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Antiprogesteronic and Estrogenic Effect of *Mangifera indica* in Female Rabbits

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ABSTRACT

Preparations from *M. indica* are commonly used for herbal concoctions. This study aimed at investigating the effect of methanol extract of *M. indica* on female sex hormones and ovary in Chinchilla rabbits with the view to finding its pharmacodynamics. A total of 24 female Chinchilla rabbits aged 10 to 14 weeks was used for this study. The experimental groups designated group A (control), B, C and D. Group A, B, C and D were orally administered the doses of 00, 500, 1000 and 1,500 mg kg^{-1} body weight of the methanol *M. indica* extract, respectively. Serum concentrations of estradiol and progesterone were estimated using the microplate enzyme immunoassay method. Result showed significant increase in the serum concentration of estradiol in a dose dependent manner ($p<0.05$) while insignificant increase and insignificant decreases in progesterone were observed in group B and treated groups C and D ($p>0.05$), respectively when compared with the control group. Significant increase in weight gain and significant decreases in weight gain were observed in group B and treated groups C and D ($p<0.05$), respectively when compared with control group. Insignificant decreases in the relative weight of the ovary were observed in the treated groups B and C ($p>0.05$) while significant decrease was also observed in the ovaries of treated group D ($p<0.05$) when compared with the control group. Microscopy revealed cystic spaces and oedema in the ovary of group D- a dose dependent effect. Hence, *M. indica* has the potential to affect serum concentration of female sex hormones.

Key words: *Mangifera indica*, female sex hormones, body weight, female Chinchilla rabbits, ovary

INTRODUCTION

In developing countries of the world, most of the people depend on herbal medical care (Ekpe *et al*., 1990). Extracts of root, stem, bark and leaves of some medicinal plants have been shown to have activities against most dreaded pathogenic organisms (Singh and Pattak, 1994) while some others are cytotoxic (Prohp *et al*., 2006).

In south-western part of Nigeria *Psidium guajava* and *M. indica* are commonly used for herbal preparations in the treatment of toothache, gastrointestinal disorders, dynsentery, diarrhoea, sore gums and sore throats (Akinpelu and Onakoya, 2006). In Uganda leaf extracts of *M. indica* has been shown to possess some antibacterial activity and has been the basis for their medicinal use (Bbosa *et al*., 2007). In south America and other parts of the world the ashes of the leaves are used to treat burns, scalds, sores, cough and diarrhoea. *M. indica* is used as antidiabetic (Garcia *et al*., 2003), anti-oxidant, anti-viral, cardiotonic, hypotensive anti-inflammatory, antibacterial, anti fungal, anthelmintic, anti-parasitic (Agbe *et al*., 2010; Ojewole, 2005) anti tumor, anti HIV, anti-bone resorption, antispasmodic, antipyretic, antidiarrheal, antiallergic, immunostimulatory, hypolipidemic, anti microbial, hepatoprotective and gastro protective agent in Nigerian folk medicine (Garcia *et al*., 2003).

This study on *M. indica* leaf extract revealed the presence of steroids, flavonoid, reducing sugar and cardiac glycosides in the hexane extract; anthraquinone, tannin and reducing sugar in the ethyl acetate extracts and saponin, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides in the methanolic extract (Aiyelaagbe and Osamudiamen, 2009). Mango contains a variety of phytochemical such as the pigment antioxidants-carotenoids and polyphenol and omega-3 and 6 polyunsaturated fatty acids. Leaves also have significant polyphenol content, including xanthonoids, mangiferin and gallic acid etc (Singh *et al*., 2004). *Mangifera indica* has also been found to have aphrodisiac activity (Singh *et al*., 2012). The lethal dose (LD_{50}) of the *M. indica* extracts which was estimated to be greater than 5000 mg kg^{-1} body weight (John *et al*., 2012). Estrogen is synthesized in all vertebrates (Ryan, 1982). Estrogens are used as part of some oral contraceptives, in estrogen replacement therapy for postmenopausal women and in hormone replacement therapy for trans women. Estrogens, in females are produced primarily by the ovaries and during pregnancy, the placenta. Fat cells produce estrogen as well (Nelson and Bulun, 2001). In the female, estradiol acts as a growth hormone for tissue of the reproductive organs, supporting the lining of the vagina, the cervical glands, the endometrium and the lining of the fallopian tubes. It enhances growth of the myometrium. Estradiol appears necessary to maintain oocytes in the ovary. In humans and mice, estrogen promotes wound healing (Oh and Philips, 2006). Progesterone is a C-21 steroid hormone involved in female menstrual cycle, pregnancy (supports gestation) and embryogenesis of humans and other species. Progesterone is produced in the ovaries (by the corpus luteum), the adrenal glands (near the kidney) and during pregnancy, in the placenta. Progesterone is also stored in adipose (fat) tissue.

