Nigerian J. Anim. Sci. 2020 Vol 22 (2):90-97 (ISSN:1119-4308)

Observations with estradiol titre, genitalia and parturition time in female albino rats treated with aqueous *Adansonia digitata* leaf extract

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***Target Audience:** Livestock producers, Researchers and Practitioners*

Abstract

Folkloric claims that Adansonia digitat*a possess parturition-aiding properties were investigated by treating pre-pubertal female (n=45) and gravid (n=60) albino rats per os with aqueous-leaf-extract. Pre-pubertal rats were equally randomized viz: APP (2 mls distilled-water), BPP (250 mg/kg-extract) and CPP (2 mg/kg Cyclo-progynova®17β estradiol), treated as indicated for 7 days. Gravid rats were similarly randomized viz: A (2 mls distilled-water), B, C and D (100, 250 and 500 mg/kg-extract), respectively, treated daily as indicated from day 19 post-mating, until parturition. Feeds and water were provided ad libitum. Blood was assayed for estradiol, and genitalia harvested, post-euthanization, at 24 hours post-treatment in pre-pubertal rats. Gestation-length, parturition-time, litter-size and birth-weight were evaluated in gravid rats. Differences in mean values of estradiol-titre (pg/ml), reproductive-tract-length (cms), ovarian-weight (grams) and uterine-weight (grams) for BPP (9.44±0.88, 5.40±0.38, 0.08±0.01, 0.24±0.03,) and CPP (10.56±0.41, 5.00±0.42, 0.10±0.03, 0.25±0.04) were higher (P<0.05) than for APP (3.82±0.48, 3.66±3.95, 0.03±0.01, 0.14±0.03), respectively. Differences in mean parturition-time (minutes) for A (88.6±2.73), B (81.2±4.06) and C (78.6±3.14) were higher (P<0.05) than for D (63.4±9.30). Differences in other parameters were not significant. Findings indicate that aqueous Adansonia digitata leaf-extract increased estradiol-titre and genitalia but shortened parturition-time in albino rats. Increased estradiol-titre possibly accounted for shortened parturition-time.*

***Keywords:** Estradiol-titre; Genitalia; Parturition-time; Adansonia digitata leaf-extract; Albino rats.*

Description of problem

Available claims that the plant, *Adansonia digitata*, possesses properties that are capable of aiding parturition in pregnant subjects are frequently encountered in folkloric literature. There are reports of instances where leaf concoctions of *Adansonia digitata* were used traditionally to relieve dystocia that resulted from uterine atony, and retained placenta (1, 2). Parturition is the complex physiological process involving mechanical, nervous and hormonal factors leading to the birth/expulsion of an erstwhile *in utero* fetus that often require only little support for survival (3). The pattern of parturition varies only slightly from one mammalian species to another but substantial differences have been observed in the duration, regardless of complications, especially in litter bearing species (3). For example, it had been reported that the duration of parturition i.e. parturition time in the sow, mare, cow and rat are 1-4 hours, 1-3 hours, 4-24 hours and 1-2 hours (4, 5), respectively. The stage one in all mammalian parturition is referred to as cervical dilatation, an event that depends on two major hormones of reproduction namely, estradiol and relaxin (5). Dilatation of the cervix is a rate limiting step of the entire parturition process, just as are: expulsions of

fetus and fetal membranes (5). Post dilatation, estradiol in combination with other hormones e.g. oxytocin is required for muscular contraction needed to void the fetus (6). The actions of estradiol are not limited to parturition. Estradiol is responsible for the development of secondary sexual characteristics which are normally evident in the general body and reproductive organs of female mammalian species (7). These often culminate in onset of sexual cycles that are characteristic of puberty (8). Delays in parturition are normally associated with grave consequences ranging from fetal and/or dam morbidities or death to losses in income and replacement stock (9). These pose threats to animal production industries and by extension, man's survival. Hence, obstetric interventions are such that shorten the length of the parturition process thereby, averting the consequences of delayed parturition. *Adansonia digitata*, known as Baobab plant, kuka in Hausa and Ose in Yoruba, is mainly found in savannah areas of Sub-Saharan Africa (10). It is a priced plant in Africa for its health boosting properties (11). However, there is scant documentation on the effect of its aqueous leaf extract on reproduction in female albino rats. This study therefore investigated the effects of aqueous *Adansonia digitata* leaf extract on estradiol titre, genitalia and some parturition indices in female albino rats.

Materials and Methods

Study Site

The study was carried out at the Assisted Reproduction Techniques Unit of the Department of Theriogenology, University of Ibadan, Oyo State, Nigeria.

