PACIFIC JOURNAL OF MEDICAL SCIENCES
{Formerly: Medical Sciences Bulletin}
ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)
www.pacjmedsci.com. Email: pacjmedsci@gmail.com.

EFFECT OF *MOMORDICA CHARANTIA* ON ESTROUS CYCLE OF SPRAGUE-DAWLEY RATS

Amah C. Ifeanyi, *Yama O. Eboetse, Duru F. Ikechukwu, Osinubi A. Adewale,

Noronha C. Carmel, and Okanlawon A. Olugbenga

Department of Anatomy, College of medicine of the University of Lagos, Idi-Araba Lagos, Nigeria (*Corresponding Author: Y. O. Eboetse: Department of Anatomy, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria; E-mail: dro_yama@yahoo.com.)

EFFECT OF *MOMORDICA CHARANTIA* ON ESTROUS CYCLE OF SPRAGUE-DAWLEY RATS

Amah C. Ifeanyi, Yama O. Eboetse, Duru F. Ikechukwu, Osinubi A. Adewale,

Noronha C. Carmel, and Okanlawon A. Olugbenga

Department of Anatomy, College of medicine of the University of Lagos, Idi-Araba Lagos, Nigeria
(Corresponding Author: Y. O. Eboetse: Department of Anatomy, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria; E-mail: dro_yama@yahoo.com)

Running title: Effect of *Momordica charantia* on estrous cycle, morphology of ovary and uterus in rats

ABSTRACT

Bitter Melon (*Momordica charantia*) is a plant known widely particularly in the Indo-Malayan region for its uses in various ailments as reported in literature. However, little is known for its effect on the female reproductive system. The aim of this project was to evaluate the effect of oral administration of methanolic seed extract of *Momordica charantia* on the estrous cycle and the histology of the ovary and uterus in Sprague-Dawley (S-D) rats. A total of 20 adult cyclic female S-D rats (4-day cycles), weighing between 110– 140 g were used. These were divided into 4 groups (A, B, C and D) of 5 rats/ group. The dose of the extract administered was 25 mg/100g body weight and the route of administration was oral by gastric gavages with a metal canula. Groups A and B were both treated with a daily dose of the extract for 28 days (7cycles) and vaginal smear monitored within this period between 9.00– 10.00 am daily. Animals in Groups C and D (control) were fed distilled water and vaginal smear monitored daily throughout the duration of the experiment. The effect of withdrawal of the extract was studied in Group B which was treated with distilled water for another 28 days. The rats were sacrificed by cerebral dislocation. Groups A and C at the end of the first 28 days while Groups B and D at the end of the second 28 days. The ovaries and uterii were harvested for histological studies. Irregular changes in the phases of the estrous cycle in all the treated rats were observed. The diestrous phase was increased while the proestrous and estrous phases were decreased significantly. These effects were reversible on withdrawal of the extract. Histological sections did not show any difference between the ovarian and uterine tissues of the treated and control respectively. The extract resulted in a reversible variation in the estrous cycle pattern. Histological sections reviewed were essentially normal.

Key words: *Momordica charantia,* ovary, uterus, estrous cycle, Sprague-Dawley

Received: January 2011; Accepted March 2011

INTRODUCTION:

Momordica charantia (Bitter melon) is a monoecious climber. It grows in tropical areas including parts of the Amazon, East Africa, Asia and the Caribbean and is cultivated throughout South America as food and medicine [1, 2]. In Nigeria, especially in folkloric settings, its culinary usage is largely hinged on its relief to various ailments. Studies have demonstrated its antimicrobial (against viruses, bacteria and fungi) [3 – 6], anticancerous (cytotoxic) [7, 8], antidiabetic (hypoglycemic) [9 – 11], anti-oxidant [12, 13] and anti-ulcer actions [14, 15]. Phytochemical profile shows it contains an array of biologically active substances that include triterpenes, proteins and steroids. Its actions in the female reproductive system have also been documented [16]. The fruit and leaf have demonstrated an *in-vivo* anti-fertility effect in female animals and in male animals to affect the production of sperm negatively [17]. The seeds however have exhibited ability to induce abortions in rats; the roots shown to have uterine stimulant effect [18]. In another study, ethanolic extract of the roots of *Momordica cymbalaria fenzl* at doses 250 and 500 mg/kg body weight was found to have abortifacient and anti-ovulatory effects in rats [19]. Scanty literature exists on its effect on the estrus cycle and histology of the ovary and uterus of which this work is designed to address. The results therefore will help in assessing its suitability as an anti-fertility agent.

