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Research Article

Evaluation of maternal behavior and offspring growth throughout the weaning process in maternal rats treated with aqueous leaf extract of *Jatropha tanjorensis*

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Keywords:

Jatropha tanjorensis, Maternal behavior, Offspring growth, Neuroendocrine, Intrauterine indices

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ABSTRACT

Background: The lack of maternal behaviors represents a serious problem that endangers not only offspring survival but also offspring growth. Therefore, many mothers are seeking medication to improve maternal care and infant bonding. This study assessed maternal behavior and offspring growth throughout weaning process in dams treated with aqueous leaf extract of Jatropha tanjorensis (ALEJT).

Methods: The rats were grouped into 2 (n=10); control received 20ml/kg of distilled water and Jatropha received 500mg/kg of ALEJT orally, and were treated throughout gestation and postnatal day (PND) 15-21. Non-pregnant rats were excluded from the study. Pup assessments: intrauterine growth indices (body length, tail length and birth weight), pup growth indices (alive at PND 1-7, 8-14, 15-21 and weaning weight) and reproductive index (live birth-, viability-, pre-weaning- and weaning- indexes). Maternal (pup retrieval, licking, crouching, resting with pup, nesting and sniffing pup) and non-maternal (resting alone, gnawing, self-grooming and feeding) behavior was observed every 2nd day from PND 2-15. At expiration of weaning dams were anesthetized with 60mgkg-1 of ketamine-HCl and blood samples collected by cardiac puncture were used to assess serum levels of maternal hormones.

Results: Litter size, live birth, live birth index and pups alive at PND 1-7 differed not significantly but pups body length, birth weight, weaning weight, weaning index ($P<0.001$), pups alive at PND 15-21, pre-weaning index ($P<0.01$), tail length, viability index and pups alive at PND 8-14 ($P<0.05$) increased significantly in Jatropha compared to control. Treated dams spent more time engaged in maternal behavior compared to control. Follicle stimulating hormone (FSH) differed not significantly, but prolactin (PRL), estrogen ($P<0.05$), progesterone ($P<0.01$), luteinizing hormone (LH), oxytocin and relaxin increased significantly in Jatropha compared to control.

Conclusions: According This study has shown that ALEJT enhances neuroendocrine hormones release which facilitates maternal caregiving, pups survival and growth.

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1. Introduction

Improved maternal behaviors during postpartum is highly adaptive, since the strong drive to seek out and interact with offspring shortly after birth arouses the expression of maternal behaviors which are vital for optimal development, well-being and survival of the offspring (Rincón-Cortés and Grace, 2021; Zeevi *et al*., 2022). The lack of maternal care is a serious problem that risks not only the survival of the offspring but also the offspring growth, early socialization, cognitive development, and epigenetic changes (Coria-Avila *et al*., 2022). The expression of maternal behavior in mammals relies on the hormonal state of the mother and sensory cues (odors, vocalizations, movements) coming from the offspring (Robinson *et al*., 2011; Fuentes *et al*., 2022). Hormonal changes occurring during gestation serve a critical role in modifying maternal physiological and neuroendocrine systems to aid fetal development and it induces both short- and long-term changes in maternal brain that contribute to maternal behavior during the postnatal period (Stolzenberg and Champagne, 2016). Hormones like estrogen, progesterone, prolactin (PRL) and oxytocin which remain elevated in female rodents throughout postpartum are responsible for induction and regulation of maternal behavior (Stagkourakis *et al*., 2020). These hormones are pivotal in establishing mother-pup bonding which increases the probability of young survival and, ensure successful reproductive functions (Pérez-Torrero and Rubio-Navarro, 2015). However, from postpartum days 4–20, maternal behavior is strongly influenced by learning and by recent sensory experiences via interacting with pups (Rincón-Cortés and Grace, 2021).

Several factors have been identified to influence maternal behavior. Such include exposure to endocrine disruptors, and maternal high-fat diet in rodents (Baptissart *et al*., 2018), exposure to opium during pregnancy in rat (Fuentes *et al*., 2022) and exposure to *Valeriana officinalis* during the postpartum (Carvalho *et al*., 2021). Njoku-Oji *et al*., (2020) reported a direct relationship between maternal diet and anthropometric measures of their infants. Mcguire *et al*., (1995) showed a strong relationship between diet, maternal behavior and endocrine status of gonadotropins (LH and FSH) and PRL levels in dams. Pregnancy has been described as a stress test for life (Ukoh *et al*., 2022) and the neuroendocrine changes that occur during gestation favour the development of postpartum disorders which can also alter neuroendocrine function such as maternal care and mothers' capability to recognize their own pups (Belnoue *et al*., 2016 and Fuentes *et al*., 2022**)**. Several studies reported that periodic maternal separation, not only affect the young but also alter maternal behavior of the dam (Boccia and Pedersen, 2001; Pryce *et al*, 2001). Infant survival in typical mammalian species depends crucially on correct maternal behavior, requiring broad investment of a mother's time and energy to be successful (Robinson *et al*., 2015). However, some mothers fail to rear offspring successfully or inefficiently in energetic terms which can impair infant development. Therefore, many mothers are seeking medication to improve maternal care and mother-infant bonding (Budiono *et al*., 2023). Medicinal plants play a substantial role during pregnancy, birth and postpartum care globally (De Boer and Lamxay, 2009).

Jatropha tanjorensis is a perennial herb typically grown in Southern Nigeria and is traditionally called 'Hospital too far' (Oyewole *et al*., 2012). The leaves contain phytochemical constituents such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (Iroanya *et al*., 2018). The leaves are commonly consumed in Nigeria as soups and tonic (Danborno *et al*., 2019), and are employed traditionally in the treatment of anaemia, diabetes and cardiovascular diseases (Danborno *et al*., 2019), and to ease baby delivering process (Nindaratnasari, 2017). *Jatropha tanjorensis* increases iron level available for erythropoiesis, packed cell volume and hemoglobin levels in rats (Falodun *et al*., 2013), the leaves extract improve

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haematological indices which indicate a boost of bone marrow function, and was recommended of it usage in physiological conditions such as pregnancy and during menstruation (Iroanya *et al*., 2018; Danborno *et al*., 2019). In an earlier study we observed that consumption of ALEJT improves fertility potential and gestational outcome in virgin female rats (Ukoh *et al*., 2022), we also observed that gestational administration of ALEJT alleviated postpartum-like behaviors such as postpartum-like depression and anxiety and locomotor activity in dams (Antai *et al*., 2023)**.** Fuentes *et al*., (2022) stated that the design of pharmacological and behavioral treatments of postpartum disorders would greatly benefit from a better understanding of the neurobiological mechanisms of maternal care. Hence, this study describes the effect of gestational and postpartum administration of ALEJT on maternal behavior, offspring survival and growth during the weaning process. The findings of this study will build on our understanding of how *Jatropha tanjorensis* affects maternal care, with potential long-term consequences for offspring physiology.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Extract

The leaves of *Jatropha tanjorensis* were harvested from the botanical garden of the University of Calabar, Nigeria. A voucher specimen has been kept in the botanical garden (Herb/Bot/UCC/182). They were prepared as described by (Agarwal *et al.,* 2007). The leaves was thoroughly washed with clean water and air-dried at room temperature for two days then further dried in an oven at 40°C for 24 hours. The crispy leaves were then ground into fine powder and preserved in moisture-free, airtight laboratory containers for further use. The powdered plant material (100 g) was macerated with water (1000 ml) in ratio of 1:10 and was agitated intermittently for 48 hours, filtered into a clean glass jar. Filtration of the mixture was done first with filter cloth, Whatman filter paper of pore size 11µm, and then filtrates was evaporated to complete dryness using a thermostatically controlled water bath at 42 ^{0}C.

