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## Effects of methanolic leaf extract of *Telfairia occidentalis* (hook f.) On the reproductive indices of female albino rats

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#### ABSTRACT

This study investigated the effects of oral administration of methanolic leaf extract of *Telfairia occidentalis* (MLETO) on the reproductive indices of female albino rats. Fifty-six Sprague-Dawley albino rats (42 females and 14 males) were used for the study. The females were randomly assigned into 7 groups (designated A, B, C, D, E, F and G). Group A received only distilled water, while groups B and C received 200 and 800 mg/kg body weight of MLETO for the first 7 days of gestation; groups D and E received 200 and 800 mg/kg of MLETO for the first 14 days, and groups F and G received 200 and 800 mg/kg of MLETO for the entire 21 days of gestation respectively. The males were only used in mating the females. The number of return to estrus per pregnancy, number of implanted/developing conceptuses, gestation length, litter size at birth, litter weight at birth, crown-rump length at birth and aborting rat were assessed. Quantal pregnancy, implantation index, viability index, birth index, live-birth index and percentage aborted were calculated. Serum

progesterone was also assayed. There were no significant ( $P > 0.05$ ) variations between the groups in all the reproductive indices assessed and also on the serum progesterone level. It was concluded that administration of MLETO as used in this study had no significant effect on the reproductive indices and serum progesterone levels of pregnant rats.

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## 1. Introduction

The fluted pumpkin plant *Telfairia occidentalis* (Hook f.) is a component of the Nigerian ethnomedical pharmacopoeia as well as an ingredient in many dishes. The plant is a common leafy vegetable indigenous to West Africa (Tindall, 1983). It is the second most consumed leafy vegetable after *Amaranthus* amongst most Nigerian homes (Mensah et al., 2008). The vine stem and leftover leaves are used as animal fodder (FAO, 2007).

The medical and pharmacological importance of this plant is enormous. The aqueous extract is commonly used ethno-medically as an herbal hematinic especially by post-parturient, pregnant and post-menstrual women (Ukwuoma and Muanya, 2005; Olaniyan et al. 2005) and as anti-convulsant and antimalarial agents (Olaniyan, et al., 2005; Ukwuoma and Muanya, 2005; Ehiagbonare, 2008; Oyeyemi et al., 2008; Kayode and Kayode, 2010). Phytochemical analysis of the plant showed that it is rich in the water soluble vitamins (thiamine, riboflavin, nicotinamide ascorbic acid), carotenoids, alkaloids, flavonoids, glycosides, oxalates, phenols, resins, saponins and minerals (Oboh, et al., 2006; Omale et al., 2009; Ogunlesi et al., 2010; Kayode and Kayode, 2010).

Effect of botanical on pregnancy is limited; nevertheless women from different cultures and economic backgrounds prefer to use herbal preparations during pregnancy (Dog, 2009). Some of these plants do have adverse effect on pregnancy (Ratnasooriya et al, 2003; Marcus and Snodgrass, 2005; Aronson, 2009) while others do not to have such adverse effect (Mendes et al, 2010).

Studies had shown that the plant extract has effects on male reproduction. Oyeyemi et al. (2008) reported a decrease in spermatozoa motility and moderate interstitial degeneration as well as interstitial oedema of testicular tissue following dosing with aqueous leaf extract of the plant. On the other hand Salman et al., (2008) reported increase in sperm motility (32.4%), sperm viability (30.7%) and sperm count (21.1%) following dosing with aqueous leaf extract of the plant. However, the report of Saalu et al. (2010) demonstrated that the effect of the aqueous extract of the plant is dose-dependent. At low dose ( $\leq 200$ mg/kg body weight daily oral dosing) the extract produced near normal sperm count, histopathological profile, morphometric parameters and hyperspermatozoa formation in the seminiferous tubule. But at higher doses (400 and 800 mg/kg body weight daily oral dosing) the extract produced testicular degeneration and deranged sperm parameters.

It is necessary to evaluate the effect of these natural health products during pregnancy and its potential effects to the fertility of the recipients (Dugoua, 2010). To the best of our knowledge, there are no reports in available literature on the effects of extracts of *T. occidentalis* on the reproductive indices of females. The aim of the present study was to evaluate the effect of methanolic leaf extract of *T. occidentalis* on the reproductive indices of female albino rats.