MATERIALS AND METHODS:

Plant Materials (Collection and identification)

The procurement of fresh fruits of *Momordica charantia* seed (MC) was done in a local market at Mushin, Lagos State Nigeria. The same was identified and authenticated by Professor J. Olowokudejo, a taxonomist in the Botany Department of the University of Lagos, where the voucher specimen was deposited (ascension number FHI 108422).

Processing/Preparation of seed extract

The processes leading to the constitution of the appropriate formulation of 230 g of MC in 1000 ml of methanol was done in the Pharmacognosy Department of College of Medicine, University of Lagos (CMUL). These included drying the fruits to get seeds which were then weighed and Soxhlet extraction done using alcohol (absolute methanol) as solvent. The percentage yield of the seed extract obtained was 23.0% w/w.

Animals

This study was done with 20 healthy adult female albino rats of Sprague-Dawley strain. These rats, 8-10 weeks old, weighing 125±15 g were obtained from the Animal Breeding Laboratory Centre of CMUL. They were housed in well-ventilated plastic cages in the rat room, Anatomy Department of CMUL under standard animal house conditions (temperature: 28^{0}C to

31^0C; light: approximately 12 hours natural light alternating with 12 hours darkness per day; humidity; 50 to 55 %). The rats were allowed free access to pelleted food (Pfizer Nigeria Limited) and water *ad libitum*. Lighting in the room was by sunrays (natural light) reflecting through the glass windows. Illumination periodicity plays a dominant role in the incidence and duration of the stages of the ovarian cycle [21]. Animals were also allowed to acclimatize to the laboratory environmental condition for two weeks.

They were weighed at procurement and weekly subsequently. Each animal had a 4-day estrous cycle, confirmed through vaginal smears taken and examined daily between 9.00-10.00 am for 16 days (4 cycles).

Experimental Protocol

A total of 20 adult female Sprague-Dawley rats, weighing between 110-140 g were used. These were divided into 4 experimental groups of 5 rats per group. The groups were designated A, B, C and D. The rats were weighed weekly from the onset of the experiment. The dose of the extract administered was 25 mg/100 g/body weight of rat [19, 20]. When required, they were fed orally with the extract/distilled water. From the percentage yield of the seed extract obtained, the treatment dose (25 mg/100 g) suspended in distilled water was prepared20. Appropriate volumes based on the animal's individual weight were calculated by simple proportion and administered orally by gastric gavages with a metal canula [20].

Groups A and B were the treatment groups; C and D were the control groups. These rats showed 3 consecutive regular 4-day estrous cycles. The first day of the estrous cycle called the diestrous phase, showed predominance of leukocytes and a few large nucleated cells. This is designated "L2". The second day showed large nucleated cells with the leukocytes, called the proestrous phase and designated "N". The third day, called the estrous phase, showed large flake of squamous cells with small pyknotic nuclei. This was designated "C". The metestrous, which was on the fourth day showed leukocytes amidst remnants of large squamous cells with pyknotic nuclei and was, designated "L".