The aim of this study was to investigate the effect of methanol extract of *M. indica* on female sex hormones and the micro-architecture of the ovary, in view to finding its pharmacokinetics.

MATERIALS AND METHODS

Test sample: Fresh *M. indica* leaves were collected in Otolo Nnewi, Anambra State and taken to the Department of Botany Nnamdi Azikiwe University Awka for identification.

Sample extraction: The fresh *M. indica* leaves were collected in Otolo Nnewi, then cut into smaller pieces with knife and air-dry for three weeks. The dried leaves were grounded with milling machine and then a crude extraction was carried out using the aqueous methanolic solvent. The extraction solvent was prepared by using 800 mL of absolute methanol and 200 mL of distilled water, to obtain 80% methanol. The 100 g coarse powdered leaf were soaked in 5000 mL of the methanolic solvent (ratio1: 5), in a screw capped container and was extracted for 72 h and stirred

every 4 h. The extract/solvent mixture was filtered with Whatman No.1 filter paper. The filtrate was left to dry at room temperature, to obtain a brownish green extract. The extracts were concentrated to dryness and the residue were obtained as a greenish black solid after which, the residue were transferred into a pre-weighed sample containers and were stored in airtight container. Prior to use 30, 60 and 90 g, of the concentrate was reconstituted each in 300 mL of distilled water, respectively to obtain 500, 1000 and 1500 mg kg^{-1} of body weight which were then stored in refrigerator at 4°C.

Experimental design: A total of 24 female Chinchilla Rabbits aged 10-14 weeks and weighing 0.60-1.30 kg was used for this study. The animals were examined, treated for ectoparasites using Lymectin (Hebei New Century Pharmaceutical Co. Ltd) by a veterinarian and allowed to acclimatize for two weeks at the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus. The animals were randomly divided into five groups, each containing 6 rabbits.

The experimental group consisted of four groups, designated group A (control), B, C and D. Group B to D were orally administered the doses of 500, 1000 and 1,500 mg kg^{-1} body weight of the *M. indica* methanol extract, respectively. Group A was orally administered distilled water only. Due consideration was given to their body weight (those with greater body weights have their dose divided into two; one in the morning one at evening) and orally administered the extract (using orogastric tube) for 28 days.

Ethical approval: The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH Publication No. 85-23, Revised) (NIH, 1985) and Animal Welfare Act on the care and use of laboratory animals. All procedures were examined and approved by the Faculty's Ethics Committee.

Animal treatment: The animals were kept under standard and good laboratory conditions (12 h light and 12 h darkness, temperature (30±4.5°C), humidity and ventilation). Prior to exposure, the animals were starved overnight for solid food and their body weights taken. This was also done weekly using compression spring balance (BAW-660-M) for the duration of the study, to check for weight loss or gain which is associated with toxicity. The animals were fed grower pallets (from Vital feed Ltd, Jos, Plateau State, Nigeria), vitamins and water *ad libitum* for 28 days.

