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Abortifacient Properties of Ethanolic Leaf Extract of *Jatropha curcas* Linn. in Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study sought to investigate the abortifacient properties of *Jatropha curcas* leaf extract in female Wistar rats.
Methods: Healthy leaves of *J. curcas* were harvested, dried and extracted using soxhlet apparatus and ethanol as the solvent. Thirty fertile male and thirty female Wistar rats were acclimatized for seven days. The females were separated into individual cages and had estrus synchronization using Diethylstilbestrol dissolved in paraffin oil and administered at the dose of 1 mg/kg body weight. A male rat was then introduced into each cage for mating. After confirmation of pregnancy, the pregnant rats were grouped into four. Group A was treated with normal saline, groups B, C and D were treated with 1 g/kg body weight of *J. curcas* leaf extract for 24, 48 and 72 hours respectively. The animals were then observed daily if they littered. *In vitro* effect of the fruit on isolated rats' uteri was determined using standard method.
Results: *J. curcas* leaf extract was safe in rats at the tested oral doses (500–2000 mg/kg). There was no mortality within the study period. In the *in vitro* experiment, *J. curcas* leaf extract elicited a dose dependent multiple contractions on the pregnant rats' uteri. 60% of animals treated with *J. curcas* leaf extract for 24 hours were observed to heave littered which is suggestive that miscarriage

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had occurred in 40%. In the animals treated for 48 hours, only 20% littered which is also suggestive that miscarriage had occurred in the remaining 80%. In the group treated with *J. curcas* leaf extract for 72 hours, no animal littered.

Conclusion: The results from this study revealed that *J. curcas* leaf extract induced multiple contractions on pregnant rats' uteri following *in vitro* and *in vivo* administrations. This is an indication that it contains active agents which could be isolated and processed into pure utero-tonic agents. Therefore, care should be taken in the use of *J. curcas* leaves during pregnancy and its use in folklore medicine during pregnancy should be discouraged. However, it might serve as an effective abortifacient.

Keywords: Abortifaceous properties; Jatropha curcas; miscarriage; oxytocin; pregnancy.

1. INTRODUCTION

The abortifacient is a substance that induces abortion/miscarriage. Miscarriage, also known as spontaneous abortion and pregnancy loss, is the natural death of an embryo or fetus before it is able to survive independently [1]. Some use the cutoff of 20 weeks of gestation, after which fetal death is known as a stillbirth [2]. The most common symptom of a miscarriage is vaginal bleeding with or without pain. Miscarriage has been linked to different factors. Gestation or Pregnancy is an important part of female reproductive cycle all over the world. It normally consists of three trimesters and care should be taken during each trimester for safe delivery of the offspring into the world. At the completion of gestation period, oxytocin induces contraction for parturition to occur [3]. Oxytocin is a nine amino acid peptide hormone secreted by the posterior pituitary that elicits milk ejection in female animals and women. In pharmacological doses, oxytocin can be used to induce uterine contraction and maintain lactation [4].

Oxytocin and vasopressin are typical neural hormones. The binding protein for oxytocin is designated neurophysin I and that for vasopressin is designated neurophysin II. Both neurophysins are similar in structure. The hormone-neurophysin complex stabilizes the hormone within the neurosecretory granules. Oxytocin is stored as neurosecretory granules and released from axonal terminals by calcium-dependent exocytosis [5]. In fact oxytocin has been best known for its roles in female reproduction. It is released in large quantities during labor, and after stimulation of the nipples. It is a facilitator for childbirth and breastfeeding. However, recent studies have begun to investigate oxytocin role in various behaviors, including orgasm, social recognition, bonding, and maternal behaviors. oxytocin is believed to be involved in a wide variety of physiological and pathological functions such as sexual activity, penile erection, ejaculation, pregnancy, uterine contraction, milk ejection, maternal behavior, social bonding, stress and probably many more [2]. Oxytocin is usually administered intravenously to induce contractions during parturition. It is also available as nasal spray to induce lactation post-partum. Oxytocin infusion near term will produce contractions that decrease the fetal blood supply. It is inactive orally because it is destroyed by gastric and intestinal enzymes [3].