Groups A and B (treatment groups) were both treated with a daily dose of the extract while Groups C and D (control groups) were fed equal volumes of distilled water (5 ml), all for 28 days (7cycles). Vaginal smears from each animal were examined daily between the hours of 9.00-10.00 am during this period; while the assessment of the weight done weekly. The vaginal smears were collected using a small rubber suction manual pipette and normal saline. The normal saline was first drawn into the tip of the pipette. This was then introduced into the vaginal canal and the normal saline released. The vaginal fluid, by negative pressure is suctioned into the tip of the pipette. The fluid was then smeared on a glass slide

and examined under the light microscope immediately before drying up. It is important to note that the treatment for 7 cycles was to cover three regular estrous cycles and to exclude the possibility of irregular cycling being caused by pseudo-pregnancy [22]. At the end of this period, Groups A and C rats were sacrificed by cervical dislocation, a laparotomy done and the ovaries and uteri harvested *per* abdomen, for histological studies. The effect of withdrawal of the extract was studied in Group B rats. These rats were discontinued from the extract and together with the group D rats fed with distilled water for another 28 days (7 cycles) and vaginal smears monitored within the same period (reversal effect). These rats again were sacrificed by cervical dislocation at the end of this period and the ovaries and uteri harvested as described above for histological studies (reversal studies). It is important to mention that Groups A and C rats were sacrificed at the end of the first 28 days while Groups B and D at the end of the second 28 days.

Tissue processing for histological studies

The harvested organs were carefully dissected out, trimmed of fat and connective tissue. The tissues were processed by the method described below with slight modification [23]. The steps involved in tissue processing included fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning, and staining. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol (50%, 70%, 90%, absolute alcohol), then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and made into blocks of wax. Microtome whose sectioning size knob was adjusted to six Microns was then used to section the block, fixed on clean slides and later stained with haematoxylin and eosin.

RESULTS:

Estrous Cycle

Analysis of the Estrous cycle revealed that oral administration of 25 mg/100g body weight of Methanolic seed extract of *Momordica charantia* produced an irregular pattern of cycling in all the treated rats (100 % of the treated rats). The length of the estrous cycle was significantly increased (Tables 1) and this was marked in the diestrous phase.

Cycles up to 8 days were observed when compared with the control at $p < 0.05$. A significant decrease in the duration of the proestrous and estrous phases were also observed throughout the treatment period.

However these effects were reversible on withdrawal of the extract and it took the rats an average of 8 to 10 (2– 3 cycles) days to regain their normal 4-day cycle. No death was recorded as a result of the test extract.

Control rats showed a regular 4-day cycle.

Histology of the uterus and ovary

Histological analysis of the uteri of the treated groups showed a well differentiated serosa, muscularis and endometrial layers. The glands were numerous and well developed. There were no overt histoarchitectural (uterotrophic) changes compared to those of the control groups (Figures 1-3).

The ovaries of the treated groups were characterized by the presence of growing follicles, matured follicles, numerous ruptured follicles and some atretic follicles (Figures 4-6).

Body Weight

There was a general downward trend in the weights of all the rats treated with Methanolic seed extract of *Momordica charantia* at the specified dose (25mg/100g body weight). This is shown in table 2 which depicts the mean body weights of all the animals while on treatment with the extract for 28 days and on withdrawal. There was a significant mean loss of weights ($p < 0.05$) in the animals while on treatment with the extract for 28 days compared to control. In the mean weights of the animals for reversal studies; animals were initially treated with the extract for 28 days. On withdrawal of the extract, a slight weight gain was observed although this was not statistically significant (p □ 0.05). The difference in weight after experiment was not statistically significant in the treatment group but was significant ($p < 0.05$) when compared to the control group.

Table 1: The estrous cycle analysis in experimental and control Sprague-Dawley rats

Estrous cycle phase (%)	Treatment with *Momordica charantia extract/distill water*			
	Before administration	During administration	Withdrawal of extract	Control
Normal	100.0	0.0	0.0	100.0
Irregular	0.0	100.0	100.0	0.0
Metestrous	24.4	16.5	16.5	24.3
Diestrous	25.8	35.5	35.5	25.9
Proestrous	24.6	20.5	20.5	24.7
Estrous	25.2	27.5	27.5	25.1

Table 2: Effect on the mean body weights during extract administration and following extract withdrawal (Reversal effect)