The dosages were calculated from stock solution of the aqueous extract of the plant dissolved in distilled water (vehicle) at 20 ml/kg (2 ml/100g) body weight (OECD, 2000).

Dose volume was calculated as follows:

$$\frac{\text{Body weight}}{1000\text{g (1kg)}} \times 20\text{ml}$$

$$\text{Dosage in mg} = \frac{\text{Body weight}}{1000\text{g (1kg)}} \times \text{dose (mg)}$$

LD50 was determined by the method of Lorke, (1983), *Jatropha tanjorensis* showed no overt signs of distress or toxicity even at a dose of 5000mg/kg body weight. Therefore, in this study the extract dosage of 500mg/kg was considered safe for pregnant and postpartum rats.

2.2. Animals

A total of 30 albino rats (20 females and 10 males) weighing between 120 -150g were obtained from the College of Medical Sciences animal house of University Calabar, Cross River State, Nigeria. The animals were kept under standard laboratory conditions and housed in well ventilated plastic cages at room temperature and relative humidity with light and dark cycles (12hr/12hr). The animals were acclimatized for a period of one week and were fed with standard rat pellet (Pfizer feed PLC, Lagos, Nigeria) and water ad libitum. Ethical approval was obtained from the University of Calabar College Ethical Committee on the use of experimental animals with protocol number (093PHY3321).

2.3 Induction of pregnancy in rats

After acclimatization, the rats were housed singly in stainless steel cages, except for mating and postpartum periods. A male rat was caged with two female rats on varying days, each female rat was returned to their cages while the next female rat was introduced. The appearance of vaginal plug was considered day 1 of gestation. The

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inseminated female rats were randomly assigned into two groups. Group 1 served as control (n=10) which received 20 ml/kg of distilled water (vehicle) orally, while Group 2 served as *Jatropha* treated (n=10) and received 500mg/kg of ALEJT orally throughout gestation and the last week of weaning (postpartum day 15-21). After two weeks of observation, the number of inseminated non-pregnant rats was excluded from the study.

2.4 Assessment of pregnancy outcome and pup growth indices

All pregnant rats were allowed to deliver. At birth, the following outcomes were recorded:

(1) Live birth: Evaluated as the total number of pups in the litter that was alive at parturition.
(2) Litter size: Evaluated as the total number of pups in the litter, dead or alive.
(3) Intrauterine growth indices: At birth (PND 0) digital balance (DSW200D; DI Delmac Instruments, Athens, Greece) was used to determine the body weight of pups. Body length and tail length of litters were measured, 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.
(4) Litter survival: The numbers of whole litters were counted daily from birth to 21 days of age. Litter survival was assessed by calculating the proportion of litters born that survived to 21 days at a scale of: Alive at PND 1-7, PND 8-14 and PND 15-21.

(9) Weaning weight: Body weight of pups alive at day 21.

2.6 Assessment of reproductive index

Reproductive indices help to assess various health outcomes of pups in a litter that was alive at parturition up to expiration of weaning.

(1) Live birth index:

$$\frac{\text{Number of live litters}}{\text{Number of litters delivered}} \times 100$$

(2) Viability index (4-Day survival index):

$$\frac{\text{Number of live litters at postnatal day 4}}{\text{Number of litters delivered}} \times 100$$

(3) Weaning index:

$$\frac{\text{Number of live litters at day 21}}{\text{Number of live litters born}} \times 100$$

(4) Pre-weaning index:

$$\frac{\text{Number of live litters born - Number of litters weaned}}{\text{Number of live litters born}} \times 100$$

2.5 Assessment of maternal behavior

Periodic (spot-check) and continuous (disturbed) observation using a time-sampling procedure was used to assess maternal behavior. Periodic observation provides a "snapshot" of the dam-litter interaction under undisturbed conditions, while continuous observation of dam-litter interaction was performed after a period of separation between the dams and the litters.

Procedure: Maternal observations occurred every second day from postpartum day 2 to day15. On each day, the home cages were placed on a table in a lit room and the dams were given 2 minutes to acclimatize before the observations. For periodic observation, the dams were observed with their litters for 600-sec (10 minute), and the presence or absence of each behavior was scored at 10-sec intervals for 60 observations. For continuous observation (postpartum day 4 and 12), the pups were removed from the home cage and kept in a flat plastic container for 10 minutes, while the dam was kept in a holding cage. At 10 minutes, the pups were returned to the home cage and scattered over the floor of the home cage covered with wood shavings. The dam was then

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returned, and the dams were observed with their litters for 600-sec (10 minute), and the presence or absence of each behavior was scored at 10-sec intervals for 60 observations. Each behavior was recorded once within any single interval. Thus, one or more behaviors were scored in each interval.

Behaviors recorded as "maternal behaviors" were as reported by Brown *et al.,* (1999):

(1) Retrieval (maximizing contact with young): carrying pup usually at the back of the neck from one point to another.
(2) Pup-licking and grooming: The rat licks any region of a pup's body.
(3) Crouching (active nursing): Assuming the arched-back nursing position over the pups. Sucking at least one pup
(4) Resting with pups: Inactive, but in contact with a part of her body other than the tail to the body of at least one pup
(5) Sniffing pups: Touching pups with nose and sniffing
(6) Nesting: wood shavings for bedding were observed for nest building behavior throughout the days of observation. A dam was recorded as having a nesting behavior if it carried or pushed strips of wood shavings from various locations in the cage and placing them around the pups or covered them with it (Brown *et al.,* 1999).
(7) Nest building: On PPD 5 cotton wool was provided as nesting material. Five gram (5g) of the nesting material was placed in the home cage on PPD 5 and examined after 24 hours (PPD6) for nest building behavior.

2.7 Nest scoring

a. Nestlet not noticeably touched (90% or more intact)
b. Nestlet partially torn (50-90% intact)
c. Less than 50% of nestlet remains intact, but not gathered into a nest site but spread throughout cage.
d. More than 90% of the nestlet is torn into a flat nest
e. More than 90% of the nestlet is torn, nest is fairly even, the nest is a crater

Behaviors recorded as "non-maternal behaviors" were as reported by Brown *et al.,* (1999):

(1) Resting alone (inactivity without contacting any pups),
(2) Gnaw (gnawing on wood shavings or on bars of cage),
(3) Self-*grooming* (wiping, licking, or scratching of the dam's own body),
(4) Feeding (ingestion of food or water)

2.8 Sample collection

At the end of the weaning process, the dams were euthanised with 60 mg kg^{-1} of ketamine-hydrochloride (#50155, Rotex Medica, Trittau, Germany) using a 5 ml syringe and a 21 G needle. Blood samples were collected via cardiac puncture into plain sample bottles and allowed to stand for 2 hours, after which they were centrifuged at 1,000 rpm for 5 minutes using a bucket centrifuge (B-Bran Scientific and Instrument Company, England). After centrifugation the serum samples were stored at -20 °C until assayed for respective hormones. This was in adherence to the College of Medical Science, University of Calabar, Nigeria standards.