Sample collection and light microscopy: On the 29th day, the animals were anaesthetized using cotton wool damped in chloroform with due consideration of their body weights. The blood samples, obtained by marginal ear vein puncture, were drawn into tubes using 22 gauge sterile needles. For biochemical analyses, blood samples collected into plain test tubes were centrifuged (Rotofix 32®-Hettich) at 3000 g for 10 min the serum was collected and kept at -20°C until analysis. Animals were sacrificed; the ovaries excised, blotted dry to remove traces of blood and fluid and weighed using an electronic weighing balance (using 210/0.1 mg digital balance ESJ-210-4). The ovaries were excised, grossed, fixed in 10% formal saline, processed through paraffin wax, sectioned and slices of 3 μm thickness were stained using Haematoxylin and Eosin (H and E) technique (Avwioro, 2002). Photomicrograph of the stained tissue sections were taken (by a microscope which had a camera attached to it) for comparison and documentation. The processing of the ovaries was carried out at Histopathology Unit in the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Biochemical analysis: Serum concentrations of estradiol and progesterone were determined using the microplate enzyme immunoassay kit (from MONOBIND Inc., USA) and measured using ELISA machine (MR 96 USA). The hormonal assay was carried out using the facilities of Nnamdi Azikiwe University Teaching Hospital, Anambra State, Nigeria.

Statistical analysis: Mean±SD of the sex hormones, body and ovary weights were taken for analysis. The data was tested for homogeneity of variance and significantly different results were established by one-way ANOVA using the SPSS software application (version 16). Pair-wise comparisons were made using the *post hoc* test. The accepted level of significance was set at $p<0.05$. The Pearson's correlation was carried out to correlate the body weights before and after the experiment, with the accepted level of significance set at 0.01.

RESULTS

The Table 1 show significant increase in the serum concentration of estradiol in the treated groups ($p<0.05$), especially in group D. The effect of the *M. indica* extract on estradiol level was in a dose dependent manner. Insignificant increase in serum concentration of progesterone was observed in group B with insignificant decreases in treated groups C and D ($p>0.05$). Insignificant dose dependent decrease in the weight of the ovary was also observed in the treated groups ($p>0.05$), except in group D which had significant decrease ($p<0.05$) when compared with the control group (A) one-way ANOVA and *post hoc* test.

The Table 2 shows the paired samples mean and correlation of the weight of the control with treated groups in relation to their weight before and after the experiment. The statistics shows strong and significant correlation between the mean weight before and after the experiment for treated group B ($p<0.05$), with strong but insignificant correlation between the mean weight before

Table 1: Mean and paired comparison of the sex hormones between the control and test groups

	Groups				
Parameters	A (control)	B (500 mg kg^{-1})	C (1000 mg kg^{-1})	D (1,500 mg kg^{-1})	p-value
Estradiol (pg mL^{-1})	1014±3.80	1019±1.20 (0.883)	1030±11.1 (0.218)	1045±14.7 (0.016)**	0.017
Progesterone (ng mL^{-1})	56.70±1.30	58.00±0.15 (0.769)	55.50±1.30 (1.070)	55.60±0.95 (0.942)	0.055
Ovary weight (g)	0.034 ±0.003	0.034 ±0.002 (0.931)	0.029±0.006 (0.101)	0.027±0.003(0.034)*	0.078

p is significant at $p<0.05$. Values are expressed as Mean±SD, n = 6, *: Significant, **: Most significant

Table 2: Mean and paired samples correlation of the weight of the groups before and after the experiment

