as number of female rats housed with male rats
(2) Mated females: numbers of cohabited with appearance of vaginal plug
(3) Pregnant females defined as number of female rats with appearance of vaginal plug that become pregnant
(4) Not pregnant females defined as number of female rats with appearance of vaginal plug that was not pregnant
(5) Mated index: number of female mated/number cohabited x 100
(6) Fertility index: number of cohabited that become pregnant/number cohabited x 100
(7) Fecundity index: number of females cohabited that become pregnant/number of mated female rats x 100
(8) Gestational index: number of rats that delivered live rats/number of pregnancy rats x 100.

Gestational assessment
Food intake: the inseminated females were fed with 40g of animal feed daily in metabolic cages. The amount of food consumed was determined by subtracting the grams of uneaten food after 24-hours period from the grams of food placed in the cage.

Water intake: the inseminated females were fed with 50ml of distilled water daily in metabolic cages. The volume of water consumed was determined by subtracting the volume of un-drank water after 24-hours period from the volume of water placed in the cage.

Weight gain; pregnant rats were weighed daily from Day 0 to Day 21 with a weighing balance.

Assessment of pregnancy outcome
All pregnant rats delivered and at birth, recorded were:
(1) Gestation days defined as length of pregnancy
Live birth: total number of pups in the litter alive at parturition
Litter size: total number of dead/alive pups in the litter
Pup birth weight defined as body weight of pups at post-natal day (PND) 0
Pup's body and tail length defined as body length and tail length of litters; were measured at PND 0. Each litter was placed on a flat transparent plastic cover on a table, the litter was allowed time to be stable. Markers (black and blue) were used to make dots at the beginning and end of each segment, and then a ruler was used to measure the dotted lines.

Statistical Analysis
Results obtained were presented as mean ± standard error of mean. The statistical investigation was done using independent T-test and chi-square test. A difference between means was deemed significant at $p<0.05$. The software used include SPSS version 20 and graph pad prism version 8.

RESULTS

Acute toxicity study: Table 1 showed that aqueous extract of *J.T* caused no death or adverse effects following observation during and beyond 24 h even at the highest dose of 5000 mg/kg.

Table I:
Acute toxicity study on aqueous extract of *Jatropha tanjorensis*

Dose (mg/kg bwt)	No. of rats	No. of deaths	Survival	Mortality ratio
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
1600	1	0	1	0/1
2900	1	0	1	0/1
5000	1	0	1	0/1

Mortality ratio: No. of death divided by No. of survival.
Oral LD_{50}>5000mg/kg body weight

Serum luteinizing hormone (LH): The mean LH concentration (MIU/ml) for control and treated dams of unmated groups are 10.75 ± 0.18 and 10.26 ± 0.94 respectively. The mean LH concentration differ not significantly in treated versus control (Figure 1*I*).

Serum follicle stimulating hormone (FSH): The mean FSH concentration (MIU/ml) for control and treated dams of unmated groups are 6.23 ± 0.52 and 7.94 ± 0.21 respectively. The mean FSH concentration was significantly (P<0.001) higher in the treated group compared to control (Figure 1*II*).

Serum estrogen: Mean estrogen concentration (pg/ml) for control and treated dams of unmated groups are 24.67 ± 1.10 and 29.50 ± 1.07 respectively. The mean estrogen concentration was significantly (P<0.01) higher in the treated group compared to control (Figure 1*III*).

Serum progesterone: Mean progesterone concentration (ng/ml) for control and treated dams of unmated group are 10.75 ± 0.18 and 10.26 ± 0.94 respectively. Did not differ significantly among groups (Figure 1*IV*).