## 2. Materials and methods

### 2.1. Plant material

*Telfairia occidentalis* vines were procured from within Nsukka in Enugu State, Nigeria and authenticated by a plant taxonomist. The leaves were hand-picked off the vine and then air-dried under shade at room temperature. The dried leaves were pulverized and the weight determined. The pulverised leaves were then macerated in 80% methanol for 48 hours (with intermittent agitation) and afterwards filtered using Whatman No.1 filter papers. The filtrate was concentrated to obtain a crude extract (Asuzu, 2010; Omale and Okafor, 2009). The percentage yield was determined, and the extract was preserved in a refrigerator (4o C) throughout the duration of the study.

### 2.2. Animal

Fifty-six nulliparous Sprague-Dawley strain of albino rats (42 females and 14 males) obtained from the Laboratory Animal House of the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The rats were acclimatized for two weeks before the study commenced. Pregnancy was induced in the rats by introduction of proven sexually viable males overnight. Confirmation of pregnancy was by positive identification of seminal coagulate from vaginal swab (Ochiogu et al., 2006). The pregnant rats were separated and housed in stainless steel cages in the Experimental Animal House of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria. Commercial pelleted feed (Vital®, GCOML, Nigeria) containing 14.5% crude protein and 2,500 Kcal/kg metabolizable energy, and water were provided ad libitum. The rats were handled humanely throughout the duration of the study in accordance with the ethics for animal handling and research (Canadian Council on Animal Care, 2003; NHMRC, 2008).

### **2.3. Experimental design**

The study was done in two phases. Phase 1 investigated the effect of the methanolic extract of plant leaf on conception while the second phase studied the effect of the plant on gestation and the health of the resultant neonates. The 42 female rats were randomly assigned into 7 groups - designated A, B, C, D, E, F and G of 6 rats each. The 14 male rats were used in mating the females. They males were introduced to the females at a ratio of 1:3. The day of mating was designated the first day of gestation. Group A served as untreated control while groups B and C received 200 and 800 mg/kg body weight of the methanolic leaf extract *Telfairia occidentalis* (MLETO) respectively for the first 7 days of gestation (first trimester). Groups D and E received 200 and 800 mg/kg body weight of MLETO respectively for the first 14 days (first and second trimesters). Groups F and G received 200 and 800 mg/kg body weight of MLETO respectively for the entire gestation period. The extract was first emulsified in 5% volume of Tween 20® (to enhance dissolution in distilled water) before daily oral dosing with the aid of orogastric cannula. Daily cage-side examination following dosing was done to detect overt signs of toxicity, morbidity or mortality.

### **2.4. Acute toxicity test**

The acute toxicity was determined according to the method of Lorke (1983).

### **2.5. Progesterone assay**

On the first, tenth and nineteenth days of gestation blood samples were collected from the rats in groups A, F and G (groups F and G were the only two that received MLETO throughout the entire gestation which span the days progesterone assay were to be determined). Serum samples were harvested from the blood samples. The mean concentration of progesterone was assayed using direct enzyme immunoassay method of Monobind Inc® (2011).

### **2.6. Determination and calculation of the reproductive indices**

Between the 10th – 15th days of gestation, laparotomy was performed under anaesthetic aseptic conditions on the rats of each group; the uteri were examined in situ. The sites of implantation/developing conceptuses, site of resorption, dead foetus(es) as well as number of corpora lutea on the ovaries were recorded. The laparotomy sites were then sutured and antibiotic administered, the rats were allowed to carry the pregnancy to term to give birth. The number of return to estrus(es) per pregnancy, number of implantated/developing conceptuses, gestation length, litter size at birth, litter weight at birth, crown-rump length at birth and number of aborting rat were also recorded.

From the determined reproductive indices, the following remaining indices were calculated using standard formulae (Ratnasooriya, 2003): quantal pregnancy = (number of pregnant rats ÷ number mated) X 100; implantation index = (total number of implantations ÷ number mated) X 100; viability index = (number of live pups on day 4 post-partum ÷ number of live-born pups) 100; birth index = (number of pups born ÷ number of implantations) ÷ 100; foetal survival ratio = (number of surviving pups on day 4 ÷ number of implantations) X 100; live birth index = (number of live-born pups ÷ total number of pups born) X 100; gestation index = (number of live pups ÷ number of pregnant rats) X 100; resorption index = (total number of resorption site ÷ total number of implantation sites) X 100 and percentage aborted = (number of rats that aborted ÷ number of rats) X 100.

Following parturition, the neonates were monitored closely for any gross external congenital abnormalities such as deformed tail, clubfoot, oligodactyly or syndactyly; day of opening of the eye and day of appearance of fur and also neonatal mortalities/survival up to the sixth day postpartum.

