IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 5 Ver. II (May. 2016), PP 57-62
www.iosrjournals.org

The Aqueous Methanolic Extract of Unripe *Carica Papaya* (Pawpaw) Fruit Distrupts Oestrous Cycle in Albino Rats

Ebere O. Odirichukwu[1], Nneka V. S. Uchechukwu[1] and David Ogwu[2]
[1]*Department of Veterinary Surgery and Theriogenology, Michael Okpara University of Agriculture, Umudike, Nigeria*
[2]*Department of Veterinary Theriogenology and Animal Production, Ahmadu Bello University Zaria, Nigeria*

Abstract: *This study investigated the effects of aqueous methanolic extract of unripe Carica papaya (AMEUCP) on cyclicity of female rats. Fresh mature unripe pawpaw fruits were obtained from Nsukka, Nigeria and extracted by cold marceration. The effect of the extract on the estrous cycle was studied using twenty (20) matured non pregnant female rats (160 -195 g) randomly assigned into four groups (A, B, C and D) of five rats each. Rats in group A served as the control and received 0.5 ml of distilled water orally while those in groups B, C and D received 450 mg/kg, 900 mg/kg and 900 mg/kg AMEUCP respectively for 15 days. During this period, vaginal smears were evaluated daily for oestrous cyclicity. The extract was further investigated for estrogenic effect using twenty five immature (30-40 days old; 35-40 g) female rats following standard procedures. Data obtained from the study were subjected to one-way analysis of variance (ANOVA). Variant means were separated using the Duncan's multiple range test and significance was accepted at $p < 0.05$. The study demonstrated that although the AMEUCP does not possess estrogenic properties, it caused irregularity in the estrous cycle which was not reversible even after withdrawal of the treatment. It was therefore concluded that the unripe fruits or its products may have a negative influences on the reproductive health of animals and probably man.*
Keywords: *Carica papaya, oestrous cycle, oestrus, estrogenic, cyclicity, albino rats.*

I. Introduction

Numerous plants have been reported historically to aid child delivery, stimulate menstrual flow or alter fertility [1, 2]. Modern scientific studies in experimental animals have confirmed the effect of some of these herbs on the reproductive system [3-6]. Most of these plants cause infertility in females by acting as anti-implantation, abortifacient, estrogenic or anti-estrogenic agents and also by disruption of the estrous cycle [7]. The estrous cycle; one of the indices of fertility in the female is a rhythmic reproductive cycle in sexually matured female mammals often influenced by the release of gonadotropin releasing hormones, gonadotropins and gonadal hormones [8]. Normal cyclicity is an index of good functioning of the neuroendocrine and reproductive systems whereas abnormality in the estrus cycle indicates the disruption in the functions of any of these systems and may invariably affect fertility. The female rat has a characteristic short estrus cycle of 4 to 5 days in phases [9] which make them ideal for reproductive studies [10], and the presence or absence of four cell types of the vaginal epithelium and the relative proportion of each cell type, determines the stages of the estrous cycle.

Carica papaya a tree of the Caricaceae family is one of the indigenous plants widely cultivated throughout the world for food and traditional medicine as all parts of the plant are beneficial and are attributed with different medicinal values [11-16]. Several researchers have reported the antifertility properties of various parts of *Carica papaya* in males and females. *Carica papaya* seed has been considered as a safe approach to male anti-fertility [5, 17-20]. In the female, crude papaya latex from unripe fruits induced spasmodic myometrial contractions similar to oxytocin and prostaglandin $F_{2\alpha}$ in rats [14] and guinea pig [21] while ethanol extracted papaya seed (EEPS) caused concentration dependent tocolysis of uterine strips isolated from gravid and non-gravid rats. More so, anti-implantation activity, embryotoxicity and increased post implantation loss has been attributed to papain present in *Carica papaya* latex [22]. There is however a paucity of information on the effects on female cyclicity. The objective of this study is therefore to determine the effects of aqueous methanolic extracts of unripe *Carica papaya* (AMEUCP) on the cyclicity of normal cycling sexually mature female rats.

