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Reproductive Impact of Aqueous Leaf Extract of *Magnifera indica* (Mango) Leaves on Some Reproductive Functions in Female Sprague-Dawley Rats

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Abstract: The reproductive impact of oral administration of aqueous leaf extract of *Magnifera indica* at a dose of 500 mg kg^{-1} was investigated. The first sets of non-gravid rats were used to study hormonal and estrous cycling pattern after extract for 4 weeks. 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 the extract throughout the pregnancy period was used for the pregnancy study. Weekly weights were recorded while numbers of viable foetus and resorption sites were counted on gestational day 19 after laparotomy. In group that carried their pregnancy to term, number and weight of litter delivered were recorded. Both gravid and non-gravid rats treated with the extract experienced significant reduction in weight gained while there was also disruption of estrous cycling. Serum FSH level and litter birth weights were also significantly reduced. There was no effect on the number of viable foetus and duration of pregnancy. These results revealed that the aqueous extract of *Magnifera indica* disrupted oestrous cycling in female rat. The extract also interfered with weight gain with resultant reduction litter birth weight. It also produced a reduction in the FSH while increasing the estradiol level in non-pregnant rats. However, it has no significant effect on duration of pregnancy.

Key words: *Magnifera indica*, oestrous cycle, FSH, estradiol, viable foetus, resorption, birth weight

INTRODUCTION

There is an increasing awareness about the beneficial effects of medicinal plants worldwide (Dahanukar *et al*., 2000). Mango leaf (*Magnifera indica*) use as a medicinal plant is dated back to as early as 327 BC (Morton and Miami, 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), stem-bark by Selles *et al*. (2002) and even the seed by Schieber *et al*. (2003). The antioxidant properties of *Magnifera 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; anti-inflammatory and analgesic effect (Islam *et al*., 2010), antimicrobial activities of the leaves and stem-back (Islam *et al*., 2010; Mada *et al*., 2012), neuroprotective effect (Kawpoomhae *et al*., 2010) 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^{-1}. Other researchers have also worked on animals using a dose of 600 mg kg^{-1} (Pratil and Ranjit, 2012).

The leaves and other various 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 were very few or no report 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 *Magnifera indica* on female reproductive functions as well as induction and maintenance of pregnancy and survival of the foetuses there in.

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MATERIALS AND METHODS

Plant material: The extract was prepared by using sun dried leaves of *Magnifera indica* harvested during the raining season (March to 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 and treatment: Forty pubertal female Sprague-Dawley rats with relatively regular estrus 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 6:00 am (Olatunji-Bello and Aliu, 2000), in a clean laboratory environment. Food and water were provided *ad libitum*. They were divided into two sets; set A and B each containing 20 rats. Each set is 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 30 days during which the test group received 500 mg kg^{-1} BW of aqueous leaf extract of *Magnifera indica* (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 foetus 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^{-1} BW/day orally from days 1-19 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 19 day of pregnancy to assess the number of viable foetus 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 was carried out using Enzymes Immuno-Assay kit (EIA) by Immunometrics UK. All rats used were anesthetized before collection of terminal data.

Anesthesia used was urethane-chloralose, dose 5 mL kg^{-1} BW intraperitonially (urethane 6.25 g, chloralose 0.25 g). 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 AND DISCUSSION

Body weight changes: Extract treated non-gravid rats showed a significant reduction in weight gain after 3 weeks of oral administration of aqueous leaf extract of magnifera indica at a dose of 500 mg kg^{-1} body weight. The body weights at the end of 4 weeks in control and extract treated rats were 183.33±2.10 and153.89±2.02 kg, respectively. Similar results was recorded in gravid rats where weight gain during pregnancy was significantly reduced at 3rd week of pregnancy (3rd week control = 61.54%, treated 47.97% of initial body weight) (Fig. 1 and Table 1).

Estrous cycle: Oral administration of aqueous leaf extract of *Magnifera indica* at a dose of 500 mg kg^{-1} body weight for a period of thirty days significantly ($p<0.05$) alters the normal estrous cycling in pubertal female rats. Estrus phase occurrence in extract treated rats was reduced to 1.80±0.42 days while the control was

Table 1: Effect of aqueous leaf extract of *Magnifera indica* (500 mg kg^{-1} BW) on maternal weight before and during pregnancy

Period of pregnancy	Control		MIL extract treated	
	Weight (g)	Increase (%)	Weight (g)	Increase (%)
Day	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±10.0	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 $p<0.05$

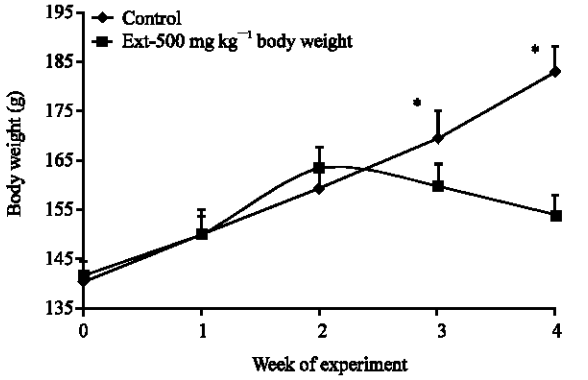

Fig. 1: Effect of aqueous leaf extract of *Magnifera indica* (500 mg kg^{-1} BW) on body weight of pubertal non-pregnant female Sprague-Dawley rats. $p<0.002$

6.40±0.04 days. Diestrus phase occurrence was significantly increased in treated rats (19.40±0.65 days) compared to control (13.10±0.38 days) (Table 2).