### 2.7. Data analysis

Data obtained from the study were subjected to one way analysis of variance. Variant means were separated post-hoc using the least significant difference method. Probability less than 0.05 was considered significant.

### 3. Results

The percentage yield of the extract was 14.57% weight per weight. The crude extract was observed to be oily, brownish-green in colour and not readily soluble in water, with some crystalline precipitates after cooling (which however disappeared on stirring).

Acute toxicity test showed that MLETO was well tolerated by the rats even beyond 5000mg/kg body weight. Following dosing of the pregnant animals, no signs of toxicity, morbidity and mortality were observed.

None of the rats in the groups returned to estrus after mating was confirmed (Table 1). Following laparotomy, the number of implanted conceptuses and implantation sites were highest in group A, followed by group G, then F and was lowest in group D but these differences were not found to be statistically significant ( $P > 0.05$ ) (Table 1). The mean gestation length (days) of the rat groups ranged from  $21.40 \pm 0.24$  recorded in groups B and G to  $21.83 \pm 0.10$  recorded for rats in group D and there were no significant variations ( $P > 0.05$ ) between the groups (Table 1). The mean litter size of birth was highest in group A rats (9.0) followed by group F (8.8) and then G, and the lowest values of 5.5 was recorded for groups B rats (Table 1). The mean litter weight of birth (g) ranged from  $5.48 \pm 0.13$  recorded for group B rats (Table 1). There were no significant variations ( $P > 0.05$ ) in the mean litter weight at birth of all the rat groups (Table 1).

**Table 1**

The measured reproductive indices ( $\pm$  standard deviation) of female rats given varied doses of methanolic leaf extract of *Telfairia occidentalis* for varied periods during pregnancy.

Indices	A (untreated control)	B (200 mg/kg for 7 days)	C (800 mg/kg for 7 days)	D (200 mg/kg for 14 days)	E (800 mg/kg for 14 days)	F (200 mg/kg for 21 days)	G (800 mg/kg for 21 days)
Number of return to estrus per pregnancy	0	0	0	0	0	0	0
Number of implanted/ developing conceptuses	9.0 $\pm$ 0.58	7.2 $\pm$ 0.66	7.5 $\pm$ 1.23	7.1 $\pm$ 0.70	7.8 $\pm$ 0.86	8.1 $\pm$ 0.94	8.2 $\pm$ 1.32
Mean number of implantation sites	9.0 $\pm$ 0.58	7.2 $\pm$ 0.66	7.5 $\pm$ 1.23	7.1 $\pm$ 0.70	7.8 $\pm$ 0.86	8.1 $\pm$ 0.94	8.2 $\pm$ 1.32
Mean gestation length (days)	21.66 $\pm$ 0.21	21.40 $\pm$ 0.24	21.33 $\pm$ 0.21	21.83 $\pm$ 0.10	21.2 $\pm$ 0.20	21.6 $\pm$ 0.24	21.4 $\pm$ 0.24
Mean litter size at birth	9.0 $\pm$ 0.58	6.6 $\pm$ 0.93	7.2 $\pm$ 1.23	7.2 $\pm$ 0.70	7.8 $\pm$ 0.86	8.8 $\pm$ 0.86	8.0 $\pm$ 1.10
Mean litter weight at birth (g)	5.48 $\pm$ 0.11	5.80 $\pm$ 0.13	5.83 $\pm$ 0.11	5.58 $\pm$ 0.07	5.66 $\pm$ 0.11	5.54 $\pm$ 0.11	5.60 $\pm$ 0.16
Mean crown-rump length at birth (mm)	36.7 $\pm$ 0.20	37.3 $\pm$ 0.14	36.9 $\pm$ 0.26	37.2 $\pm$ 0.11	37.2 $\pm$ 0.11	36.8 $\pm$ 0.27	36.9 $\pm$ 0.28
Number of aborting rats	0	0	0	0	0	0	0

There was also no significant variation ( $P > 0.05$ ) in the mean crown-rump length at birth of the rat groups, which ranged from  $36.70 \pm 0.27$  recorded for rats in group A to  $37.30 \pm 0.14$  recorded for rats in group B (Table 1). None of the rats in any of the groups aborted the pregnancy (Table 1).

**Table 2**

The calculated reproductive indices ( $\pm$  standard deviation) of female rats given varied doses of methanolic leaf extract of *Telfairia occidentalis* for varied periods during pregnancy.