Oxytocin is also used to stop postpartum bleeding. For this purpose, it is given intravenously or intramuscularly. It is released into the bloodstream as a hormone in response to stretching of the cervix and uterus during parturition and with stimulation of the nipples during lactation [6]. In estrogen and progesterone primed rodents, injections of prolactin cause the formation of milk droplets and their secretion into the ducts but oxytocin causes contraction of the myoepithelial cells lining the duct walls which results in ejection of milk through the nipple [2]. Membrane receptors for oxytocin are found in both uterine and mammary tissues. These receptors are increased in number by estrogens and decreased by progesterone. The concomitant rise in estrogen and fall in progesterone occurring immediately before parturition probably explains the onset of lactation in some individuals prior to delivery [2,7]. The primer for commencement of parturition in humans is secretion of oxytocin by certain cells of the fetus. The oxytocin secreted in turn activates some cells of the placenta to produce and release prostaglandins. Oxytocin and prostaglandins synergize to stimulate the uterine myometria leading to more vigorous and more frequent contractions. At this point, the increasing emotional and physical stresses

caused by these contractions activate the mother's hypothalamus which signals for oxytocin release by the posterior pituitary. The elevated levels of oxytocin and prostaglandins trigger the rhythmic expulsive contraction of true labour [8].

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 [9]. 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 [10,11]. 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 [12].

J. curcas has been reported to have a lot of health benefits because of its wide range of medicinal uses [13]. The name *Jatropha curcas* meaning (Doctor's nutrient) was related to its numerous medicinal uses [14]. The leaves are regarded as antiparasitic, applied to scabies, rubefacient for paralysis, rheumatism and also applied to hard tumor [15]. 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 [13]. The oil from *J. curcas* seeds is used in helping with rashes and parasitic skin diseases [16]. When the oil is mixed with benzyl benzoate, it becomes effective against scabies and dermatitis [17]. 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 [18]. 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 pile. The latex is also applied topically to bee and wasp stings, boils and sores. The latex is also used to treat tooth ache, ringworm. Latex is use to dress sores, ulcers and inflamed tongues [13]. This present study therefore sought to investigate the abortifaceous properties of *J. curcas* leaf extract in female Wistar rats.

2. METHODOLOGY

2.1 Collection and Extraction of Plant Material

Fresh and healthy leaves of *J. curcas* free from diseases 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. [19,20]. About 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°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°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. [2]. 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

Thirty fertile male and thirty virgin female Wistar rats weighing between 170 and 200 g were

purchased from the Imrat Animal House, Ibadan, Nigeria. They were acclimatized for seven (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. After the acclimatization period, the female rats were separated into its individual cages and had estrus synchronization using Diethylstilbestrol dissolved in paraffin oil and administered at the dose of 1 mg/kg body weight. A male was then introduced into each cage for mating. On the 7th day, vaginal smear of each of the female rats was made on a clean glass slide by carefully inserting a cotton-tipped swab moistened with normal saline into the vaginal cavity of the rats and rolled gently against the wall before withdrawal. The smear was stained with Giemsa and observed under microscope to check for presence of protein coagulates. After confirmation of pregnancy, the pregnant rats were grouped into four and treated according to Airaodion et al. [21] as follows:

Group A: Administered normal saline 8 hourly (3 times a day). This served as the control group.

Group B: Administered 1 g/kg body weight of *J. curcas* leaf extract 8 hourly (3 times a day) for 24 hours (1 day)

Group C: 1 g/kg body weight of *J. curcas* leaf extract 8 hourly (3 times a day) for 48 hours (2 days)

Group D: Administered 1 g/kg body weight of *J. curcas* leaf extract 8 hourly (3 times a day) for 72 hours (3 days)

The animals were then observed daily till they littered.