2.9 Serum Hormonal profile assessment

Hormonal assay were done as described by Khaki *et al*., (2009) as follows:

2.9.1 Biological principles of the procedure for the ARCHITECT estradiol assay

ARCHITECT Estradiol Reagent Kit (7K72) was used to assay for estradiol. The ARCHITECT Estradiol assay is a delayed one step immunoassay to determine the presence of estradiol in serum and plasma. In the first step, the sample, estradiol specimen diluent (10.0 mL) containing TRIS buffer with protein (bovine) stabilizers, estradiol assay diluent (5.9 mL)

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containing surfactant in citrate buffer and anti-estradiol (9.9 mL) coated paramagnetic micro-particles were combined. Estradiol present in the sample bound to the anti-estradiol coated micro-particles. After incubation, estradiol acridinium labeled conjugate was added to the reaction mixture. After a second incubation, and washing (Wash Buffer containing phosphate buffered saline solution), Pre-Trigger solution containing 1.32% (w/v) hydrogen peroxide and Trigger Solutions containing 0.35N sodium hydroxide were then added and the resulting chemiluminescent reaction was measured as relative light units (RLUs). An inverse relationship exists between the amount of estradiol in the sample and the RLUs detected by the ARCHITECT i optical system.

2.9.2 Biological principles of the procedure for ARCHITECT FSH assay

ARCHITECT FSH Reagent Kit (7K75) was used to assay for FSH. The ARCHITECT FSH assay is a two-step immunoassay to determine the presence of FSH in serum and plasma. In the first step, sample and anti-β FSH (6.6 mL/27.0 mL) coated paramagnetic micro-particles were combined. FSH present in the sample binds to the anti-β FSH coated micro-particles. After washing, anti-α FSH acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of FSH in the sample and the RLUs detected by the ARCHITECT i optical system.

2.9.3 Biological principles of the procedure ARCHITECT LH assay

ARCHITECT LH Reagent Kit (6C25) was used to assay for LH. The ARCHITECT LH assay is a two-step immunoassay to determine the presence of LH in serum and plasma. In the first step, sample and anti-β LH coated paramagnetic micro-particles were combined. LH present in the sample bound to the anti-β LH coated micro-particles. After washing, anti-α LH acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship exists between the amount of LH in the sample and the RLUs detected by the ARCHITECT i optical system.

2.9.4 Biological principles of the procedure for ARCHITECT prolactin assay

ARCHITECT PRL Reagent Kit (7K76) was used to assay for PRL. The ARCHITECT PRL assay is a two-step immunoassay to determine the presence of PRL in serum and plasma. In the first step, sample and anti-PRL (mouse, monoclonal) coated paramagnetic micro-particles were combined. PRL present in the sample bound to the anti-PRL (mouse, monoclonal) coated micro-particles. After washing, anti-prolactin (mouse, monoclonal) acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship exists between the amount of PRL in the sample and the RLUs detected by the ARCHITECT i optical system.

2.9.5 Determination of progesterone levels

Twenty µl of standards, control and extract samples were dispensed into their respective wells. 200 µl Progesterone-HRP conjugate was added to each well. Substrate blank was dispensed into well A1. The wells were covered with foil. The wells were incubated for 1 hour at 37º C. After 1 hour the foil was removed and well contents aspirated. The wells were washed 3 times with 300 µl diluted wash solution. The soak time between each wash cycle was more than 5 seconds. The remaining fluid was carefully removed by tapping the strips on tissue paper. 100 µl TMB substrate solution was added into all wells. The wells were incubated for 15 minutes at room temperature in the dark. 100 µl stop solution was dispensed into all wells in same order and same rate as for the substrate. The absorbance of the specimen was read at 450nm within 30 minutes after addition of stop solution.

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The ELISA kit product used was DNOV006, (NovaTec Immunodiagnostica GmbH, Germany).

2.9.6 Determination of oxytocin levels

Fifty µ1 each of standard, control and samples were dispensed into their respective wells. Then 50µL of Detection Reagent A was added to each well immediately. The wells were covered with a plate sealer. The wells were incubated for 1 hour at 37º C. After 1 hour the plate sealer was removed and well contents aspirated. The wells were washed three time with 350µL of 1X Wash Solution. The soak time between each wash cycle was 1-2 minutes. The remaining fluid was carefully removed by tapping the strips on tissue paper. Then 100µL of Detection Reagent B working solution was added to each well and incubated for 30 minutes at 37º C after covering it with the Plate sealer. Stop solution of 50 µl was dispensed into all wells in same order and same rate as for the substrate. Then the microplate reader was run and measurement conducted at 450nm immediately. The ELISA kit product used was CEB052Ge, (NovaTec Immunodiagnostica GmbH, Germany).

2.9.7 Determination of relaxin levels

Fifty µ1 each of standard (read Reagent Preparation), control and samples were dispensed into their respective wells. Then 50µL of Detection Reagent A was added to each well immediately. The wells were covered with a plate sealer. The wells were incubated for 1 hour at 37º C. After 1 hour the plate sealer was removed and well contents aspirated. The wells were washed three time with 350µL of 1X Wash Solution. The soak time between each wash cycle was 1-2 minutes. The remaining fluid was carefully removed by tapping the strips on tissue paper. Then 100µL of Detection Reagent B working solution was added to each well and incubated for 30 minutes at 37º C after covering it with the plate sealer. Stop solution of 50 µl was dispensed into all wells in same order and same rate as for the substrate. Then the microplate reader was run and measurement conducted at 450nm immediately. The ELISA kit product used was CEB216Po, (NovaTec Immunodiagnostica GmbH, Germany).

2.10 Statistical Analysis

The results obtained were expressed as mean ± SEM. The statistical analysis was done using the independent T-test. A difference between means was considered significant at $p < 0.05$. The statistical software was SPSS version 20.

3. RESULTS

3.1. Effect of aqueous leaf extract of *Jatropha tanjorensis* on pregnancy outcome and pups growth in rats

Table 1 illustrates indices of pregnancy outcome and pups growth indices in treated and control dams. There were no significant differences in live births and litter size. There was significant increase in intrauterine growth indices following delivery in the pups exposed to *Jatropha* prenatally compared to control pups. Body length was 5.38 ± 0.05 in the exposed pups and was significantly higher than the control pup body length 5.03 ± 0.04 ($P<0.001$). This was the trend for tail length ($P<0.05$), birth weight ($P<0.001$). There was no significant difference of litters alive at postnatal days (PND) 1-7 between the pups exposed to *Jatropha* prenatally and during postpartum compared to control. However there was a highly significant difference between litters alive at PND 8-14 ($P<0.01$) and 15-21 ($P<0.01$) between the pups exposed to *Jatropha* prenatally and during postpartum compared to control. Weaning weight was significantly higher ($P<0.001$) in pups exposed to *Jatropha* prenatally and during postpartum compared to.

Results of reproductive indices of dams treated with *Jatropha* and control are shown in Table 2. There was significant increase in the viability index ($P<0.05$) and weaning index ($P<0.001$) in the treated dams compared to the control, while pre-weaning index was significantly reduced in the treated group compared to the control.

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Table 1: Pregnancy outcome and pups growth indices of control and treated dams

Parameters	Control (n=53)	Jatropha (n=79)	P-Value
Live birth	7.6 ± 1.2	8.8 ± 0.5	NS
Litter size	8.1 ± 1.1	8.9 ± 0.5	NS
Body length (cm)	5.0 ± 0.0	5.4 ± 0.1	P<0.001
Tail length (cm)	1.5 ± 0.4	1.6 ± 0.0	P<0.05
Birth weight (g)	5.59 ± 0.1	6.09 ± 0.1	P<0.001
Alive at PND 1-7	6.9 ± 1.3	8.7 ± 0.6	NS
Alive at PND 8-14	6.0 ± 1.1	8.6 ± 0.6	P<0.05
Alive at PND 15-21	5.00 ± 0.9	8.3 ± 0.6	P<0.01
Weaning weight (g)	73.1 ± 0.9	93.1 ± 1.1	P<0.001

Caption: n= number of live birth in each groups, PND= postnatal day, NS= not significant

Table 2: Reproductive index of control and *Jatropha tanjorensis* treated dams

Parameters	Control (n=10)	Jatropha (n=10)	P-Value
Live birth index (%)	92.4 ± 4.1	98.9 ± 1.1	NS
Viability index (%)	82.1 ± 5.4	97.4 ± 1.7	P<0.05
Pre-weaning index (%)	38.8 ± 5.2	6.1 ± 3.2	P<0.01
Weaning index (%)	67.3 ± 7.0	94.9 ± 2.7	P<0.001

Caption: n= number of animals in each groups, NS= not significant

3.2 Effect of aqueous leaf extract of *Jatropha tanjorensis* on maternal behavior in rats

3.2.1 Retrieval

The dams retrieval of litters at seven specified days of observation for control and *Jatropha* group are as shown in Figure 1. Litters retrieval on "Days 1-5" increased significantly (P<0.01) in the treated group when compared to the control, while on "Days 6-7" there was significant (P<0.05) increase in the treated group when compared to the control (Fig. 1).