Mean body weight	Experimental	Control	Withdrawal	Control
Before experiment	127.5±12.5	128.6±11.0	124.2 ±11.5	128.6 ±11.0
After experiment	113.6 ±11.5*	137.4±11.0	121.6 ±11.5	146.6 ± 8.5
Difference	-13.90*	+ 08.8	- 2.6	18.0

Values are expressed in mean ± SD; Weight in grammes; *Level of significance is at $p < 0.05$; Significant difference between values of experimental and control groups

DISCUSSION

The anti-fertility potentials of many plants have been investigated [19, 24 – 26]. In this study, oral administration of *Momordica charantia* methanolic seed extract caused irregular changes in the phases of the estrous cycle in all the treated rats.

The duration of the diestrous phase was significantly increased while the proestrous and estrous phases were decreased. These effects were reversible on withdrawal of the extract. This disruption of the estrous cycle may be due to the effect of this extract on the ovary which controls ovarian functions and estrous cycle via ovarian and extra ovarian hormones [27, 28].

The diestrous phase is maintained by the activities of the corpus luteum which produces progesterone in the absence of pregnancy and terminates with the regression of the corpus luteum. Earlier studies with extracts of *Abrus precatorius* seed, castor bean and cotton seed have all shown to cause a significant but reversible alteration in the estrous cycle of Sprague-Dawley rats [25, 26, 29].

Histologically, there is no difference between the ovarian and uterine tissues of the treated and control respectively. This could be due to the anti-oxidant and anti-inflammatory potentials of MC.

The results in this study also indicated that oral administration of methanolic extract of MC seeds to adult cyclic female Sprague-Dawley rats have effects on the mean body weight which was seen to be decreasing with administration of the extract.

On withdrawal of the extract, there was a slight trend towards weight gain although this was not

statistically significant.

In conclusion *Momordica charantia* (25 mg/100g body weight) caused a reversible alteration in the estrous cycle pattern. Histological sections reviewed were essentially normal.

These properties, among others will be helpful in assessing the suitability of MC as a good anti-fertility agent. However, further studies are recommended to look at the histology at the level of electron microscopy.

The potential to cause weight loss will be beneficial in combating metabolic disorders such as obesity which is becoming an epidemic and posing serious health problem.

ACKNOWLEDGEMENTS

We wish to acknowledge Prof. J. Olowokudejo (Taxonomist, Botany Department of the University of Lagos Nigeria) for his identification and authentication of the plant. Special thanks to Mr Adeleke (Pharmacognosy Department Faculty of Pharmacy University of Lagos Nigeria) for his help with the preparation of the herbal decoction and also for his support for this work.

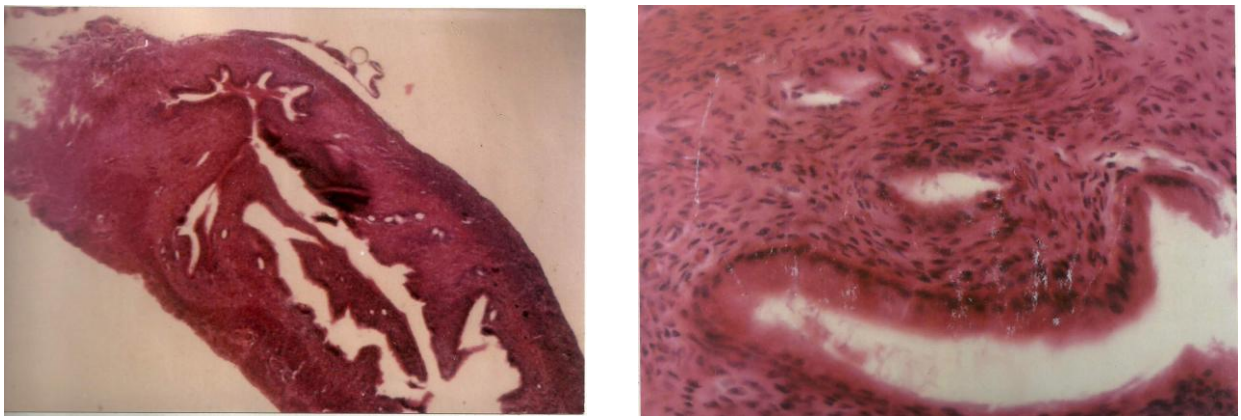

Figure 1: Photomicrograph of the uterus of a control rat stained with Haematoxylin and Eosin. Magnifications; ×40, x 100