2.4 *In vitro* Effect of *J. curcas* Leaf Extract on Isolated Rats' Uteri

The method described by Airaodion, et al. [22] was adopted: Briefly matured pregnant female rats were sacrificed by stunning and decapitation. The lower abdomen was opened and the two uterine horns were harvested and transferred into De Jalon solution that continuously bubbled with air and maintained at 37°C (pH 7.4). The De Jalon solution was constituted such that each liter contained: NaCl (9 g), KCl (0.42 g), $CaCl_2$ (0.06 g), $NaHCO_3$ (0.5 g), and glucose (0.5 g). Each uterine horn was suspended vertically in a 35 mL organ bath by means of ligatures attached at one end to a tissue holder and at the other end to an isometric force displacement transducer connected to a digital physiological recorder (Medicaid Physiopac) for displaying isometric contractions. Resting tension in the muscle strip was readjusted, just sufficient to remove the slack. The preparation was allowed to equilibrate within 30 minutes of mounting. After recording regular rhythmic contractions, dose-response relationships were established for *J. curcas* leaf extract and other standard drugs used. For all administrations, a minimum time of 1 minute was allowed for individual tissue responses before being washed 3 times with De Jalon solution. The test substances were administered as final bath concentration (FBC).

Percentage (%) rise in Amplitude of Contraction was calculated as:

$$\text{Percentage (\%) rise in Amplitude of Contraction} = \frac{\text{Amplitude of Contraction with } J.\ curcas \text{ leaf extract} - \text{Basal Amplitude of Contraction}}{\text{Basal Amplitude of Contraction}} \times 100$$

2.5 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) multiple comparison test. 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.

Table 1. *In vivo* effect of *J. curcas* leaves on pregnant rats' uteri

Treatment groups	Number of rats per group	Pregnancy test	Type of treatment	Number that littered	Percentage (%) that littered
A	5	Positive	Normal Saline	5	100
B	5	Positive	*J. curcas* leaf extract for 24 hours	3	60
C	5	Positive	*J. curcas* leaf extract for 48 hours	1	20
D	5	Positive	*J. curcas* leaf extract for 72 hours	0	0

Table 2. *In vitro* effect of *J. curcas* leaf extract on isolated pregnant rats' Uteri

Volume administered (mL)	Basal amplitude of contraction (mm)	amplitude of contraction with *J. curcas* leaf extract (mm)	Percentage (%) rise in amplitude of contraction
0.05	5.00	23.43 ± 1.89	368.60±13.83
0.10	5.00	28.00 ± 0.00	460.00±0.00
0.20	5.00	31.36 ± 3.23	527.20±16.02
0.30	5.00	32.21 ± 4.78	544.20±22.53
0.40	5.00	34.23 ± 2.73	584.60±12.84