3.2.2 Pup licking and grooming

The dams pup licking at seven specified days of observation are shown in Fig. 2. Pup licking on day 1 and 2 was significantly (P<0.05) increased in the treated group when compared to the control. Other days were not significant.

3.2.3 Crouching

Crouching at seven specified days of observation for control and Jatropha groups are as shown in Fig. 3. Crouching on day 1-3 was significantly (P<0.001) higher in the treated group when compared to the control, while day 4 and 7 was significant at P<0.01 as shown in (Fig. 3).

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3.2.4 Resting with pup

Resting with pups at seven specified days of observation are as shown in Fig. 4. Resting with pup in *Jatropha* treated dams were significantly higher than the control dams. Resting with pups on day 1, 2 and 7 was significantly (P<0.01) higher in the treated group when compared to the control, while days 3, 4, 5 and 6 was significantly (P<0.05) higher in the treated group when compared to the control (Fig. 4).

3.2.5 Sniffing pup

Sniffing pup at seven specified days of observation are as shown in Fig. 5. Sniffing pup in treated dams was significantly higher than in control dams. Sniffing pup was on days 1, 2 and 3" significantly (P<0.001) increased in the treated group when compared to the control, while day 4 was significantly (P<0.01) increased in the treated group when compared to the control and day 5 and 6 was significantly (P<0.05) increased in the treated group when compared to the control (Fig. 5).

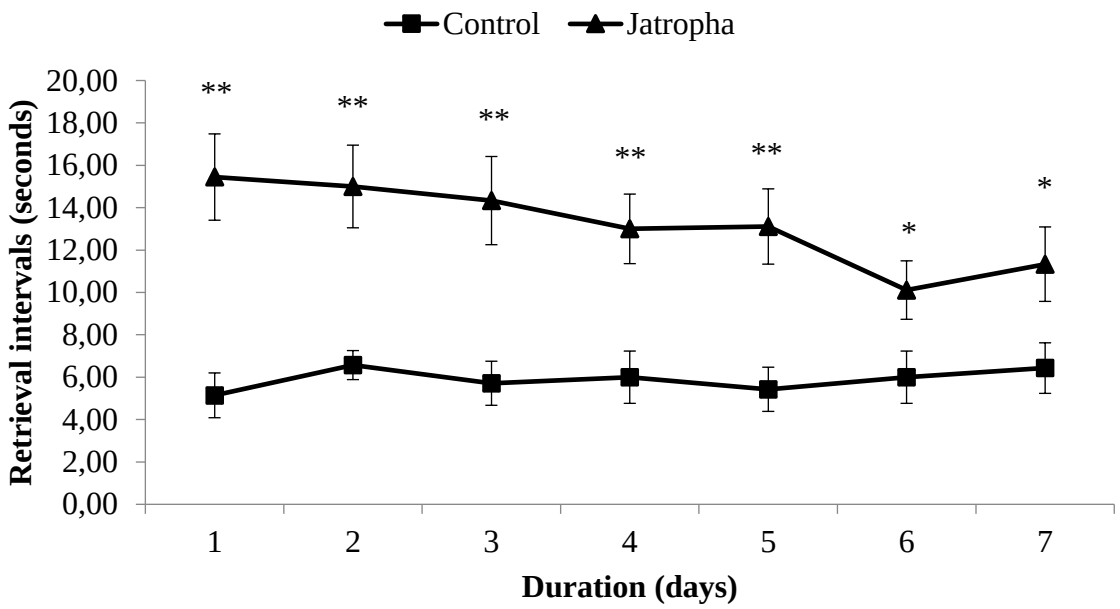

Fig. 1: Maternal pup retrieval intervals in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs control; **p<0.01 vs control

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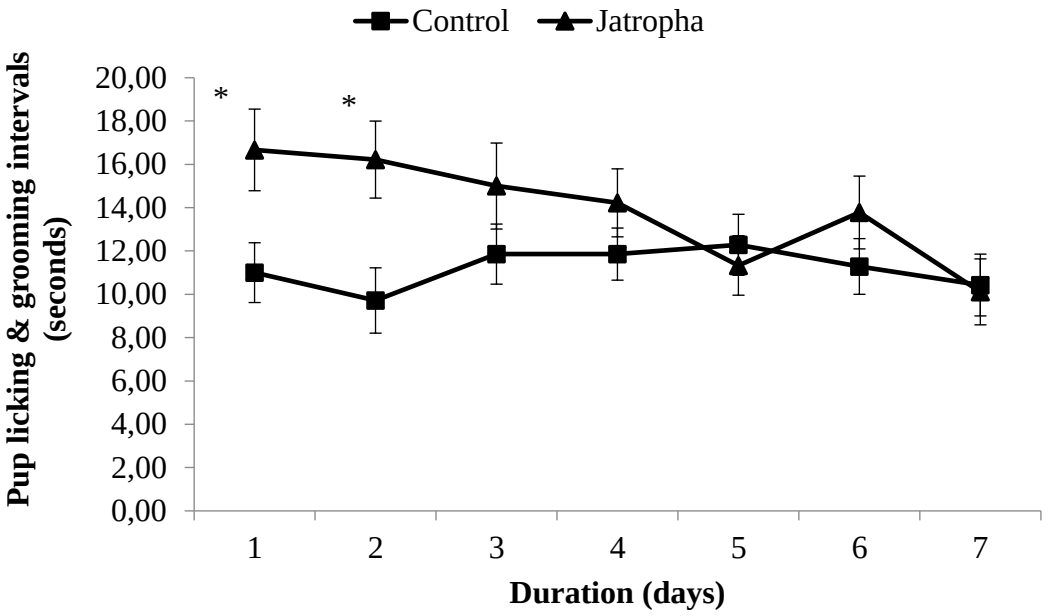

Fig. 2: Maternal pup licking and grooming intervals in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs control

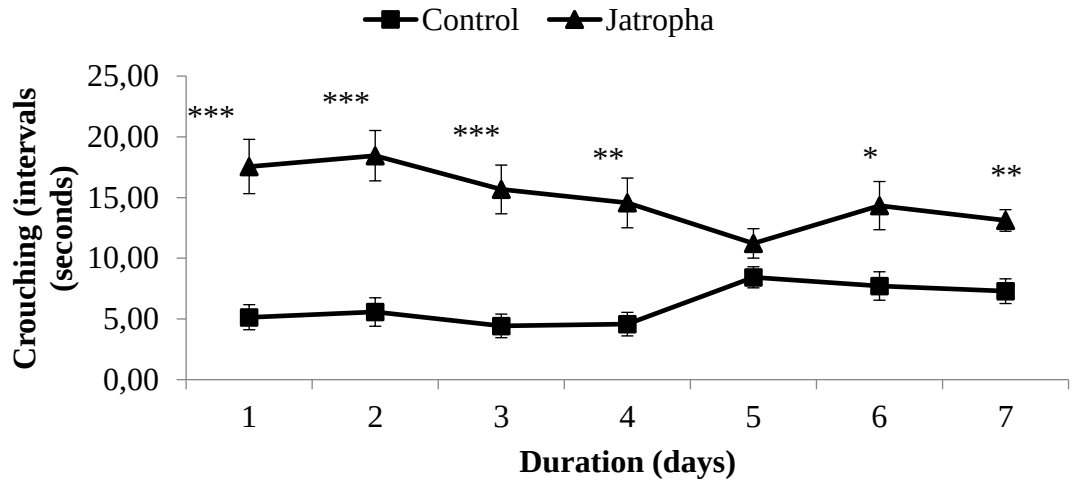

Fig. 3: Maternal crouching intervals in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs control; **p<0.01 vs control; ***p<0.001 vs control

