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Reproductive impact of aqueous leaf extract of *Mangifera indica* (Mango) on some reproductive functions in female Sprague-Dawley rats

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Abstract
Reproductive impact of oral administration of aqueous leaf extract of *Mangifera indica* (MILE)at a dose of 500 mg/kg was investigated. The first set of non-gravid rats was used to study hormonal and estrous cycling pattern after four weeks of extract administration. Estrous cycle was monitored by vagina smear technique, weekly weight recorded, and serum collected at the end of treatment period. The second set of gravid rats treated with extract during pregnancy was used to study effects on pregnancy and its outcome. Weekly weights were recorded and the number of viable fetus and resorption sites were counted on gestational day 19 after laparotomy. Number and weight of litter delivered were also recorded. The extract significantly reduced weight gained while there was also disruption of estrous cycling. Serum follicle stimulating hormone (FSH) level and litter birth weights were also significantly reduced. There was no effect on the number of viable fetus and duration of pregnancy. These results revealed that oral administration of aqueous MILE reduced weight gain, disrupted estrous cycling, reduced serum FSH while increasing estradiol level in non-pregnant rats. It also reduced maternal weight and litter birth weight. However, it has no significant effect on duration of pregnancy.

Keywords: *Mangifera indica*; oestrous cycle; FSH; estradiol; viable fetus; resorption; birth weight.

Introduction

There is an increasing awareness about the beneficial effects of medicinal plants worldwide (Dahanukar *et al.*, 2000). Mango leaf (*Mangifera indica*) use as a medicinal plant is dated back to as early as 327 B.C. (Morton, 1987). Various parts of the tree are reported to be used in traditional or folk law medicine. The leaves were reported to be used by Muruganandan *et al.*, (2005), the fruits by Schieber *et al.*, (2003), the stem-bark by Núñez Sellés *et al.*, (2002), and even the seed by Schieber *et al.*, (2003). The antioxidant properties of *M. indica* have been well documented (Kondo *et al.*, 2005). Aqueous decoction of the flower was also reported to have among other properties antiulcerative effect in piroxicam-induced gastric lesions (Lima *et al.*, 2006; Severi *et al.*, 2009). Hypoglycemic effect of the aqueous extract of the leaves has been reported (Muruganandan *et al.*, 2005; Morsi *et al.*, 2010; Pratul and Ranjit, 2012). Other reported activities includes, antiinflammatory and analgesic effect (Islam *et al*., 2010), antimicrobial activities of the leaves and stem-bark (Islam *et al*., 2010; Mada *et al*., 2012), neuroprotective effect (Kawpoomhae *et al.*, 2010), and antioxidative potentials (Olabinri *et al*., 2010; Kawpoomhae *et al.*, 2010). Phytochemical screening of the leaf extract revealed the presence of high concentration of phenols and flavonoids (Aiyelaagbe and Osamudiamen, 2009; Olabinri *et al*., 2010; Morsi *et al.*, 2010). Lima *et al*., (2006) reported a wide oral dose safety margin of the aqueous extract of the leaves, with no sign of toxicity at dose up to 5 g/kg. Other authors have also worked on animals using a dose of 600 mg/kg (Pratul and Ranjit, 2012).

The leaves and various other parts used are used indiscriminately, as pregnant women and children are usually made to drink from the

concoction in folk medicine in treating malaria (Odugbemi *et al.*, 2007; Dike *et al.*, 2012). There is dearth of information on the possible effect of this widely used medicinal plants on maternal and foetal physiology, especially likely effect on safety during pregnancy. Hence, this research is intended to evaluate possible effects of the leaf extract of *M. indica* (MILE) on female reproductive functions as well as induction and maintenance of pregnancy and survival of the fetuses there in.

Materials and Methods

Plant material

The extract was prepared by using sun dried leaves of *M. indica* harvested during the raining season (march–april). The dried leaves were squeezed into powdery form after which aqueous extraction was carried out using Soxhlet extractor. The extract was stored in a sterile container at –4°C. Fresh extract was always prepared when needed.