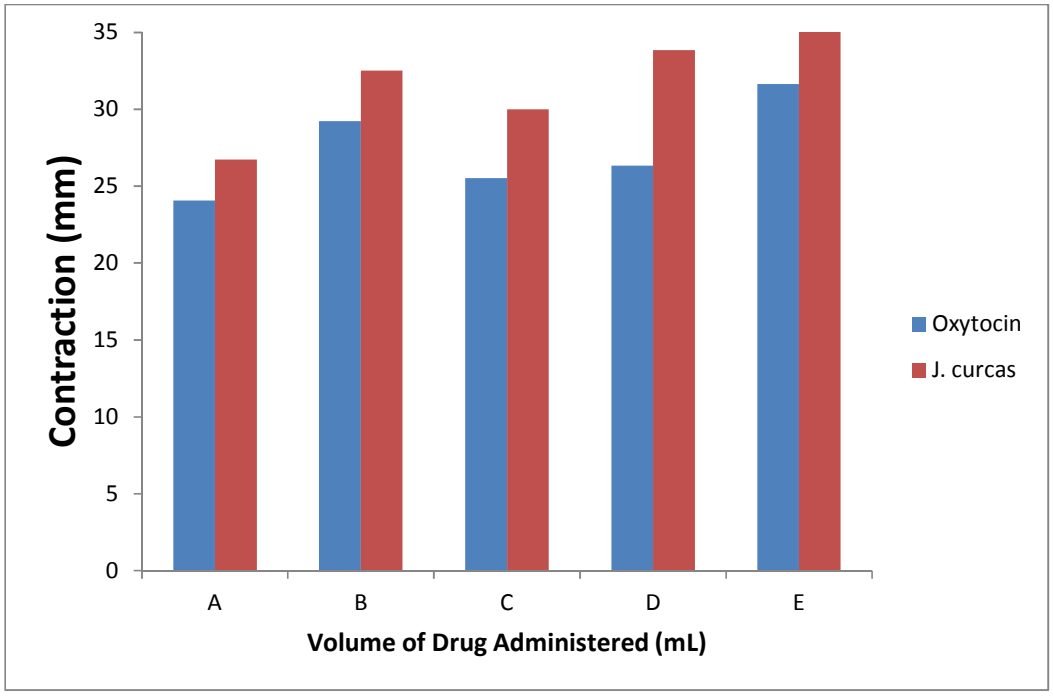

Fig. 1. Comparative effects of oxytocin and *J. curcas* leaf extract on an isolated pregnant rats' uterus

4. DISCUSSION

The nutrition of a pregnant woman plays a major role in the status of the fetus. Thus, during the gestation period, women must be careful of what they eat and pay special attention to their medications especially from plant materials. *J. curcas* have been reported to possess several health benefits [13]. Consequently, it is used for this purpose even in pregnancy. This study therefore, sought to investigate if it has the ability to induce miscarriage in early pregnancy.

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.

Throughout this experiment, all the pregnant rats administered ethanolic leaf extract of *J. curcas* appeared physically healthy. All animals in the control group littered which is an indication that no abortion occurred in this group. 60% of animals treated with *J. curcas* leaf extract for 24 hours were observed to have littered while 40% did not litter. This is an indication that administration of *J. curcas* for 24 hours caused miscarriage in 40% of the population. Further administration of *J. curcas* for 48 hours caused miscarriage in 80% of the population as only 20% was observed to have littered. When the treatment was extended to 72 hours, *J. curcas* caused miscarriage in the entire population as no animals was observed to have littered. This result is similar to the findings of Alwi and Sukardi [23] who reported the anti-implantation effects of *Jatropha curcas* crude oil when fed to pregnant Sprague dawley rats during the early gestation period. It has been reported that administration of low concentrations of compounds with estrogenic activity to many species during early pregnancy resulted in rapid passage of ova through oviducts and expulsion of ova and fetus from the uterus [24]. The reduction in the number of animals that littered in this study might be due to such properties of *J. curcas* extract. Falodun et al. [25] reported a similar study when they investigated the smooth muscle relaxant evaluation of *Jatropha curcas* Linn. and isolation of triterpenes.

In this study, administration of *J. curcas* was observed to significantly ($p<0.05$) induced contractions of isolated rats' uteri with 0.05, 0.1, 0.2. 0.3 and 0.4 mL raised the amplitudes of contractions from 5 mm to 23.43, 28.00, 31.36, 32.21 and 34.23 mm respectively. These contractions compared favourably with that of the standard drug, oxytocin.

Observations from the *in vitro* experiment showed that *J. curcas* leaf extract elicited a dose-dependent multiple contractions of pregnant rats' uteri. These effects were significantly different ($p<0.05$) from the basal contractions, with 0.05 mL of extract eliciting the least increase in amplitude of 368.60% while 0.40 mL of extract eliciting the highest increase in amplitude of 584.60% (Table 2).

The effect of *J. curcas* leaf extract on isolated pregnant rats' uteri compared with that of a standard utero-tonic agent (oxytocin) showed that *J. curcas* leaf extract was giving slightly higher effect than oxytocin at all doses (Fig. 1). *J. curcas* leaf extract when administered to the isolated pregnant rats' uteri induced multiple uterine contractions in a manner similar to that of oxytocin. This result suggests that *J. curcas* leaf extract may contain bioactive agents capable of inducing uterine contractions and as such could be used to facilitate labor or as an abortifacient. This might be due to the ability of *J. curcas* leaves to bind to histaminergic (H_2) receptors present in the uterus [26], promoting calcium flux in the smooth muscles [2,27].

5. CONCLUSION

Results from this study revealed that *J. curcas* leaf extract induced multiple contractions on pregnant rats' uteri following *in vitro* and *in vivo* administrations. This is an indication that it contains active agents which could be isolated and processed into pure utero-tonic agents. Therefore, care should be taken in the use of *J. curcas* leaves during pregnancy and its use in folklore medicine during pregnancy should be discouraged. However, it can serve as an effective abortifacient.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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