Animals and Management

One hundred and twenty albino rats (15 adult males, 45 pre-pubertal and 60 adult females) were used for the study. They were purchased from a reputable rat breeder, kept in specially constructed cages and acclimatized for 2 weeks. They were fed standard rat pellets and water was provided *ad libitum.*

Source and authentication of *Adansonia digitata*

Fresh leaves of *Adansonia digitata* (Baobab) were obtained from apparently healthy trees in Ibadan, Oyo State, Nigeria. The leaves were authenticated and assigned the herbarium number UIH-22588 at the Department of Botany and Microbiology, University of Ibadan, Oyo State, Nigeria.

Preparation of Extract

Fresh leaves of *Adansonia digitata* were liberally rinsed in distilled water and wiped using a clean towel. The leaves were then oven-dried (25^0C) in the laboratory for 14 days. The dried leaves were pulverized to a coarse powder using wooden pestle and mortar, and stored in an air-tight container. A certain quantity (100 grams) of the pulverized *A. digitata* leaves was suspended in 1 litre of distilled water. The mixture was vigorously shaken and allowed to stand for 48 hours at 25^0C. Thereafter, the mixture was filtered with Whatman filter paper No.1. The filtrate/extract was stored in labelled bottles at 4^0C for subsequent use.

Study Groups

Animals were randomly assigned into two (2) studies as follows:

Study 1

Pre-pubertal female albino rats (n=45) weighing 50-60 g and aged 26-28 days were randomly assigned into three equal groups of fifteen (15) rats each as shown below:

APP: Control group. Rats were administered 2 mls distilled water *per os*

BPP: Rats were administered aqueous *Adansonia digitata* leaf extract at 250 mg/kg *per os*

CPP: Rats were administered 2 mg/kg Cyclo-progynova® 17β estradiol as reference drug *per os*

Duration of treatment
All treatments were administered daily for seven (7) days using an oral gavage.

Sample collection
Samples (blood and genitalia) were collected 24 hours post treatment from pre-pubertal rats as follows:

Blood collection
A volume (1 ml) of blood was collected from the media canthus, following restraint, through a capillary tube directed into a plain vacutainer tube. Vacutainer tubes were placed in slanting position for about 45 minutes to separate the serum.

Harvest of genitalia
Following sedation in a chloroform chamber, entry was made into abdomino-pelvic region via laparotomy and the entire genitalia from the ovaries to the vulva were carefully dissected out.

Evaluation of Parameters
Assay for Estradiol
Serum was centrifuged at 1000G for 10 minutes**.** The supernatant was thereafter decanted in Eppendorf tubes and kept at -20^{0}C. Estradiol (pg/ml) assay was carried out using ELISA kit (CALBIOTECH lnc. USA). Absorbance was measured with the aid of spectrophotometer at 450nm.

Reproductive tract length
Each complete genitalia were placed on a white smooth board and carefully straightened out without stretching. With the aid of a graduated tape placed adjacent to it, the entire length (centimeters) of the genitalia was obtained.

Weight: Ovaries and Uteri
The ovaries were carefully dissected along the broad ligament-ovarian boundary. The uterus was also dissected out by severing its cranial (utero-ovarian junction) and caudal (utero-cervical junction) borders. The ovaries and uteri were weighed using a digital electronic top loading scale (SF-400, China).

Study 2
Matured albino rats (60 females and 15 males, 200-250 g, aged 3-4 months) were used for this study. To achieve mating, females were introduced to males at a ratio of 4:1 for a period of 24 hours. Mating was confirmed to have occurred if sperm cells were observed in vaginal smears under X40 microscope. Post mating, rats were randomly assigned to 4 equal groups (15/group) as follows:
A- Control group. Rats were administered 2 mls distilled water *per os*
B- Rats were administered aqueous *A. digitata* leaf extract at 100 mg/kg *per os*
C- Rats were administered aqueous *A. digitata* leaf extract at 250 mg/kg *per os*
D- Rats were administered aqueous *A. digitata* leaf extract at 500 mg/kg *per os*

Duration of treatment
All treatments were administered to rats using an oral gavage starting from day 19 post mating until parturition.

Evaluation of parameters:
Gestation length
Gestation length was taken as the number of days between the day of mating and parturition.