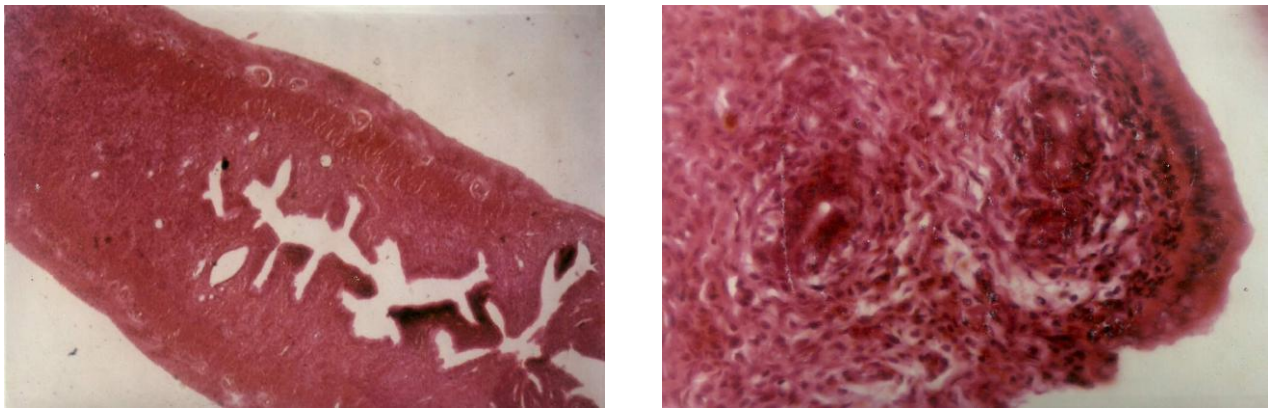

Figure 2: Photomicrograph of the uterus of a treated rat stained with Haematoxylin and Eosin. Magnifications; ×40 x100

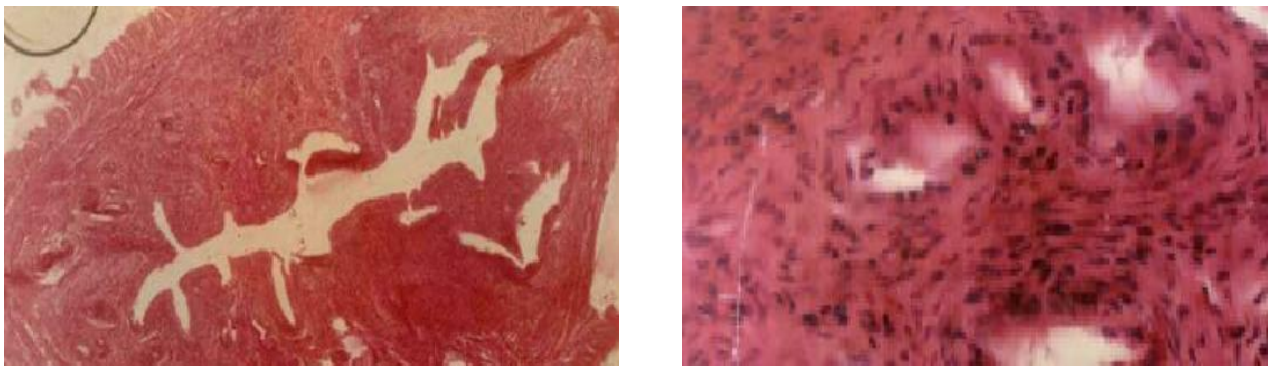

Figure 3: Photomicrograph of the uterus for withdrawal rat stained with Haematoxylin and Eosin. Magnifications; ×40 x100