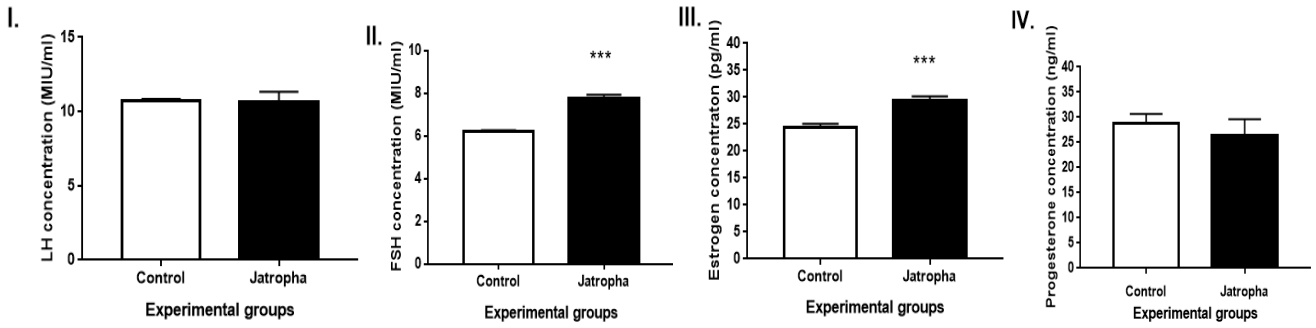

Figure 1:
Comparison of the concentration of (I) Luteinizing hormone, (II) Follicle stimulating hormone, (III) Estrogen and (IV) Progesterone between experimental groups. Values are mean ± SEM, n = 10. ***p < 0.001 versus control.

Reproductive performance for mated dams: Table 2 shows the summary of reproductive performance for rats treated with *Jatropha tanjorensis* when compared with control. There was positive variation in the reproductive performance indices of the *J.T* treated group compared to control.

Table 2:
Reproductive performance for mated dams

Indices	Control	*Jatropha*
Female (n)	10	10
Females cohabited	10	10
Mated females	10	10
Pregnant females	7	9
Not pregnant females	3	1
Mated index (%)	100%	100%
Fertility index (%)	70%	90%
Fecundity index (%)	70%	90%
Gestation index (%)	100%	100%

Daily food intake: The daily food intake (g) of the dams for twenty-one (21) days during gestation are shown in Figure 2I. Daily food intake on day 1 of the treated group significantly (P<0.01) reduced compared to control, while days 2, 3, 4, 5, 6 and 16 differed not significantly. Daily food intake on day 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19 and 20 (P<0.05), as well as day 21 (P<0.01) improved significantly in treated group when compared to control.

Food intake per trimester: Food intakes in the first trimester (Day 1-7) of gestation are: 15.94 ± 0.35 and 15.44 ± 0.57 (g); second trimester (Day 8-15) are: 20.78 ± 1.00 and 28.30 ± 0.79 (g); and third trimester (Day 16-21) were: 21.30 ± 1.11 and 29.38 ± 0.82 (g). Food intake differed not significantly in first trimester, while in second and third trimester, it significantly (P<0.001) increased in treated when compared to control (Figure 2II).