Table 1: Comparism of estradiol titres (Mean ± S.E.M) in pre-pubertal female albino rats across treatment groups

Parameter	APP (2 mls distilled water) n = 15	BPP (250 mg/kg aqueous *A. digitata* leaf extract) n = 15	CPP (2 mg/kg Cyclo-progynova® 17β Estradiol) n = 15	P-value
Estradiol titre (pg/ml)	3.82 ± 0.48[b]	9.44 ± 0.88[a]	10.56 ± 0.41[a]	0.02

[a,b] Values with different superscripts in the same row differ significantly (P< 0.05)

Table 2: Comparism of mean values of reproductive tract length, ovarian weight and uterine weight in pre-pubertal female albino rats across treatment groups

Parameters	APP (2 mls distilled water) n = 15	BPP (250 mg/kg aqueous *A. digitata* leaf extract) n = 15	CPP (2 mg/kg Cyclo-progynova® 17β Estradiol) n = 15	P-value
Reproductive tract length (cm)	3.66 ± 3.95[b]	5.40 ± 0.38[a]	5.00 ± 0.42[a]	0.01
Ovarian weight (g)	0.03 ± 0.01[b]	0.08 ± 0.01[a]	0.10 ± 0.03[a]	0.04
Uterine weight (g)	0.14 ± 0.03[b]	0.24 ± 0.03[a]	0.25 ± 0.04[a]	0.04

[a,b] Values with different superscripts in the same row differ significantly (P< 0.05)

Parturition time

Gravid rats were transferred into separate well labeled cages for the purpose monitoring from the point treatment commenced. A common sign that was looked out for was the level of restlessness which normally increases towards parturition. Parturition time (minutes) was taken as the time elapsed from the birth of the first to the last pup.

Litter size

This is the total (n) number of pups birthed by gravid rats per group.

Birth weight

The birth weight of each pup was measured using a digital electronic top loading scale (SF-400, China).

Ethical consideration

Ethical guidelines for laboratory animal use and care as described by the Canadian Council on Animal use was adhered to (12).

Data Analysis

Data obtained were expressed as Mean ± S.E.M. Means of the parameters were compared using One-way analysis of variance (ANOVA) and Tukey Post-hoc test using Statistical Package for Social Science (SPSS) version 20. Values of P<0.05 were considered significant.

Result

The results obtained are presented in Tables 1-3. Table 1 shows the estradiol titres (Mean ± S.E.M) of pre-pubertal female albino

rats across the treatment groups. The differences in the mean values for BPP (9.44±0.88 pg/ml) and CPP (10.56±0.41 pg/ml) were higher (P<0.05) compared with APP (3.82±0.48 pg/ml). The difference in mean estradiol titres between BPP and CPP was however not significant (P>0.05).

Table 3: Comparism of mean values of gestation length, parturition time, litter size and birth weight of pups in gravid albino rats across treatment groups

Parameters	A	B	C	D	P-value
	(2 mls distilled water) n=15	(100 mg/kg aqueous *A. digitata* leaf extract) n=15	(250 mg/kg aqueous *A. digitata* leaf extract) n=15	(500 mg/kg aqueous *A. digitata* leaf extract) n=15	
Gestation length (days)	20.2±0.84[a]	19.6±0.55[a]	19.4±0.55[a]	19.6±0.89[a]	0.36
Parturition time (minutes)	88.6 ±2.73[a]	81.2 ± 4.06[a]	78.6 ± 3.14[a]	63.4 ± 9.30[b]	0.03
Litter size (n)	6.0 ± 0.32[a]	6.0 ± 0.32[a]	6.6 ± 0.87[a]	5.8 ± 0.49[a]	0.75
Birth weight (g)	4.32±0.11[a]	4.40±0.10[a]	4.23±0.12[a]	4.27±0.09[a]	0.68

[a,b] Values with different superscripts in the same row differ significantly (P< 0.05)

Table 2 shows the differences in the mean values of reproductive tract length, ovarian weight and uterine weight across the treatment groups**.** The differences in the reproductive tract lengths (Mean ± S.E.M) for pre-pubertal rats in BPP (5.40±0.38 cm) and CPP (5.00±0.42 cm) were higher (P<0.05) compared with APP (3.66±3.95 cm). The difference in the reproductive tract lengths between BPP and CPP was not significant (P>0.05). The differences in the ovarian weights (Mean ± S.E.M) for pre-pubertal rats in BPP (0.08±0.01) and CPP (0.10±0.03 g) were higher (P<0.05) compared with APP (0.03±0.01 g). The difference in mean ovarian weights between BPP and CPP was not significant (P>0.05). The differences in uterine weights (Mean ± S.E.M) for pre-pubertal rats in BPP (0.24±0.03 g) and CPP (0.25±0.04 g) were higher (P<0.05) compared with APP (0.14±0.03 g). The difference between BPP and CPP was also not significant (P>0.05).