II. Materials And Methods

2.1 Collection of plant material

Fresh sample of mature unripe pawpaw fruits were obtained from Nsukka urban and authenticated by Mr A. Ozioko, a taxonomist at the International Centre for Ethnomedicine and Drug Development (INTERCEDD), Nsukka.

2.2 Preparation of Extracts and Determination of Plant Yield

The fruits were sliced into thin sheets, sun-dried and pulverized into a fine powder using a dry grinder. The powder was stored in an air tight container at 23-25°C until required. About five hundred (500) grams of the dried sample were subjected to cold maceration with 80% methanol for 48 hours, stirring at intervals. The extract was concentrated to dryness in a hot air oven at a reduced temperature of 40^{o}C and then stored at 4^{o}C throughout the period of the study.

2.3 Animals

Adult female Wistar albino rats (175-200g) were obtained from the Animal House Unit of the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in rat cages in a well ventilated house, temperature of 27 – 30^{o}C, 12 hour natural light and 12 hour darkness. The animals were provided commercial feed (Grand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria) and clean water *ad libitum*. Protocol for this experiment was in accordance with the guidelines on the care and well being of research animals [23]. They were also allowed to acclimatize for two weeks before the commencement of the experiment.

2.4 Evaluation of Aqueous Methanolic Extract of Unripe *Carica papaya* (AMEUCP) on oestrus cycle in rats

Part I: Pre-treatment phase

Three pre-treatment oestrous cycles were established by vaginal smears as described by Marcondes [10, 24] to determine the rats with regular estrous cycle. Vaginal smears were prepared everyday at a constant interval of 9.00-10.00 am for 16 days. Briefly, a moistened swab sticks was used for smear collection from the vaginal lumen by introducing the swab stick gently into the vagina and gently rotating it along the floor of the lateral walls after which it was used to make an impression smear on a clean microscope slide.

The smeared slides were stained using the Giemsa's staining technique after which the stained slides were observed under a light microscope with 10 and 40x objective lenses. The relative proportions of recognized cells were used to determine the phases of the estrous cycle [10]. The female rats that had undergone three successive 4 or 5 day cycles were selected for this study.

Part II: Treatment phase

Twenty (20) matured female rats were used for this study and were randomly divided into four groups; (A, B, C and D) with each group consisting of five rats. Rats in group A served as the control and received 0.5mls of distilled water (the vehicle) while those in groups B and C received 450mg/kg BW and 900mg/kg BW of the aqueous methanolic extracts of unripe *Carica papaya* (AMEUCP) respectively for 15 days. Rats in group D also received 900 mg/kg BW of AMUCPE for the same period of time. Administration of the extract was done orally. The vaginal smears were evaluated daily as previously described in the pre- treatment phase throughout the treatment phase for all the groups. Rats exhibiting a 4 -stage and 4-5 day estrous cycle of proestrus-estrus-metestrus-diestrus were classified as normal while any deviation from this pattern in terms of duration and sequence was categorized as abnormal. The weight of the animals was monitored daily. At the end of this study, the rats were humanely sacrificed by cervical dislocation. The ovaries and uteri were dissected out and surrounding tissues were carefully trimmed of fat. They were blotted on filter papers and weighed quickly on a sensitive balance and recorded. The weight was later expressed as the ratio of the live body weight.

2.5 Investigation of AMEUCP for Possible Reversibility effects

At the end of the estrous cycle study the animals in group D were allowed free access to feed and water for another 15 days. During this period, the vaginal smears were also prepared and evaluated daily as previously described by Marcondes [10] to check for possible reversibility effects if any.

2.6 Investigation of AMEUCP for Oestrogenic activity

The extract was further investigated for oestrogenic activity. Twenty five (25) sexually immature female Wistar rats, 25–30 days old, were used for this study. Twenty (20) of these animals were prepared for ovariectomy. Anaesthesia was achieved using Xylaxine (5mg/kg) and Ketamin (60mg/kg) administered intraperitonially. The uterine horns were exteriorized and the ovaries excised via a laparotomy incision. The laparotomy incision was routinely closed. Fifteen days post surgery; the animals were divided into four groups (A - D) of five rats each.