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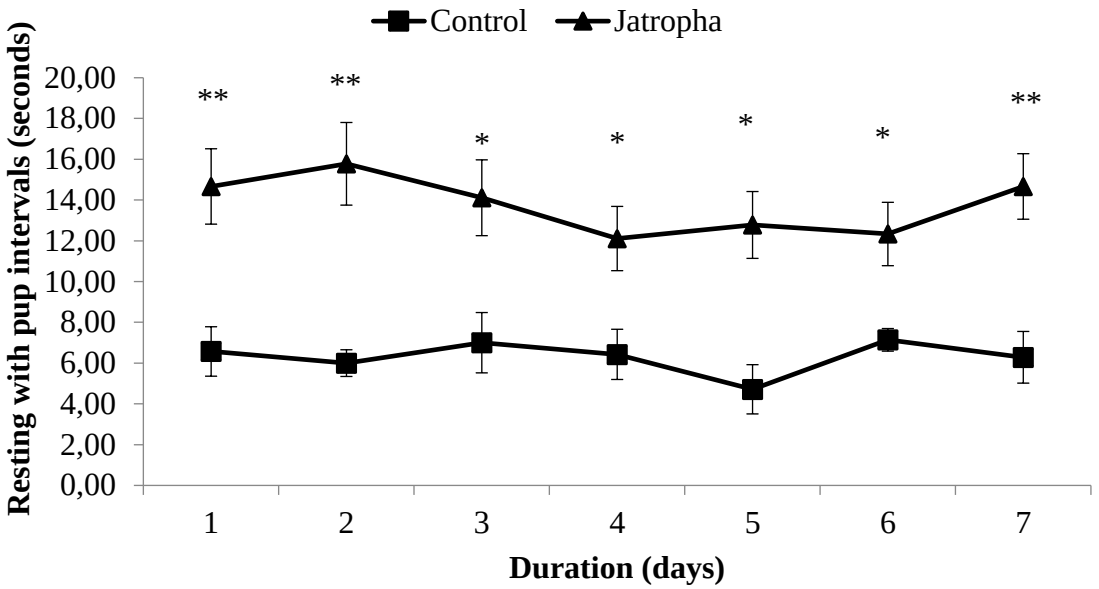

Fig. 4: Maternal resting with pups interval in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs control; **p<0.01 vs control

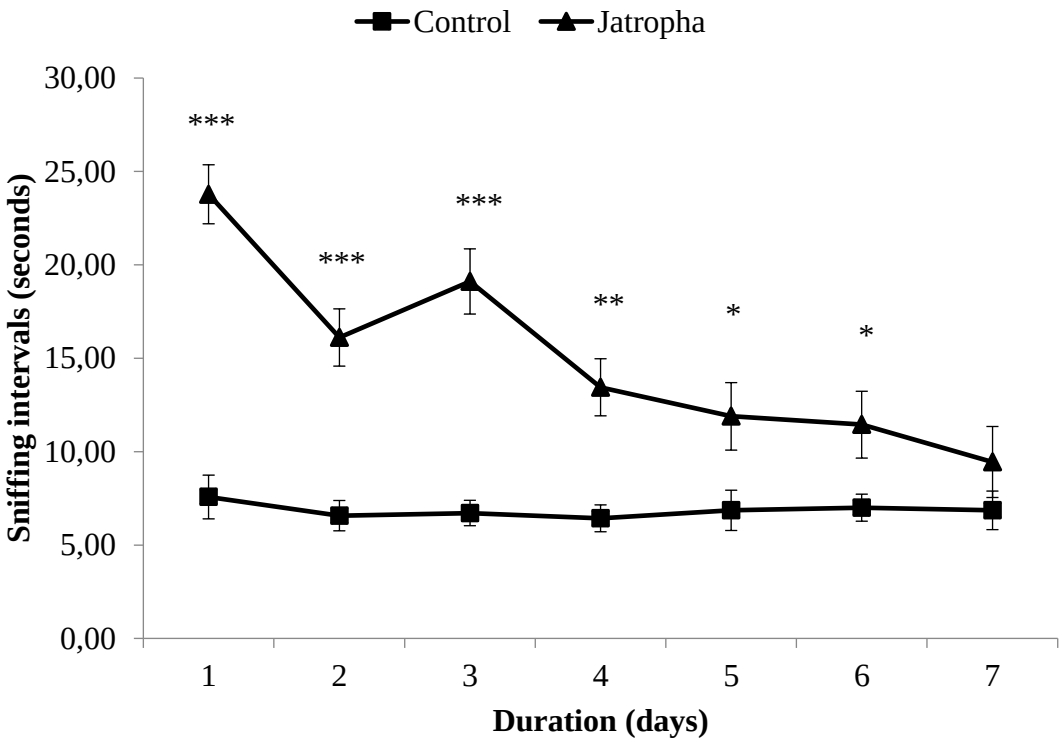

Fig. 5: Maternal pups sniffing intervals in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10.*p<0.05 vs control; **p<0.01 vs control; ***p<0.001 vs control

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3.2.6 Nesting

Nesting at seven specified days of observation are as shown in Fig. 6. Nesting was on day 1-7 significantly (P<0.001) increased in *Jatropha* when compared to the control (Fig. 6).

3.2.7 Nest building

Observations of nest building in Fig. 7 showed that nest building increased significantly (P<0.05) in *Jatropha* (4.11 ± 0.26) compared with control (2.71 ± 0.47).

3.3 Effect of aqueous leaf extract of *Jatropha tanjorensis* on non-maternal behavior in rats

3.3.1 Resting alone

Dams resting alone at seven specified days of observation for control and Jatropha groups are as shown in Fig. 8. Dams resting alone in *Jatropha* were significantly reduced compared to control dams. Resting alone was on day 1 and 2 significantly (P<0.001) reduced in the treated group when compared to the control, while day 3, 4 and 5 (P<0.01) and day 6 and 7 (P<0.05) were significantly reduced in the control when compared to the treated group (Fig. 8).

3.3.2 Gnawing

Dams gnawing at seven specified days of observation for control and Jatropha groups are as shown in Fig. 9. Dams gnawing in rats were significantly reduced in the *Jatropha* compared to the control. Dams gnawing was on day 1 significantly (P<0.001) reduced in *Jatropha* compared with control, while day 2, 3 and 4 (P<0.01) and day 6 (P<0.05) were significantly reduced in the JT group compared with control (Fig. 9).

3.3.3 Self-grooming

Dams self-grooming at seven specified days of observation are as shown in Fig. 10. Dams self-grooming was on day 1, 2, 3, 5 and 7 significantly (P<0.001) reduced in *Jatropha* when compared to the control, while day 4 (P<0.01) and day 6 (P<0.05) were significantly reduced in *Jatropha* compared to the control.