Animal grouping and treatment

Forty pubertal female Sprague-Dawley rats with relatively regular estrous cycle were used for this study. All animals used were housed in plastic cages under a 12 h light/dark cycle with lights on at 6 am (Olatunji-Bello and Aliu, 2000), in a clean laboratory environment and provided food and water *ad libitum.* They were divided into two sets, Set A and Set B each containing 20 rats. Each set was further divided into two groups, control and test. Set A rats were used for weight monitoring study, estrous cycle study, and hormonal assay. Estrous cycle study was carried out on group A rats for a period of thirty days during which the test group received 500 mg/kg b.w./day of aqueous MILE orally while the control group received equal volume of the vehicle distilled water (Ojewole, 2005). Weight of each animal was recorded daily throughout the experimental period. Daily vaginal smear of female rats were collected in the morning on a clean slide and score under the microscope according to Marcondes *et al*., (2002). Blood sample was collected via cardiac puncture after cervical dislocation at diestrous phase, centrifuged at 3000 rpm and serum collected into a sterile bottle for hormonal assay.

Set B rats with normal estrous cycle were allowed to mate freely with adult male of proven fertility, and were divided into test and control groups, once mating was confirmed with the presence of vaginal sperm plug. They were used for assessment of viable fetus and resorption studies as well as the effects on pregnancy outcome. The test group which is made up of ten pregnant rats received 500 mg/kg b.w./day orally from day 1 to day 19 of pregnancy while control received only equivalent of the vehicle (distilled water) daily for 19 days. Daily weight of each animal was recorded. Half of the animals in each group were sacrificed after cervical dislocation on the day 19 of pregnancy to assess the number of viable fetus and resorption site. The rest were allowed to carry the pregnancy to term and, the weight and number of litter delivered recorded against the duration of the pregnancy.

Hormonal assay

Hormonal assay was carried out using enzyme immunoassay kit (EIA) by Immunometrics, UK. Duplicate analyses of the samples were performed for accuracy.

Statistical analysis

All results were presented as mean ± SEM and analyzed using ANOVA. Bar chart and line graph were used for graphical presentation. Level of significance was placed at $p < 0.05$.

Results

Body weight changes (Figure 1 and Table 1)

Extract treated non-gravid rats showed a significant reduction in weight gain after three weeks of oral administration of aqueous MILE at a dose of 500 mg/kg b.w./day. The body weights at the end of four weeks in control and extract treated rats were 183.33 ± 2.10 g and 153.89 ± 2.02 g, respectively (Figure 1). Similar results was recorded in gravid rats where weight gain during pregnancy was significantly reduced at third week of pregnancy (control = 61.54% and treated 47.97% of initial body weight).

Estrous cycle (Table 2)

Oral administration of aqueous MILE at a dose of 500 mg/kg b.w./day for a period of 30 days, significantly ($p < 0.05$) alters the normal estrous cycling in pubertal female rats. Estrous phase occurrence in extract treated rats was reduced to 1.80 ± 0.36 days while that control to 6.40 ± 0.36 days. Diestrous phase

Figure 1: Effect of aqueous MILE (500 mg/kg b.w./day) on body weight of pubertal non-pregnant female Sprague-Dawley rats.

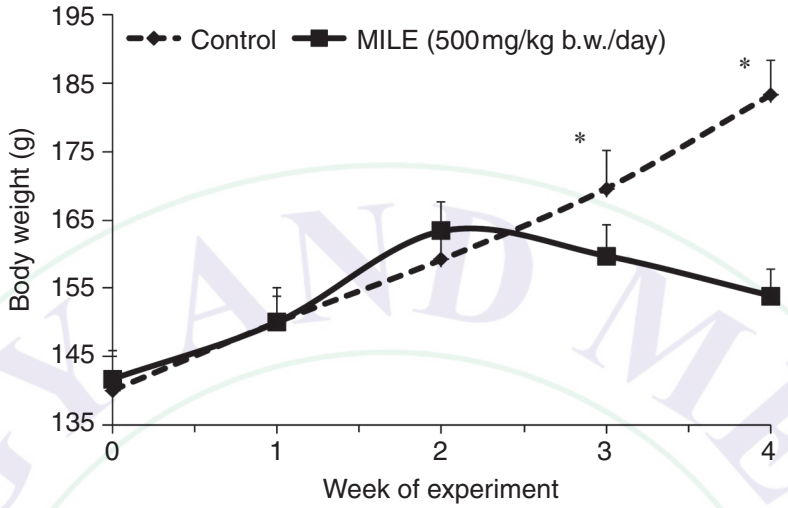

Table 1: Effect of aqueous MILE (500 mg/kg b.w./day) on maternal weight before and during pregnancy.