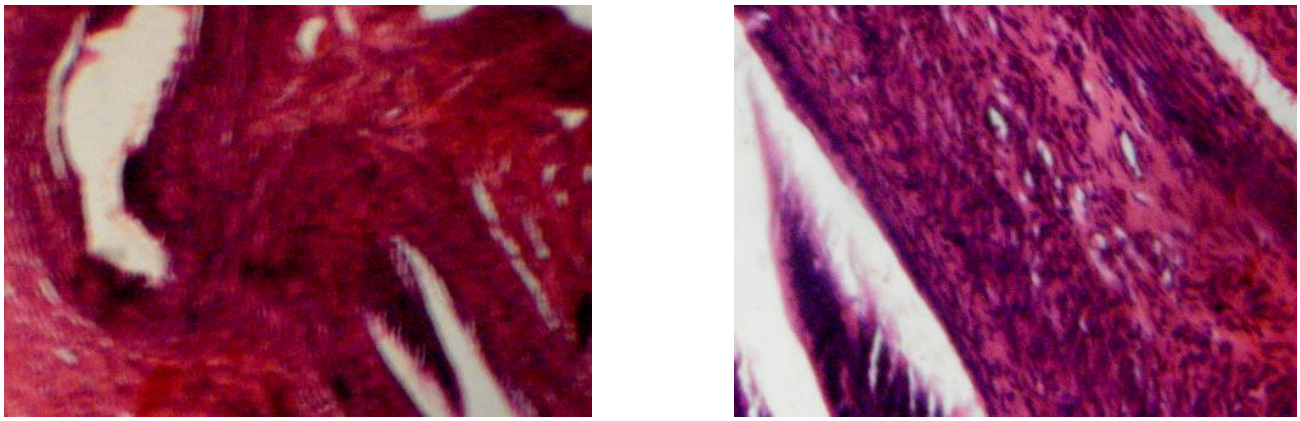

Figure 4: Photomicrograph of the ovary of a control rat stained with Haematoxylin and Eosin. Magnifications; ×40; ×100

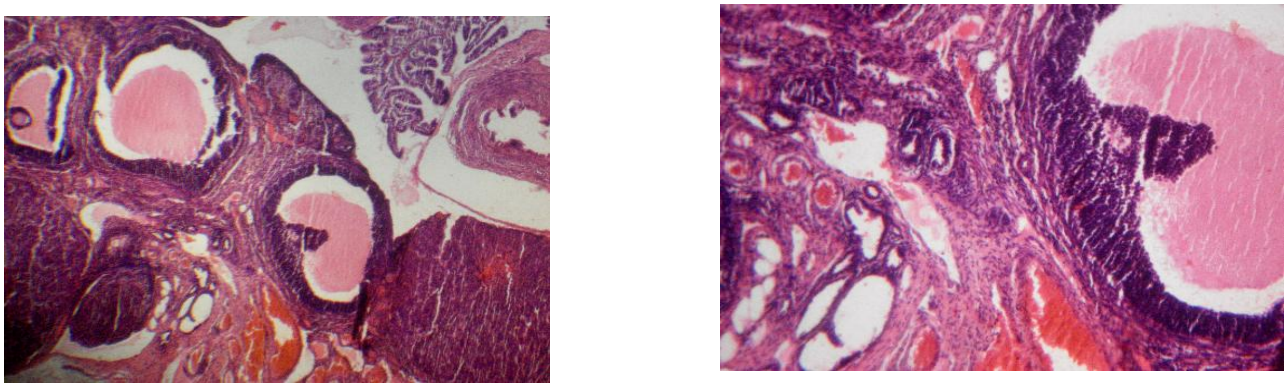

Figure 5: Photomicrograph of the ovary of a treated rat stained with Haematoxylin and Eosin. Magnifications; ×40 x100