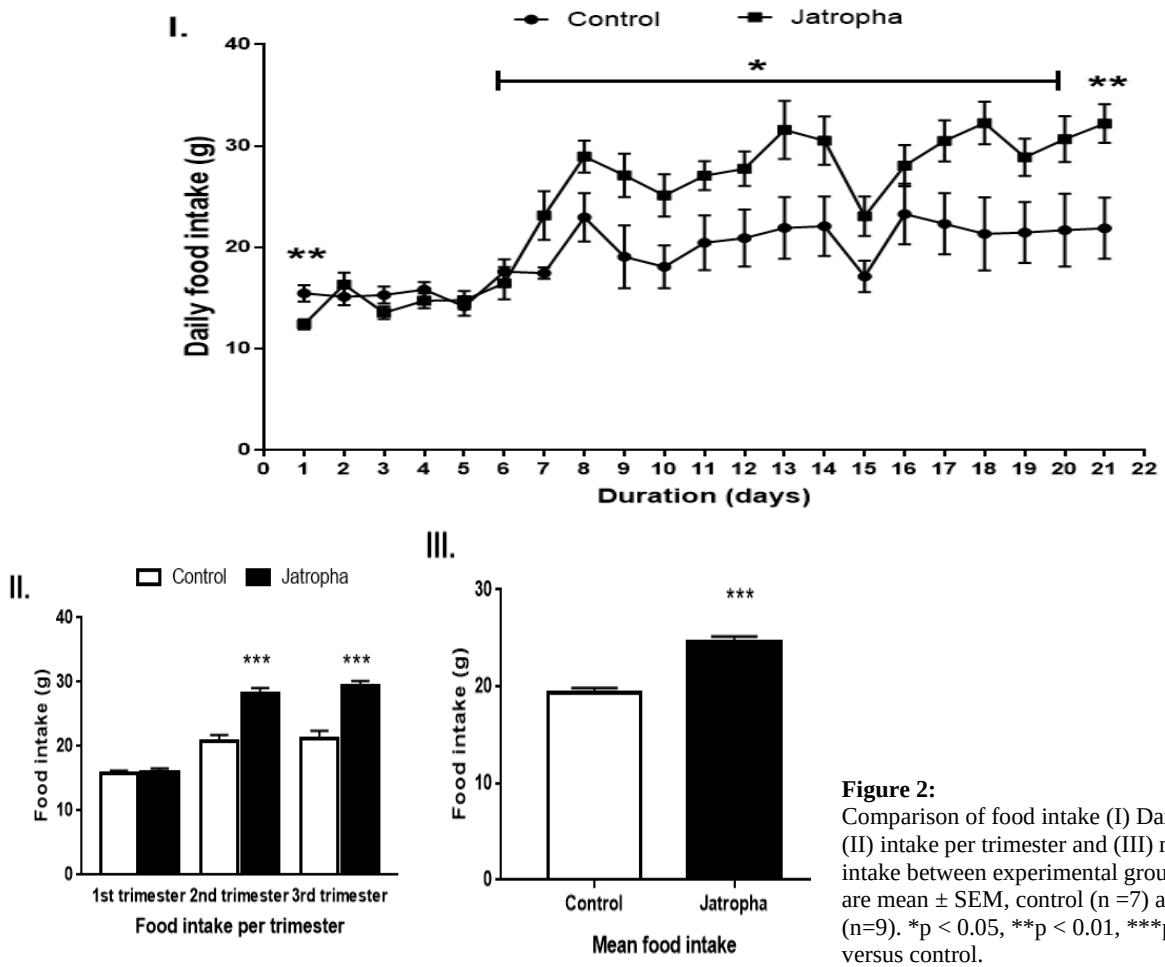

Figure 2:
Comparison of food intake (I) Daily intake, (II) intake per trimester and (III) mean food intake between experimental groups. Values are mean ± SEM, control (n =7) and treated (n=9). *p < 0.05, **p < 0.01, ***p < 0.001 versus control.