Period of pregnancy	Control		MILE treated	
	Weight (g)	% increase	Weight (g)	% increase
Days	146.25 ± 0.56	–	153.75 ± 3.08*	–
1st week	160.00 ± 0.00	9.40	172.31 ± 2.82	12.07*
2nd week	195.00 ± 10.00	33.33	203.34 ± 3.55	32.25
3rd week	236.25 ± 8.75	61.54	227.49 ± 3.43	47.97*

*Significant compared to control group result at $p < 0.05$.

Table 2: Effect of aqueous MILE (500 mg/kg b.w./day) on estrous cycle phases in pubertal female Spraque-Dawley rats.

Phase of cycle	Control		Mile treated	
	Frequency (days)	% of phase in a cycle	Frequency (days)	% of phase in a cycle
Diestrous	13.10 ± 0.38	46.80	19.40 ± 0.65*	69.30
Proestrous	6.80 ± 0.25	24.30	6.20 ± 0.57	22.10
Estrous	6.40 ± 0.36	22.90	1.80 ± 0.42*	6.40
Metestrous	1.70 ± 0.47	6.10	0.70 ± 6.21	2.50

*Significant compared to control group result at $p < 0.05$.

occurrence significantly increased in treated rats (19.40 ± 0.65 days) compared to control (13.10 ± 0.38 days).

Female hormonal profile (Figure 2 and Figure 3)
Out of the four female hormones measured, only follicle stimulating hormone (FSH) and estradiol recorded a significant reduction and increase, respectively. Serum FSH level in extract treated rats and in control were 0.50 ± 0.10 and 0.04 ± 0.01 iu/L, respectively. Estradiol level was also significantly increased in extract treated rats, of 0.08 ± 0.01 nmol/L compared with control that recorded a value of 0.05 ± 0.01 nmol/L.

Figure 2: Effect of aqueous MILE (500 mg/kg b.w./day) on FSH and LH.

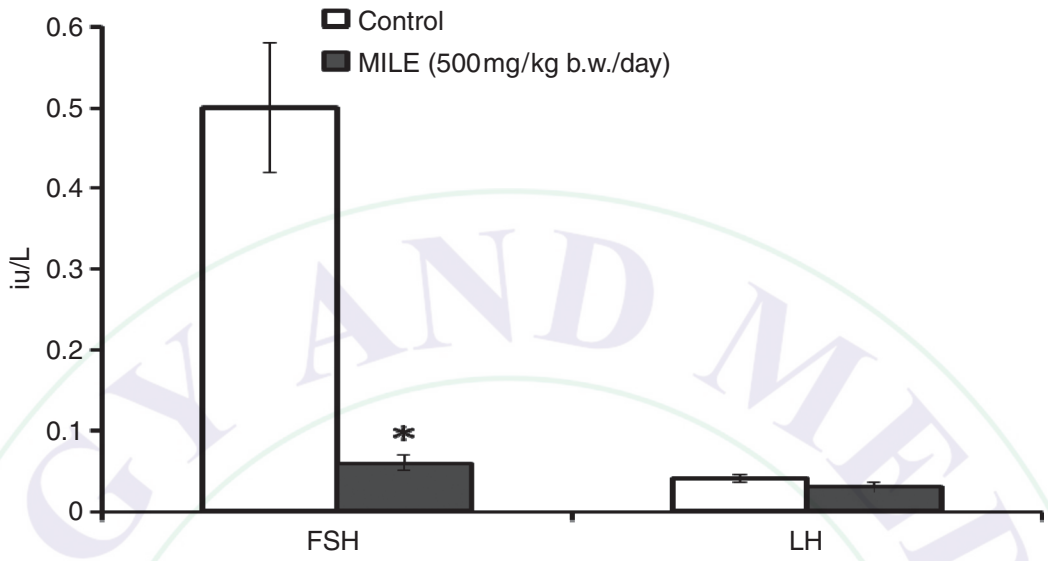

Figure 3: Effects of oral administration of aqueous MILE (500 mg/kg b.w./day) on serum estradiol and progesterone level in pubertal female rats compared with the control.

Table 3: Effect of aqueous MILE (500 mg/kg b.w./day) on number of viable fetuses and number of resorption sites, number and weight of litter delivered at term and duration of pregnancy compared with control rats.