Table 3 shows the differences in mean values of gestation lengths, parturition times, litter sizes and birth weights, of pups, across the treatment groups. The differences in gestation lengths (Mean ± S.E.M) for gravid/pregnant albino rats in A (20.2±0.84) days, B (19.6±0.55) days, C (19.4±0.55) days and D (19.6±0.89) days were not significant (P>0.05). The differences in parturition times (Mean ± S.E.M) of pregnant albino rats in D (63.4±9.30) minutes was lower (P< 0.05) compared with A (88.6±2.73) minutes, B (81.2 ±4.06) minutes, and C (78.6±3.14) minutes. The differences in parturition times observed in groups A, B and C were not significant (P>0.05). The differences in litter sizes (Mean ± S.E.M) in A (6.0±0.32), B (6.0±0.32), C (6.6±0.87) and D (5.8 ± 0.49) were not significant (P>0.05). The differences in birth weights (Mean ± S.E.M) of pups in A (4.32±0.11 g), B (4.40±0.10 g), C (4.23±0.12 g) and D (4.27±0.09 g) were not significant.

Discussion

The observation from Table 1 showed that aqueous *Adansonia digitata* leaf extract increased estradiol to a level that is comparable to the reference drug (Cyclo-progynova® 17β estradiol) and higher than the control. This suggests that the extract could have stimulated follicular development leading to an increase in estradiol production. This increase could also be attributed to flavonoids which are a component of the leaf of *A. digitata* (13) since earlier reports have not only shown that flavonoids have estrogenic activities but have affinity for estrogen receptors, thereby initiating estrogen synthesis (14, 15). The comparability of the effect produced by the reference drug to that of the extract suggests the potency of the extract at increasing estradiol titre, and thereby its bioactivity, in pre-pubertal rats. To a large extent, this singular observation suggests that aqueous *A. digitata* leaf extract may be used in instances where estrogens are required. The observations in the groups of pre-pubertal rats with regard to genitalia parameters (Table 2) were not only similar but comparable with present findings on estradiol titre. In all of these genitalia parameters, observations with aqueous *A. digitata* leaf extract and the reference drug were comparable. The changes (increases) observed with reproductive tract length, ovarian and uterine weights in the rats are normal physiological responses to increased estradiol in the circulation. This is because estradiol and other estrogens have been known to produce morphological and histological changes leading to growth and development of the genitalia (16, 17). Such changes are also normally associated with the follicular phase of the estrous cycle in pubertal and adult females (18). Having observed an increase in estradiol titre following the administration of the extract, it is then only normal or expected that the extract would cause increases in reproductive tract length, ovarian and uterine weights in treated rats, as the case was in this study. Present observations are similar to earlier reports that aqueous extracts of *Holarrhena floribunda* (19) and *Senecio biafrae* (20) increased uterine and ovarian weights. Observations from the present study further suggest that aqueous *A. digitata* leaf extract may be deployed to hasten puberty attainment in animals. The observation with parameters investigated in gravid albino rats (Table 3) was somewhat interesting. While various dosages of the extract produced no significant changes in gestation length, litter size and birth weight in the groups of rats, only the highest (500 mg/kg) produced a reduction ($P<0.05$) in the duration of parturition. Recalling the roles of estradiol in parturition (5), the effects of administered extract on parturition time apparently indicate that neither the dosage (250 mg/kg) which produced significant estradiol titre in pre-pubertal rats nor the lower (100 mg/kg), exerted significant influence on parturition time. Although, their effect on parturition time was not significant, both dosages have shown clinical significance with respect to the observed reduction ($P>0.05$) in parturition times compared to control rats. The administration of estradiol in pregnancy is a common cause of abortion (21). Abortion was not recorded in this study likely because of the point in gestation during which the extract was administered. Additionally, all parturitions occurred shortly after treatment with extract around day 19 and 21 post mating. This may have equally accounted for our observation of no significant differences in gestation length, litter size and birth weight in the study.

Conclusion and Applications

The study showed that:

1. Aqueous *Adansonia digitata* leaf extract increased estradiol titre in pre-pubertal female albino rats.
2. Aqueous *Adansonia digitata* leaf extract

increased reproductive tract length, ovarian and uterine weights in pre-pubertal albino rats.

3. Aqueous *Adansonia digitata* leaf extract shortened parturition time in gravid albino rats.
4. Findings of this study would be applicable to livestock production researchers and practitioners especially, those dealing with puberty control, parturition managers or obstetric veterinarians.

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