3.3.4 Feeding

Dams feeding at seven specified days of observation for control and Jatropha groups are as shown in Fig. 11. There was no significant difference in feeding behavior observed between the control and treated group.

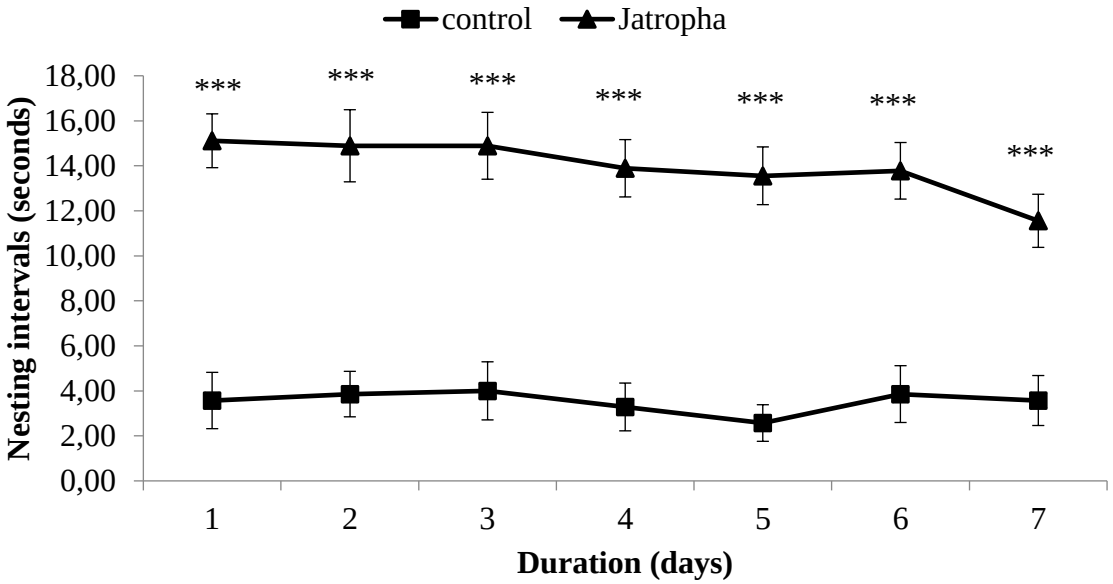

Fig. 6: Maternal nesting intervals in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. ***p<0.001 vs control

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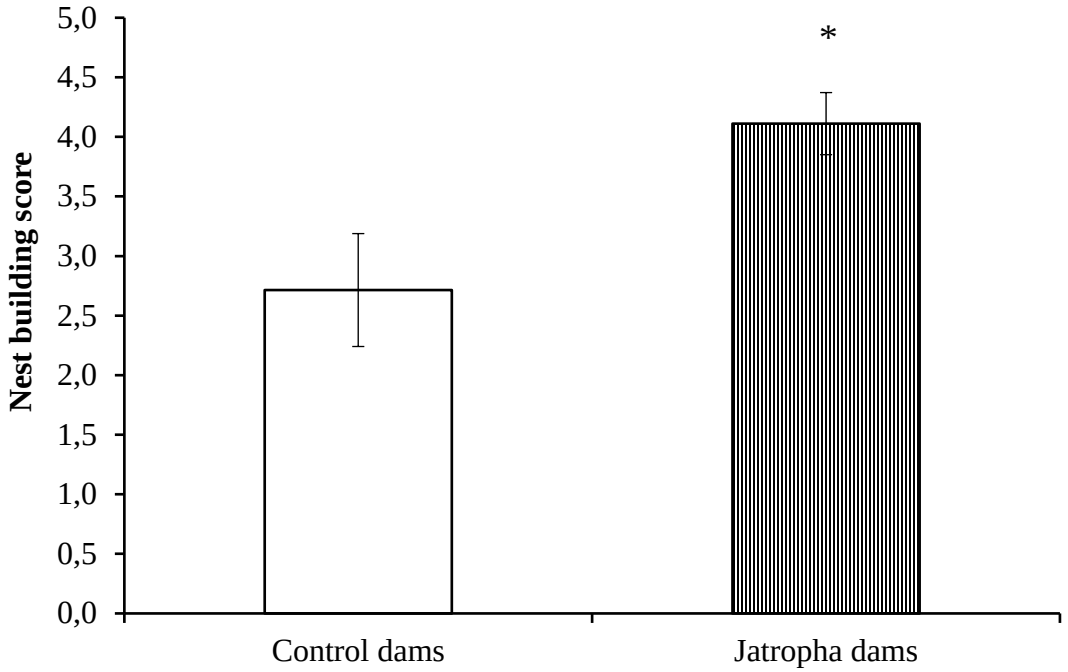

Fig. 7: Maternal nest building score in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs. control

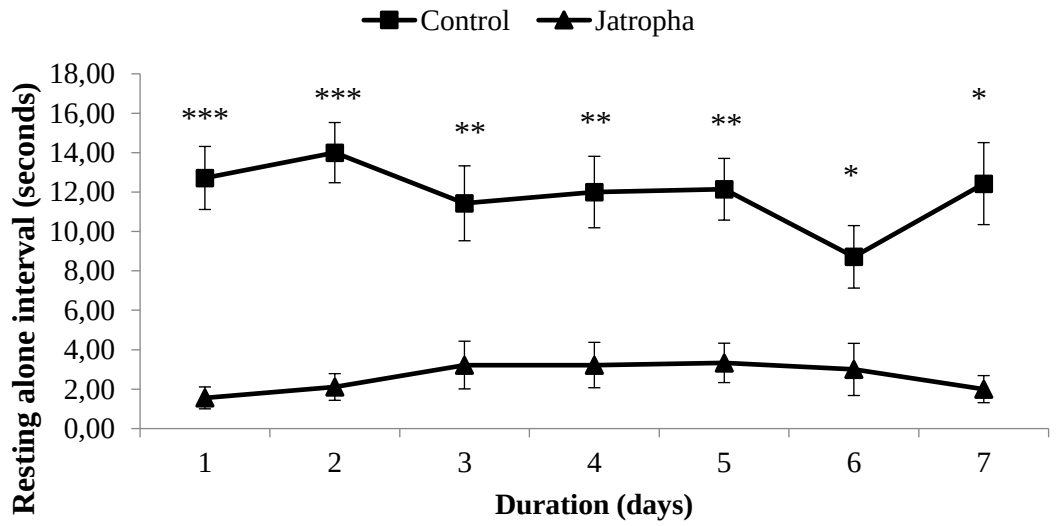

Fig. 8: Maternal resting alone interval in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs control; **p<0.01 vs. control; ***p<0.001 vs. control

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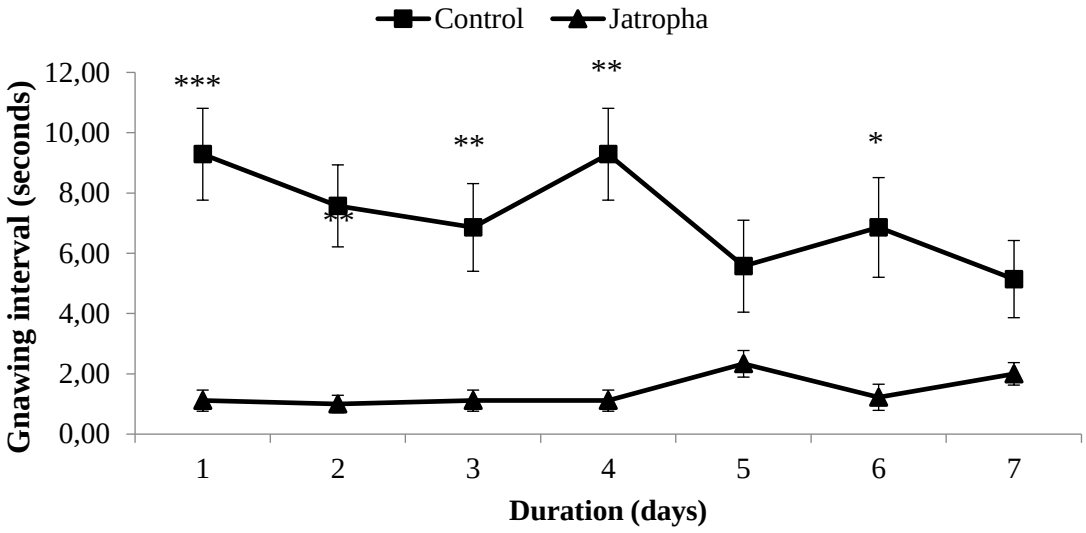