Pregnancy outcome parameter	Control	MILE treated
Number of viable fetuses	10.00 ± 0.00	10.00 ± 0.00
Number of resorption sites	0.00 ± 0.00	0.00 ± 0.00
Number of litter delivered	10.00 ± 0.00	10.10 ± 0.00
Litter weight (g)	3.07 ± 0.03	1.34 ± 0.21
Duration of pregnancy (days)	21.00 ± 0.00	21.00 ± 0.00

Pregnancy and pregnancy outcome (Table 3)
There was no significant difference between the number of viable fetuses and the number of litter delivered between control and treated groups. No resorption was recorded in either control nor extract treated group at day 19 of pregnancy. However, the weight of litter delivered was significantly reduced in extract treated group (1.34 ± 0.21 g) compared to control (3.07 ± 0.03 g). The pregnancy duration was also not affected by extract administration.

Discussion

The present study showed that oral administration of aqueous MILE to pubertal female rats at a dose of 500 mg/kg b.w./day for a period of four weeks significantly disrupted the estrous cycle. This was evident by the significant increase in the average number of days for the diestrous compared to the control and a significant reduction in the average number of days for estrous compared to the control. The fertile period in female rats is between proestrous and estrous phase (Marcondes *et al.*, 2002). Environmental influences which includes diet especially those containing phytoestrogens are known to adversely affect female reproductive cycle and their fertility (Burton and Wells, 2002). The oestrous cycle is functionally under the direct regulation of the pituitary ovarian hormone; FSH, estrogen, pregesterone, and Luteinizing hormone (LH) which usually peaks during the oestrous phase of the cycle (Campbell, 2009). Serum hormones of the treated animals revealed a significant reduction in the FSH secreted from the pituitary compared to control. Estradiol level was also significantly increased compared to control while other hormones such as progesterone and LH were not affected. FSH is responsible for stimulating the growth of the graffian follicles indicating that the MILE influenced the cycle via the pituitary ovarian axis hormones, as a reduction in the FSH level will also potentate a decrease in growth of the follicles which are ultimately released during ovulation. However, no significant alteration was recorded in the number of implants or viable fetus at day 19 of pregnancy in extract treated rats compared to control. Further work will be required to investigate the effect of the extract on ovulation and fertilization of the oocyte.

Studies on implantation resorption activities showed no difference between the treated compared with the control as no resorption was recorded in all the treated rats at the day 19 of pregnancy giving an indication that the extract does not possess abortificient activities at the dose used. In addition, the number of implants was not significantly different between control and treated rats suggesting that the extract does not induce ovulation at the dosage used. Thus the average numbers of litter delivered by the treated rats were not significantly different from that of the control. Mango leaves is part of decoction used in folk law medicine as antimalarial decoction.

However, litter delivered by pregnant rats that were treated with the extract recorded a significantly reduced weight relative to the control litter. This corroborates previous report in which low weight gain at any of the trimester was associated with a significant decrease in birth weight (Abrams and Selvin, 1995). The significant reduction in birth weight may be associated with the earlier reported hypoglycemic activities of the leaf extract (Muruganandan *et al*., 2005). Decrease in energy availability for placenta fetal transport during pregnancy will reduce energy available for fetuses. This has been well linked to the onset and development of intrauterine growth restriction (IUGR) and low birth weight. Low birth weight, preterm birth, and IUGR which are adverse birth outcomes represent the leading causes of neonatal death among children born without congenital anomalies (Bhutta *et al.*, 2005; Abu-Saad and Fraser, 2010). Similar observation was recorded by Ogata *et al.*, (1987) and Lueder *et al.*, (1992) in which pregnant rats that suffered from insulin induced hypoglycemia delivered litters with significantly reduced weight compared to the control.

The present study further revealed that although, pubertal non-pregnant female rats treated with the extract registered a weight gain like the control rats within the four weeks treatment period, a significant weight loss was however recorded at the third and fourth weeks of treatment. Previous work on nutritional utilization of the *M. indica* leaves by rabbits had reported poor acceptance and reduced intake of the leave foliage because of its high fiber content (Aduku *et al.*, 1989).

Conclusion

Oral administration of aqueous MILE at a dose of 500 mg/kg b.w./day significantly disrupted the

estrous cycling of matured female rats as it alters the hormonal profile responsible for the synergy between the phases of the estrous cycle and ovulation. It also significantly reduced maternal body weight gained and litter birth weight.

Ethical Approval

This study was approved by the ethics committee of the College of Medicine, University of Lagos, Lagos, Nigeria.

Conflict of Interests

We declare that there is no conflict of interest whatsoever concerning this research work.

Authors' Contributions

All authors contributed equally to this study.

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