Pair (mg kg^{-1})	Groups	Mean±SD	Correlation	p-value
00	A weight before	0.94±0.21		
	A weight after	1.20±0.23	0.904	0.096
500	B weight before	0.74±0.17		
	B weight after	1.01±0.19	0.965	0.035*
1000	C weight before	1.06±0.11		
	C weight after	1.29±0.09	0.638	0.362
1500	D weight before	1.20±0.08		
	D weight after	1.38±0.12	0.857	0.143

p-value is significant at $p<0.05$. Values are expressed as Mea±SD, n = 6, Group A: Control; 00 mg kg^{-1} methanol extract of *M. indica*, B: 500 mg kg^{-1} methanol extract of *M. indica*, C: 1000 mg kg^{-1} methanol extract of *M. indica* and D: 1,500 mg kg^{-1} methanol extract of *M. indica*, After: Post experiment weight, Before: Pre-experimental weight

and after the experiment for the groups A, C and D ($p>0.05$), respectively. This indicates that there are increases in weight gain in groups A (control) and B but decreases weight gain in groups C and D.

The Table 3 shows significant differences in their mean weight before and after the experiment. There was a significant increase in weight gain in groups A and B with a significant decrease in weight gain in treated groups C and D ($p<0.05$) using paired sample t-test.

DISCUSSION

The methanolic leaf extract of *M. indica* have been shown to possess aphrodisiac activity (Singh *et al*., 2013) and anti-fertility effect in male rats (Ibraheem *et al*., 2007). Hence, it is pertinent to investigate its effect on female sex hormones and micro-architecture of the ovary.

Progesterone treatment has been found to increase food intake body, weight gain and carcass adiposity in rats (Schwartz and Wade, 1981; Gonzalez *et al*., 2000). In this study, it was observed that the treated groups (especially group D) had reduction in food intake (they had remnants which was about 1/3 of the total given feed) when compared with the control group while the control group had no food remnants. This could be linked to the decrease in weight gain observed in groups C and D (Table 2 and 3). This agrees with the reports of Awobajo *et al*. (2013). Furthermore, the change in weight gains of the treated animals was found to be concurrently affected with their serum concentrations of progesterone (Table 1, Fig. 1). It could be adduced that observed changes in the serum concentration of progesterone may have stimulated the observed differences in weight gain in the treated animals. This supports the earlier reports that progesterone plays a role in weight gain. The decrease in progesterone could be as a result of the decrease in the number of

Table 3: Paired samples test of the mean weight of the groups before and after the experiment

Pair (mg kg^{-1})	Groups	Mean±SD	t	df	p-value
00	A weight after-A weight before	0.26±0.10	5.326	3	0.013
2500	B weight after -B weight before	0.28±0.05	11.000	3	0.002
31000	C weight after-C weight before	0.23±0.09	5.196	3	0.014
1500	D weight after-D weight before	0.18±0.06	5.422	3	0.012

p-value is significant at $p<0.05$. Values are expressed as Mean±SD, n = 6, Group A: Control, 00 mg kg^{-1} methanol extract of *M. indica*, B: 500 mg kg^{-1} methanol extract of *M. indica*, C: 1000 mg kg^{-1} methanol extract of *M. indica*, D: 1,500 mg kg^{-1} methanol extract of *M. indica*, After: Post experiment weight, Before: Pre-experimental weight

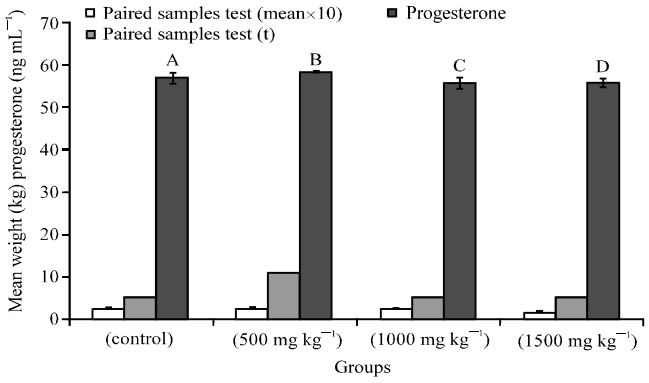

Fig. 1: Mean±SD association and paired samples comparison of progesterone and weight of the control group and treated groups

functional hormone producing cells of the ovary. This is supported by the cystic spaces and oedema observed in the ovarian section of treated group D (Fig. 2-3) when compared with that of the control group, though there was no significant decrease in the weight of the ovary (Table 1).