Female hormonal profile: Out of the four female hormones measured, only FSH and estradiol recorded a significant reduction and increase, respectively. Serum FSH level in extract treated rats was 0.50±0.10, 0.04±0.01 iu L^{-1} in control and extract treated group, respectively. Estradiol level was also significantly increased in extract treated rats with a value of 0.08±0.01 nmol L^{-1} compared with control that recorded a value of 0.05±0.01 nmol L^{-1} (Fig. 2 and 3).

Pregnancy and pregnancy outcome: There was no significant difference between the number of viable fetus and the number of litter delivered between control and extract treated groups. No resorption wasrecorded in either control nor extract treated group at 19 day 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 (Table 3).

Table 2: Effect of aqueous leaf extract of *Magnifera indica* (500 mg kg^{-1} BW) on oestrous cycle phases in pubertal female Spraque-Dawley rat

	Control		Treated	
Phase of cycle	Frequency (day)	Percentage of phase in a cycle	Frequency (day)	Percentage of phase in a cycle
Diestrus	13.10±0.38	46.80	19.40±0.65*	69.30
Proestrus	6.80±0.25	24.30	6.20±0.57	22.10
Estrus	6.40±0.36	22.90	1.80±0.42*	6.40
Metestrus	1.70±0.47	6.10	0.70±6.21	2.50

*Significant difference

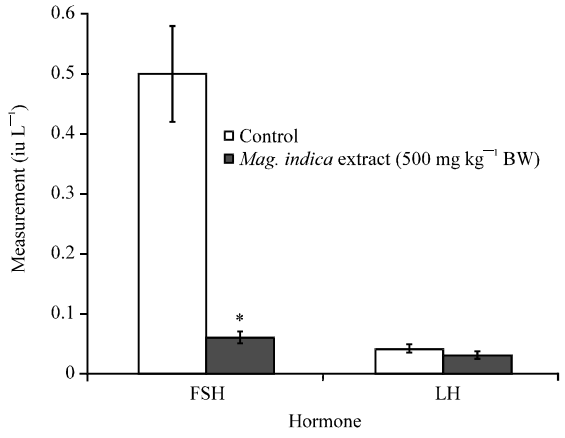

Fig. 2: Effect of aqueous leaf extract of *Magnifera indica* (500 mg kg^{-1} BW) on follicle stimulating hormone and Luteinizing hormone. * = Significant different compared with control at $p<0.05$

The present study showed that oral administration of aqueous leaf extract of *Magnifera indica* to pubertal female rats at a dose of 5000 mg kg^{-1} body weight for a period of 4 weeks significantly disrupted the estrous cycle. This was evident by the significant increase in the average number of days for the diestrus compared to the control and a significant reduction in the average number of days for estrus compared to the control. The fertile period in female rats is between proestrus and estrus 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 oestrus cycle is functionally under the direct regulation of the pituitary-ovarian hormone; FSH, estrogen, pregesterone and LH which usually peaks during the oestrus phase of the cycle (Campbell, 2009). Serum hormones of the treated animals revealed a significant reduction in the Follicular Stimulating Hormone (FSH) secreted from the pituitary compared to control. Estradiol level was also significantly increased compared to control while other hormones such as progesterone and Luteinizing hormone were not affected. FSH is responsible for stimulating the growth of the graffian

Table 3: Effect of aqueous leaf extract of *Magnifera indica* (500 mg kg^{-1} BW) 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

Characteristics	Control	MIL extract treated rats
No. of viable foetus	10.00±0.00	10.00±0.00
No. of resorption sites	0.00±0.00	0.00±0.00
No. 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	21.00±0.00	21.00±0.00

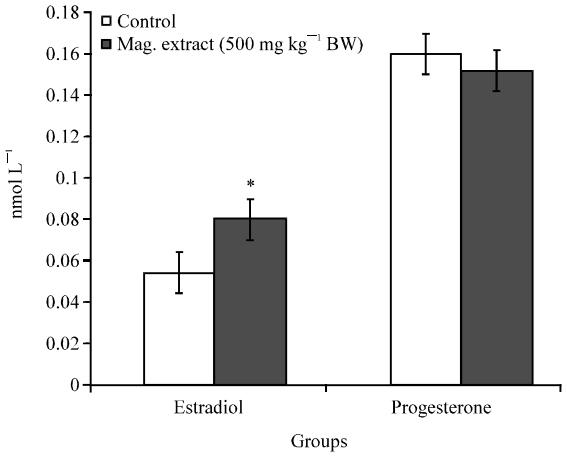

Fig. 3: Effects of oral administration of aqueous leaf extract of *Magnifera indica* (500 mg kg^{-1} BW) on serum estradiol and progesterone level in pubertal female rats compared with the control

follicles. This gave an indication that the leaf extract of *Magnifera indica* 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 foetus at nineteen day of pregnancy in extract treated rats compared to control. Further, research 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 19 day 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 earlier 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 foetal use. This has been well linked to the onset and development of Intra Uterine Growth Restriction (IUGR) and low birth weight. Low birth weight, preterm birth and Intrauterine Growth Restriction (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 4 weeks treatment period, a significant weight loss was however recorded at the third and 4th weeks of treatment. Earlier research on nutritional utilization of the *Magnifera 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 leaf extract of *Magnifera indica* at a dose of 500 mg kg^{-1} body weighs 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.

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