Fig. 9: Maternal gnawing intervals in control and *Jatropha tanjorensis* dams.Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs control; **p<0.01 vs control; ***p<0.001 vs control

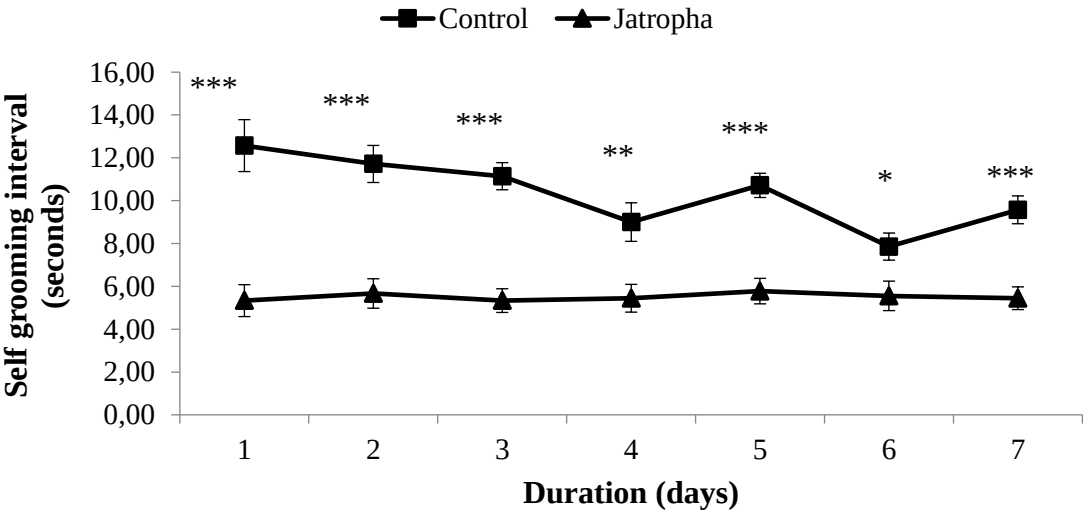

Fig. 10: Maternal self-grooming intervals in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10. *p<0.05 vs control; **p<0.01 vs control; ***p<0.001 vs control

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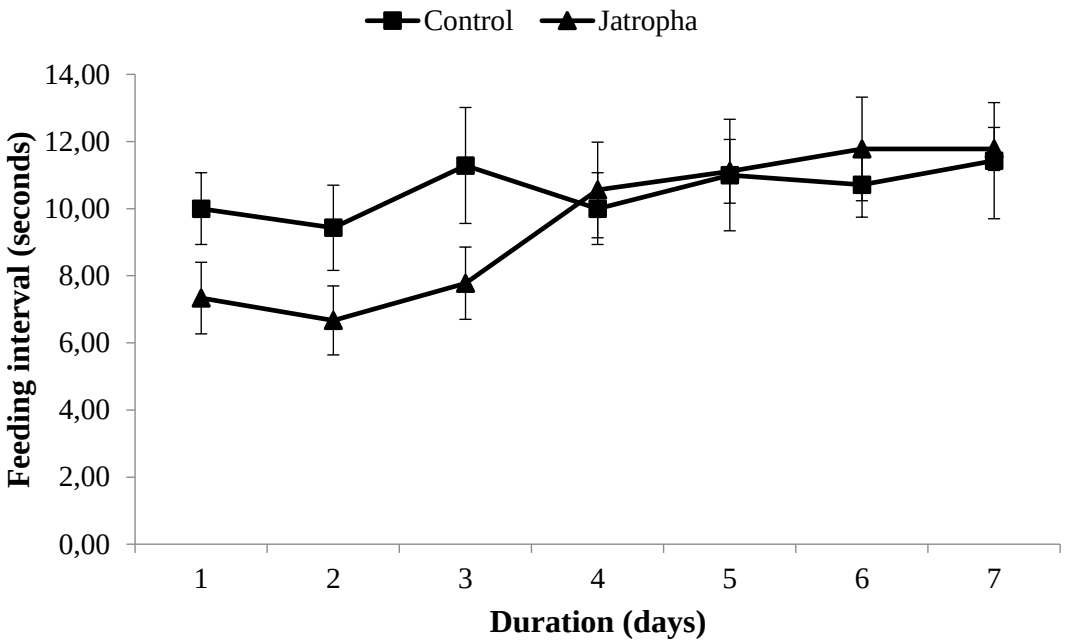

Fig. 11: Maternal feeding intervals in control and *Jatropha tanjorensis* dams. Values are mean ± SEM, control (n) = 10; *Jatropha* (n) = 10.

3.4 Effect of aqueous leaf extract of *Jatropha tanjorensis* on hormonal concentrations of dams after weaning

The results of hormonal concentration of postpartum dams immediately after weaning is as shown in Table: 4. The concentration of oxytocin, relaxin and LH was significantly (P<0.001) higher in treated the group versus control. The concentration of estrogen and progesterone was significantly (P<0.01) higher in treated the group versus control. The concentration of PRL was significantly (P<0.05) higher in treated the group versus control. There was no significant different in the concentration of FSH treated group versus control.

Table 3: Hormonal concentration of postpartum dams

Hormonal concentration	Control (n=10)	Jatropha (n=10)	P values
Luteinizing hormone	4.6 ± 0.6	13.9 ± 0.7	P<0.001
Follicle stimulating hormone	1.7 ± 0.8	1.6 ± 0.6	NS
Estrogen	44.2 ± 1.1	48.6 ± 1.1	P<0.05
Progesterone	26.1 ± 2.2	36.9 ± 2.4	P<0.01
Prolactin	0.4 ± 0.9	0.6 ± 0.3	P<0.05
Oxytocin	30.1 ± 2.1	104.1 ± 3.3	P<0.001

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Relaxin	82.9 ± 1.6	102.7 ± 3.7	P<0.001

Caption: n= number of animals in each groups, NS= not significant

4. Discussion

The significance of plants or their active principles in treatment of diseases can be traced to prehistoric times and herbs are being progressively studied by medical researchers (Igbayilola *et al*., 2021). Hence, this study describes the effect of gestational and postpartum administration of ALEJT on maternal behavior, offspring survival and growth during the weaning process.

From the results, consumption of ALEJT during pregnancy did not affect litter size and live birth but was effective for fetal growth. The weight of a child at birth is a critical determinant of the risk of intrauterine growth retardation (Freemark, 2010). However, our result shows that intrauterine growth indices (body length, tail length and birth weight) assessed at birth was higher in pups exposed to ALEJT compared to control pups. Hilaire *et al*., (2021) and Tessema *et al*., (2021) reported that birth weight is determined by intrauterine growth rate at birth. While Taofeeq *et al*., (2005) reported that fetal growth in rodents and humans is related to maternal intake during pregnancy, herbs transport across placenta and milk. *Jatropha tanjorensis* leaves are good sources of protein, iron, vitamins and mineral elements (Bello, 2014; Mbosowo and Osim 2018), and also improve haematological indices (Iroanya et al., 2018; Falodun et al., 2013). This implies that ALEJT is a rich source of blood and nutrients required for fetal growth.