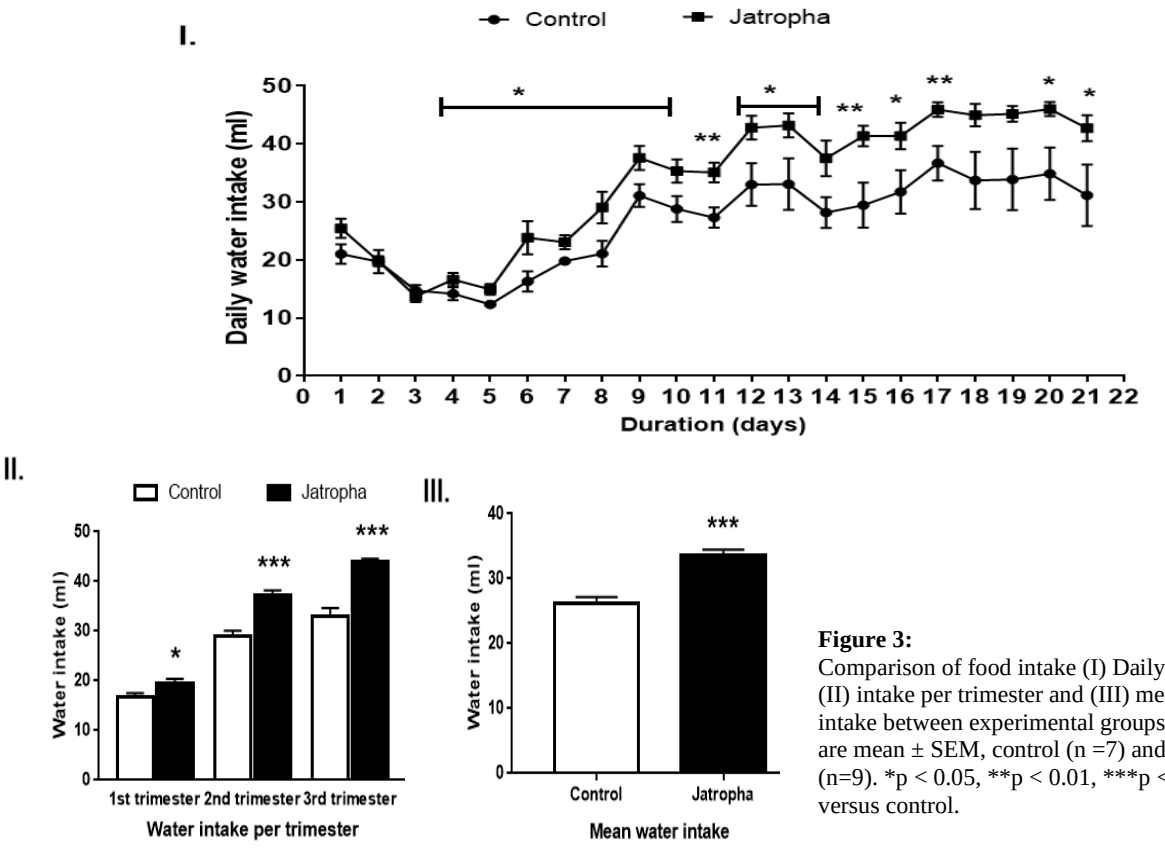

Figure 3:
Comparison of food intake (I) Daily intake, (II) intake per trimester and (III) mean food intake between experimental groups. Values are mean ± SEM, control (n =7) and treated (n=9). *p < 0.05, **p < 0.01, ***p < 0.001 versus control.

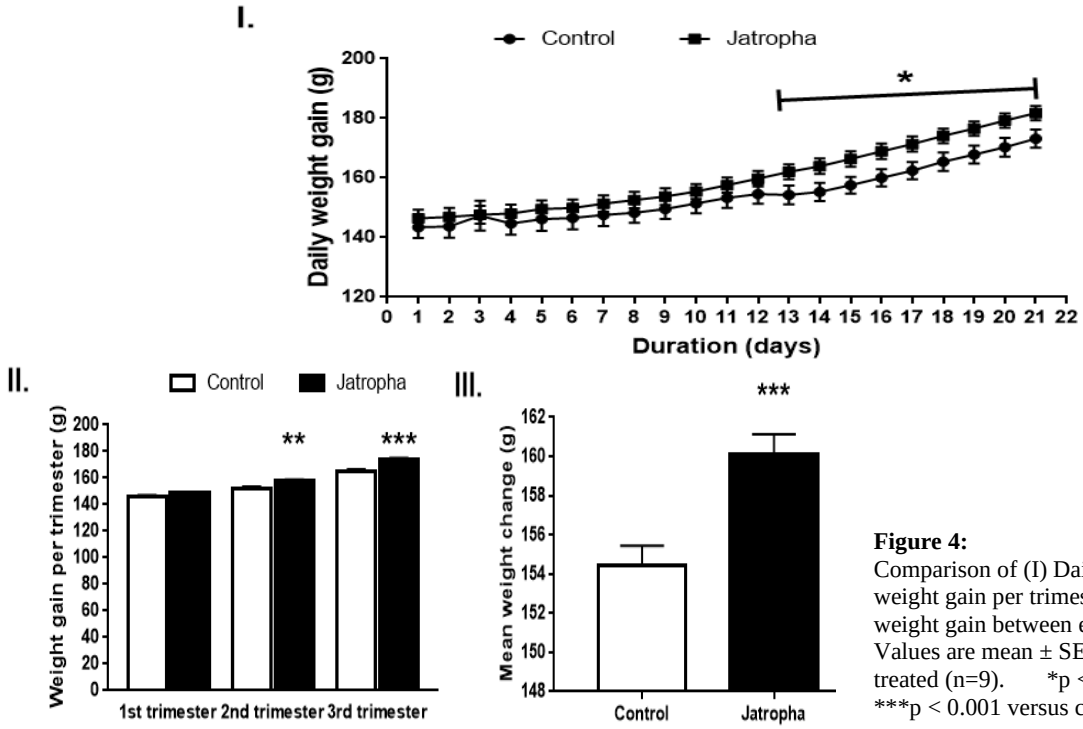

Figure 4:
Comparison of (I) Daily weight gain, (II) weight gain per trimester and (III) mean weight gain between experimental groups. Values are mean ± SEM, control (n =7) and treated (n=9). *p <0.05, **p < 0.01, ***p < 0.001 versus control.

Mean food intake: The mean food intakes for the control and treated groups during gestation are: 147 ± 0.55 and 189 ± 0.62 (g) respectively. The mean food intake increased significantly (P<0.001) in the treated group when compared to the control (Figure 2III).

Daily water intake: The daily water intakes (ml) for 21 days during gestation was generally higher in *Jatropha tanjorensis* treated group from day 5 (P<0.05) and days 11, 15 and 17 (P<0.01) against control (Figure 3I).

Water intake per trimester: Water intake at first trimester of gestation for the control and treated group were: 17.91 ± 0.72 and 19.53 ± 0.70 (ml); second trimester are: 28.91 ± 1.15 and 37.20 ± 1.00 (ml); and third trimester: 33.06 ± 1.61 and 43.91 ± 0.68 (ml) respectively. At first trimester it differed not significantly but increased significantly at second and third trimester (P<0.001) in the treated group against the control (Figure 3II).