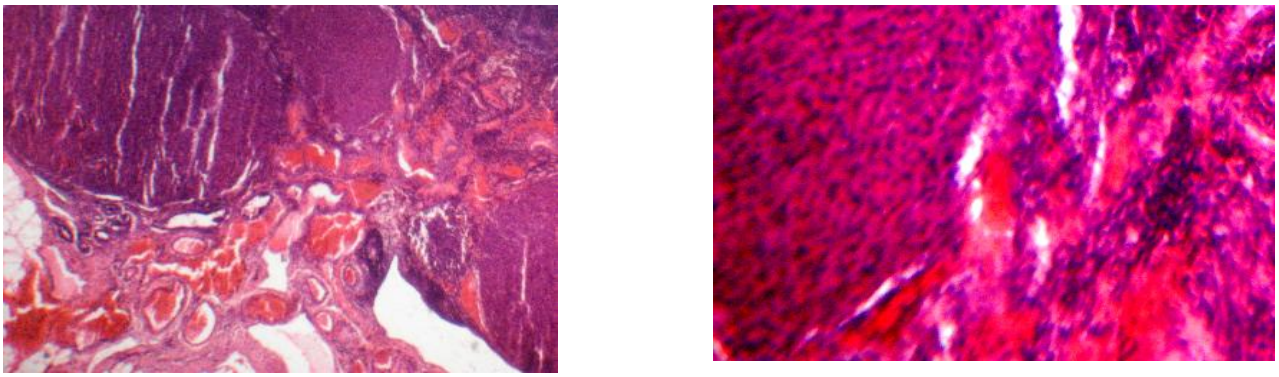

Figure 6: Photomicrograph of the ovary of a rat for withdrawal studies stained with Haematoxylin and Eosin. Magnifications; ×40, x100

REFERENCES

1. Bates DM, Merrick LC, Robinson RW. Minor cucurbits. In Smartt j and Simmonds NW (Eds.). Evolution of crop plants (John Wiley & Sons, New York) 1995, Pg. 110.
2. Momordica charantia, (Bitter melon). Alternative Medicine Review. Monograph, 2007; Vol 12, No. 4 Pg. 360–3
3. Schmourlo G, Mendonca-Filho RR, Alviano CS, Costa SS. Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants. J Ethnopharmacol 2005; 96(3): 563
4. Das P, Sinhababu SP, Dam T. Screening of antihelminthic effects of Indian plant extracts: a preliminary report. J Altern Complement Med. 2006; 12(3): 299-301
5. Coutinho HD, Costa JG, Falcao-Silva VS, Siqueira-Junior JP, Lima EO. Effect of Momordica charantia L. in the resistance to aminoglycosides in methicilin-resistant Staphylococcus aureus. Comp Immunol Microbiol Infect Dis.2009
6. Puri M, Kaur I, Kanwar RK, Gupta RC, Chauhan A, Kanwar JR. Ribosome inactivating proteins (RIPs) from Momordica charantia for anti viral therapy. Curr Mol Med. 2009; 9(9): 1080-94
7. Grossmann ME, Mizuno NK, Dammen ML, Schuster T, Ray A, Cleary MP. Eleostearic Acid inhibits breast cancer proliferation by means of an oxidation-dependent mechanism. Cancer Prev Res (Phila Pa). 2009; 2(10): 879-86.
8. Li M. Anti-tumor activity and immunological modification of ribosome-inactivating protein (RIP) from Momordica charantia by covalent attachment of polyethylene glycol. Acta Biochim Biophys Sin (Shanghai). 2009; 41(9): 792-9.
9. Leung L, Birtwhistle R, Kotecha J, Hannah S, Cuthbertson S. Anti-diabetic and hypoglycaemic effects of Momordica charantia (bitter melon): a mini review. Br J Nutr. 2009; 102(12): 1703-8.
10. Nahas R, Moher M. Complementary and alternative medicine for the treatment of type-2 diabetes. Can Fam Physician. 2009; 55(6): 591-6.
11. Teoh SL, Latiff AA, Das S. A histological study of the structural changes in the liver of streptozotocin-induced diabetic rats treated with or without Momordica charantia (bitter gourd). Clin Ter. 2009; 160(4): 283-6.
12. Nerurkar PV, Lee YK, Linden EH, Lim S, Pearson L, Frank J, Nerurkar VR. Lipid lowering effects of Momordica charantia (Bitter Melon) in HIV-1-protease inhibitor-treated human hepatoma cells, HepG2. Br J Pharmacol. 2006; 148(8): 1156-64.
13. Chaturvedi P. Bitter melon protects against lipid peroxidation caused by immobilization stress in albino rats. Int J Vitam Nutr Res. 2009; 79(1): 48-56.
14. Dengiz GO, Gursan N. Effects of Momordica charantia L. (Cucurbitaceae) on indomethacin-induced ulcer model in rats. Turk J Gastroenterol. 2005; 16(2): 85-88
15. Alam S, Asad M, Asdaq SM, Prasad VS. Antiulcer activity of methanolic extract of Momordica charantia L. in rats. J Ethnopharmacol. 2009; 123(3): 464-9.
16. Girini MM, Ahamed RN, Aladakatti RH. Effect of graded doses of Momordica charantia seed extract on rat sperm: scanning electron microscope study. J Basic Clin Physiol Pharmacol. 2005; 16(1): 53-66.
17. Dixit VP, Sharma A, Verma PK. Effects of Momordica charantia fruit extract on the testicular function of dog. Planta Med. 1978; 34:280-86.
18. Ng TB. Investigation of ribosome inactivating protein-like activity in tissues of Cucurbitaceae plants. Indian J Exp Biol. 1989; 21(12): 1353-58.
19. Koneri R, Saraswati CD, Balaraman R, Ajeesha EA. Antiimplantation activity of the