The intensity and rates of maternal behaviors are most obvious starting at the time of birth, and these are affected by mother's nutritional status during the dependent period (De-Gabriel *et al*., 2009). From the results, the treated dams spent more time displaying maternal behaviors (pups retrieval, pup licking, crouching, resting with pups, sniffing pups and nesting) and lesser time displaying non-maternal behaviors (resting alone, gnawing and self-grooming). These are consistent with the findings of Pérez-Torrero and Rubio-Navarro (2015) who observed that lactating mother spent more of time to the pup care. Neurological changes that occur during pregnancy increase maternal nursing behavior and enable proper mother care for her newborn infant (Napso *et al*., 2018). This study suggests that ALEJT possesses active ingredients that influence maternal neuroendocrine organs to release hormones required for maternal care.

The number of pups survival after PND 1-7 did not differ, but percentage viability index, survival after PND 8-14 and PND 15-21 increased in pups exposed to ALEJT in-utero and in postnatal life compared to control pups. Childs birth weight is a critical determinant of neonatal morbidity and mortality (Freemark, 2010). Low birth weight is the major risk factor for adverse health outcomes during the neonatal period (Tessema *et al*., 2021; Hilaire *et al*., 2021). In this study, the birth weight of the exposed pups was higher. Infant survival depends crucially on correct maternal behavior, requiring ample investment of a mother's time and energy (Trillmich, 2010), as well as sensitive and contingent maternal responses properly attuned to the infant's signals which are vital for the infant's maturing capacity for self-regulation (Fonagy *et al*., 2007). Wansaw *et al*., (2018) reported that the behavior of postpartum rats towards pups changes with time and is mediated by the growth of the pups, while Pérez-Torrero and Rubio-Navarro (2015) observed that lactating mother spent most time to pup care mainly the first week and gradually decreases. In this study, the behavior of the treated dams with their pups was increased despite the growth of their pups. Also, Slomian *et al*., (2019) reported that mothers with depressive symptoms showed less closeness, warmth, sensitivity and lower level of emotional availability which has a negative effect on mother-to-infant bonding. In an earlier study we observed ALEJT alleviated

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postpartum-like depressive symptoms in dams (Antai *et al*., 2023). Pre-weaning index was reduced in the exposed pups, while weaning index was higher in the exposed pups compared to control. This result agrees with the fact that infants who are small at birth have a higher morbidity and mortality than those of normal size (Tessema *et al*., 2021).

In this study, weaning weight of the exposed pups increased which implies that pup's exposure to ALEJT in-utero and through suckling enhances pups growth rate. Hilaire *et al*., (2021) observed that low birth weight is associated with poor anthropometric growth. In this study the exposed pups had increase birth weight. Slomian *et al*., (2019) reported that infants of depressed mothers gained less weight than infants of non-depressed mothers. In an earlier study ALEJT alleviated postpartum depressive-like symptoms (Antai *et al*., 2023). The increase in the exposed pups body weight may be due to the plants phyto-constituents such as flavonoids and tannins. This is agrees with the study of Njoku-Oji *et al*., (2020) who reported that the increase in body weight of the litters of the lactating dams receiving the extract of *Phoenix dactylifera* may be due to the phytochemical constituents of the fruit – anthocyanins, flavonoids, and tannins.

Hormonal priming of the mother's brain boosts the rate of maternal behaviors that support the survival and development of the highly reliant offspring (Kohl and Dulac, 2018; Lonstein *et al*., 2015). From this study, levels of LH, oxytocin, relaxin, progesterone, estradiol and PRL in treated dams were higher. A strong suckling stimulus was reported to suppress the secretion of FSH and LH, with LH much more depressed than FSH (Taya and Sasamoto 1993; Mcguire *et al*., 1995). This study propose that ALEJT influences the pituitary gland of lactating rat to release higher amounts of LH and showed higher responsiveness to LH releasing hormone (LH-RH) which is inhibited during suckling to suppress LH secretion. This is contrary to the study of Taya and Sasamoto (1981) who reported that the pituitary of lactating rats released less LH and high FSH in response to LH-RH stimulation. In this study FSH level did not differ.

Estradiol is significant in stimulating onset of maternal behavior. The actions of progesterone, PRL and oxytocin are dependent on simultaneous exposure to estrogens (Riberio *et al*., 2012). The increase in estrogen level by ALEJT is consistent with the report of Njoku-Oji *et al*., 2020 who showed that ethanolic extract of *P. dactylifera* increase estrogen levels in lactating rats.

Progesterone in addition to its lactogenic action is vital in regulating the expression of maternal behavior. It primes the gestating brain to sensitize it to stimuli from young at parturition, and as well control the timing of increased responsivity (Zakar and Hertelendy, 2007). Studies in rodents show that estrogen and progesterone play a key role in triggering maternal responses and interfering with their function impairs pup retrieval, licking, and nursing (Fuentes *et al*., 2022).

Prolactin is a neuroendocrine hormone because its regulate breastfeeding and modulate lactation, as well as regulate mood swings and shape maternal behavior (Syam *et al*., 2021). In this study the rise in PRL can be attributed to the phytochemical constituents of ALEJT such as alkaloids, flavonoids, tannins and saponins (Ukoh *et al*., 2022). These constituents found in another plant were reported to raise PRL in lactating mothers. Alkaloids and flavonoids in Chaya leaf extract (Silawati and Zamzam 2020), steroidal saponins and flavonoids in Gongronema latifolium leaf (Ogbonna *et al*., 2022) and flavonoids, saponins, tannins, and alkaloids in ethanolic extract of *P. dactylifera* (Njoku-Oji *et al*., 2020) all raised PRL levels in lactating mothers.

Oxytocin, a neuropeptide implicated on survival and reproductive activities in vertebrate **(**Pérez-Torrero and Rubio-Navarro 2015) was reported as a modulator of maternal and social behavior, by improving maternal motivation to respond to young, in addition to its lactotropic actions (Robinson *et al*., 2015). It levels are known to rise

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during healthy mother–infant interactions (Strathearn *et al*., 2009). Fuentes *et al*., (2022) suggested an inverse relationship between oxytocin levels and depressive symptoms. While Antia *et al*., (2023) earlier reported that ALEJT alleviate depressive-like symptoms in rat. In this study ALEJT increased oxytocin level. Champagne *et al*., (2001) reported that dams that display high frequency of pups licking and grooming had higher levels of oxytocin receptor in distinct brain regions involved in the regulation of maternal behavior compared to those showing low licking and grooming behaviors.

Relaxin is required for the development of the mammary nipples that occurs during the second half of pregnancy in rodents. This effect on the mammary gland is vital in rodents (Kass *et al*., 2001). It is reported that there are specific relaxin binding sites in the nipples of rats as well as human and that relaxin acts directly on the nipples to bring about its maternal-infant stimulatory effects (Kuenzi *et al*., 1995; Kohsaka *et al*., 1998).

5. Conclusion

Following the results obtained in this study, it is believed that ALEJT is of rich nutritional value, and through modification of hormonal levels improves the functionality of the hypothalamic nuclei which facilitate maternal caregiving, pups survival and pups growth indices in rats. Assessing neurodevelopmental outcomes in these infants may help clinical decisions to be made thoughtfully for the use of ALEJT during gestation and postpartum to foster care for the neonate.

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