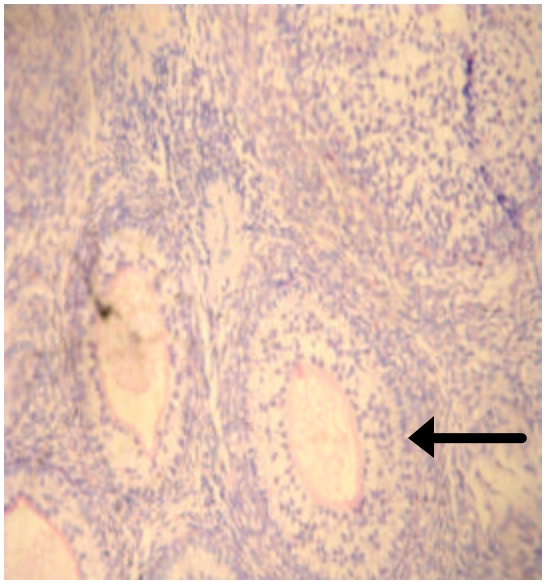

Fig. 2: Group A (control): A section of the ovarian tissue with no obvious sign of pathology, H and E stained×200

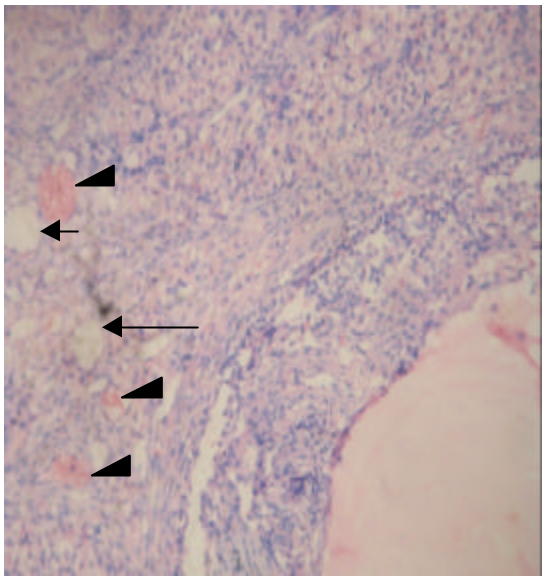

Fig. 3: Group D (1,500 mg kg^{-1}): A section of the ovarian tissue with cystic spaces (marked by arrows) and oedema (marked by arrow heads), H and E stained×200

Estradiol, a subtype of estrogen, was observed to be significantly increased in the treated groups when compared with the control group ($p<0.05$). This is in accordance with the reports of Awobajo *et al.* (2013). Estrogen appears to have a protector effect on atherosclerosis: It lowers LDL and triglycerides; it raises HDL levels and has endothelial vasodilatation properties plus an anti-inflammatory component. It has also been found to have immuno-protective and antioxidant effect. Thus, the medicinal properties associated with *M. indica* could be associated with its ability to induce estradiol increase (Table 1). More so, the significant increase in body weight observed in the treated group B indicates that at the concentration of 500 mg kg^{-1} body weight of *M. indica* there was a combine effect of estradiol and progesterone (Table 1). This observation is supportive of the report by Gonzalez *et al.* (2000), that progesterone is the key sex hormone responsible for weight gain in non-pregnant rats. Considering the fact that there was no pathological effect on the ovarian tissue (group B; 500 mg kg^{-1}), the observed effect could be considered beneficial.

Hence, the pharmacodynamics and curative effects of *M. indica* extract could be associated with its effect on hormonal concentrations in the body which is entirely dose dependent. Furthermore, its effect on estradiol could be exploited to treat infertility resulting from decrease estrogen concentration in females. However, a high dose of the extract is suggested to result in ovarian cysts.

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