Momordica cymbalaria fenzl in rats. Indian J Pharmacol. 2007; 39: 90-6.

20. Yama OE, Osinubi AA, Noronha CC, Okanlawon AO. Effect of methanolic seed extract Momordica charantia on body weight and serum cholesterol level of male Sprague-Dawley rats. Nigerian quarterly journal of Hospital Medicine.2010; Vol. 20 (4): 209-213
21. Knobil E, Neil J. The Physilogy of Reproduction, Raven Press Ltd., New York. 1988; 33-40
22. Freeman ME. The ovarian cycle of the rat in: Knobil E and Neil J. (eds) Physiology of Reproduction. Raven Press Ltd., New York. 1988; 1893-1928.
23. Animal tissue techniques. Gretchen L. Humason Los Alamos Scientific Laboratories. On open library.org. Chap. 1-3 Pg. 3-39
24. Oderinde O, Noronha C, Oremosu A, Kusemiju T, Okanlawon OA. Abortifacient properties of aqueous extract of Carcia papaya Linn seeds on Female Sprague-Dawley rats. The Nigerian Postgraduate Medical Journal. 2002; 9(2); 95-98.
25. McNeil RT, Noronha CC, Kusemiju JO, Okanlawon AO. The anti-ovualatory effect of a seed extract of Ricinus comminus linn. Nigerian Journal of Health and Biomedical Sciences. 2003; 2 (1): 31-34.
26. Olabiyi OA, Oremosu AA, Noronha CC, Okanlawon AO. Effects of cotton seed oil (Gossypol Spp.) and cotton seed meal on estrous cycle, Ovulation and histoarchitecture of female reproductive organs of adult cyclic Sprague-Dawley Rats. Nigerian Journal of Health and Biomedical Sciences. 2006; 5 (1): 21-26.
27. Prakash AO, Mathur R. Studies on oestrous cycle of albino rats. Responses to embelia ribes extracts. Planta Medica. 1979; 36:131-141.
28. Shivalingappa H, Satyanarayan ND, Purohit MG, Sharanabasappa A, Patil SB. Effect of ethanol extract of Rivea hypocrateriformis on the oestrous cycle of the rat. J Ethnopharmacol. 2002; 82:11-17
29. Khan MR. Momordica charantia and Allium sativum: Broad spectrum antibacterial activity. Korean J Pharmacog. 1998; 29(3): 155-58.