Mean water intake: The mean water intakes (ml) during gestation are: 147 ± 0.89 (control) and 189 ± 0.89 (treated). The mean water intake increased significantly (P<0.001) in the treated group against the control (Figure 3III).

Daily weight gain: The daily weight gain (g) for 21 days during gestation differed not significantly from 1 to 12 days among groups, but weight gain increased (P<0.05) significantly in treated against control on gestational days 13 to 21 (Figure 4I).

Weight gain per trimester: At first trimester of gestation, the change in weight for the control and treated group are: 145.25 ± 1.34 and 148.10 ± 1.02 (g); second trimester are: 152.39 ± 1.22 and 157.86 ± 1.07 (g); and third trimester are: 165.24 ± 1.31 and 174.03 ± 1.12 (g) respectively. In the second trimester increase (P<0.01) in weight gain was significant in the treated compared to control. In third trimester, the difference was even more pronounced in the treated (P<0.001) compared to control (Figure 4II).

Mean weight gain: The mean change in weight (g) during gestation are: 154.42 ± 1.01(control) and 160.43 ± 0.99 (treated). The mean weight gain in the treated (P<0.001) increased significantly compared to control (Figure 4III).

Dam outcome: Table 3 shows indices of pregnancy outcome in *J.T* treated and control dams. There were no significant differences in gestational days, litter size and live birth.

Pups outcome: Table 4 shows pups birth indices of dams treated with aqueous leaf extract of *J.T* treated versus that of control. The body length (P<0.001), tail length (P<0.05) and birth weight (P<0.001) increased in pups whose dams received the extract compared to control..

DISCUSSION

The goal of this study was to scrutinize the effects of consuming the aqueous extract of *Jatropha tanjorensis* leaf on reproductive performance and pregnancy assessment of female Wistar rats.

Hormones produced by the hypothalamic pituitary complex (FSH and LH) and ovarian hormones (estradiol and progesterone) regulate reproductive functions in female species (Boiti *et al*., 2005). Luteinizing hormone and progesterone levels were not affected by the extract, but the aqueous extract of *Jatropha tanjorensis* leaf increased the concentration of FSH and estrogen. The estrogenic properties of some plants are regularly linked to the stimulation of hypothalamus–pituitary complex increasing FSH concentration which in turn prompt ovarian steroidogenesis (Pelissero *et al*., 1996). The use medicinal plant with estrogenic effect is proven to ameliorate some

reproductive health issues in female species (Pelissero *et al*., 1996).

In order to provide reliable proof of the therapeutic worth claims by consumers of *Jatropha tanjorensis*, the results showed that nine (9) female rats from the treated group and seven (7) from the control got pregnant out of ten (10) from each group. Several secondary metabolites present in *Jatropha tanjorensis* has been reported to possess positive biological effects essential for reproductive health (tannin, flavonoids and saponins) and it rich mineral constituents **(**Anhwange *et al*., 2019). The rich secondary metabolites of the extract coupled with it estrogenic properties could be responsible for boosting fertility potential in rats.

Food and water intake are a vital nutrient essential during pregnancy to lessen the physiological changes, maintain fetus health in-utero and add to weight gain (Montgomery, 2002). Administration of *Jatropha tanjorensis* increased food and water intake, as well as weight gain in rats during gestation. Since pregnancy is a stressful events female mammals can assume, mostly for multiple bearing species (Engelbregt *et al*, 2001), it is assumed that increase food and water intake can improve reproductive activities required for species survival (Wade *et al*, 1996; Glass *et al*, 1986). It is not surprising that *J.T* which is nutritionally rich increases food and water intake which add to dam's body weight and provide energy for coping with the stress associated with pregnancy.

Consumption of the aqueous leaf extract of *Jatropha tanjorensis* during pregnancy did not interfere with gestation length and fecundity (litter size) but was effective for fetal growth and development. Studies have shown that mothers with poor food and water intake had disturbed fetus growth and development (Lindsay *et al.,* 2019), and gave birth to lesser birth weight babies (Sloan *et al.,* 2001; Dos-Santus *et al*., 2014). In contrast, aqueous extract of *J. tanjorensis* leaf increased food and water intake during pregnancy in dams which was essential for pups in-utero growth indices assessed on birthday one. This is because aqueous leaf extract of *J. T* contain many benefits for a positive pregnancy outcome.

In conclusion, aqueous extract of *Jatropha tanjorensis* leaf elevate reproductive hormone levels of estrogen and FSH. It also boost fertility potential, enhances gestational performance and in-utero pups growth indices.

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