Indices	A (untreated control)	B (200 mg/kg for 7 days)	C (800 mg/kg for 7 days)	D (200 mg/kg for 14 days)	E (800 mg/kg for 14 days)	F (200 mg/kg for 21 days)	G (800 mg/kg for 21 days)
Quantal pregnancy (%)	100	100	100	83.3	100	100	100
Implantation index (%)	720.0	750.0	716.7	767.0	816.0	800.0	900.0
Viability index (%)	100	100	100	100	100	100	100
Birth index (%)	100	100	100	100	100	100	100
Live-birth index (%)	100	100	100	100	100	100	100

Laparotomy revealed that all the MLETO treated rats had developing conceptuses with corresponding corpora lutea as observed in the control rats. All the rats recovered from the laparotomy, carried the pregnancy to term and experienced eutocia. There were no obvious variations in all the calculated reproductive indices of all the treated groups when compared with the control group (Table 2). There was no significant ( $P > 0.05$ ) difference in the mean serum progesterone concentration on days 1, 10 and 19 between groups A, F and G (Table 3). However, there were marked progressive increases on days 10 and 19 across the groups (Table 3).

**Table 3**

The serum progesterone concentration ( $\pm$  standard error) of female pregnant rats given varied doses of methanolic leaf extract of *Telfairia occidentalis* (ng/ml).

Gestation	Group A (untreated control)	Group F (200mg/kg)	Group G (800mg/kg)
Day 1	17.30 $\pm$ 3.93	15.00 $\pm$ 1.15	17.00 $\pm$ 7.37
Day 10	68.70 $\pm$ 6.96	71.50 $\pm$ 8.50	63.50 $\pm$ 6.50
Day 19	120.00 $\pm$ 11.55	95.00 $\pm$ 15.00	110.50 $\pm$ 19.50

#### 4. Discussion

Oral administration of *Telfairia occidentalis* methanolic leaf extract had no significant effect on the fertility of female albino rats with respect to serum progesterone of pregnancy concentration and reproductive parameters (quantal pregnancy, implantation index, viability index, birth index, foetal survival ratio, live birth index, gestation index, resorption index and percentage aborted) in the first, second and third trimesters of gestation at the different doses administered.

The quantal pregnancy was 100% in all the groups except in group D. This shows that the extract may not negatively be affecting the establishment of pregnancy. The implantation index had the highest value in the control group however, the disparity was not significant. However, the implantation site/number of conceptuses in each of the groups did not significantly differ when compared to that of the control, likewise the gestation length.

Reproductive indices such as birth index, viability index and live-birth index were 100% in all the groups. The birth index is an indicator of post-implantation survival, while the viability and live-birth indices are indicators of foetal viability or survivability (Ratnasooriya et. al., 2003). A percentage of 100 in these indicators following dosing with MLETO show that there were no post-implantation losses and also that MLETO had no effect on the foetal viability.

When laparotomy was performed on the rats between the tenth to the fifteenth day of gestation, there were developing conceptuses in the rats in all the groups. Also the presence of corpora lutea was established.

The peripheral progesterone profile of pregnancy together with the presence of developing conceptuses and corpora lutea demonstrates that pregnancy was established and progressing. The progesterone assay and laparotomy were done on days ten to fifteen, and this range of days is already beyond the first trimester and into the second trimester showing that the extract had no adverse effect on gestation in the first trimester.

Furthermore, when progesterone assay was repeated on day 19 of gestation there was increase in the level of circulating progesterone in all the rats of all the three groups. This is expected in pregnant rats in which progesterone level peaks towards term (Magness, 1988).

The findings in this present study of no significant effect on female reproductive indices is in contrast with the reports of testiculo-active effects of extracts of *Telfairia occidentalis* in males (Oyeyemi et al., 2008; Salman et al., 2008; Saalu et al., 2010). The absence of significant adverse effect of the plant leaf extract on gestation of female rat is supported by the report of Mendes et al. (2010) who demonstrated that *Bauhinia monandra* aqueous and ethanol extracts had no observable adverse effects in pregnant rats. It is thought that those testiculo-active phytochemicals do not exert any effects on pregnancy and pregnancy outcome. It may also partly be due to the fact that this present study was conducted using methanolic extract which extracted both the lipid and some of the aqueous component of the phytochemical components of the plant leaf while some of the earlier reported studies utilized aqueous extracts.

## 5. Conclusion

Based on the results of this study, it was concluded that administration of MLETO at the dose used in this study did not significantly affect the reproductive indices and serum progesterone concentration of pregnant female rats and thus did not adversely affect gestation in the rats.

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