Groups A and B received 450 and 900mg /kg of AMEUCP respectively. Group C received stilboesterol (standard) in paraffin at 0.2 mg/kg body weight subcutaneously (positive control) and group D served as negative control and received vehicle only (0.5ml paraffin oil). All the above treatments were given for four consecutive days. Group E was made up of five other rats which were neither ovariectomised nor

treated with standard drug or extract which were also kept alongside the rest. The weight of the animals was also recorded daily. On the fifth day, all the rats were sacrificed by cervical dislocation, the uteri dissected out and the surrounding fatty tissues were carefully trimmed off. The uteri were blotted on filter papers and weighed quickly on a sensitive balance and recorded. The weight was later expressed as the ratio of the live body weight.

2.7 Data analyses

All the obtained data were expressed as means ± standard deviation and analyzed using analysis of variance (ANOVA). Comparisons with the control groups were made using One-way ANOVA. Variant means were separated using the Duncan's multiple range test and differences were considered significant if P-value < 0.05.

III. Results

3.1 Plant yield

A dark brown (honey coloured) sticky paste weighing 47g (15.6% yield) was recovered following extraction by cold maceration and concentrated to dryness.

3.2 Effect on the oestrous cycle

Administration of extract produced an irregular pattern in the oestrous cycle of the extract treated rats (Table 1). Animals of the extract treated groups (450 and 900 mg/kg) exhibited a prolonged metestrus and diestrus thereby reducing the frequency of occurrence of the oestrus phase of the cycle. The reversibility study revealed that this irregularity in the oestrus cycle persisted even after withdrawal of the extract (Table 1)

3.3. Effects of AMEUCP on rat ovary and uterus.

There was a decrease in the ovarian and uterine weights of the extract treated groups (450mg/kg and 900mg/kg) when compared to those of the control group**.** There was however no significant difference ($p > 0.05$) in the ovarian and uterine ratios among the groups (Figure 1).

3.4 Oestrogenic activity of the extract

The estrogenic activity test showedno significant difference ($p > 0.05$) in the uterine ratios among the 'intact' (non ovariectomised-non treated), treated (450 and 900 mg/kg) and negative control (0.5 ml paraffin oil) groups. However, the uterine ratios of the positive control (Group C, stilboesterol – 0.2 mg/kg) differed significantly ($p < 0.05$) with those of the other groups (Figure 3). The uteri of rats in this group were inflated and fluid filled.

Table 1: Effects of AMEUCP administration on the oestrous cycle

GROUP A (control distilled H_2O) n = 5	**GROUP B (450mg/kg) n = 5**	**GROUP C (900mg/kg) n = 5**	**GROUP D R_1-900mg/kg n = 5**	**GROUP D_2 (Wdr –R_x) n =5**
+	-	-	-	-
+	-	-	-	-
+	-	-	-	+
+	-	-	-	-
+	-	-	-	-

Key;

+ = Regular oestrous cycle

- = Irregular oestrous cycle

R_1 = 900mg/kg = for reversibility study

Wdr –R_x = withdrawal of treatment

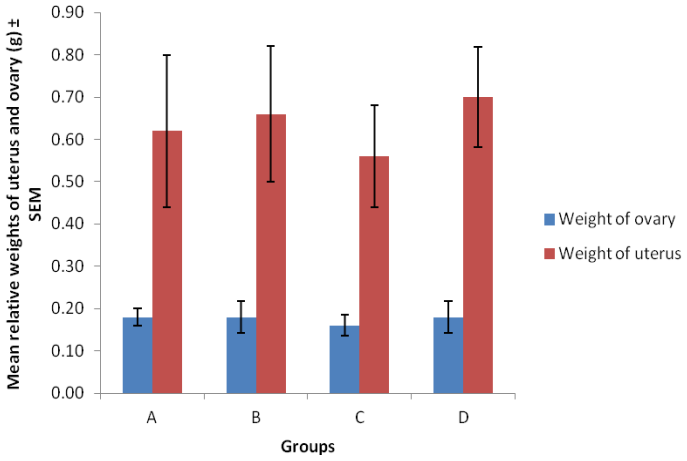

Figure 1: Effect of AMEUCP on the ovarian and uterine ratio

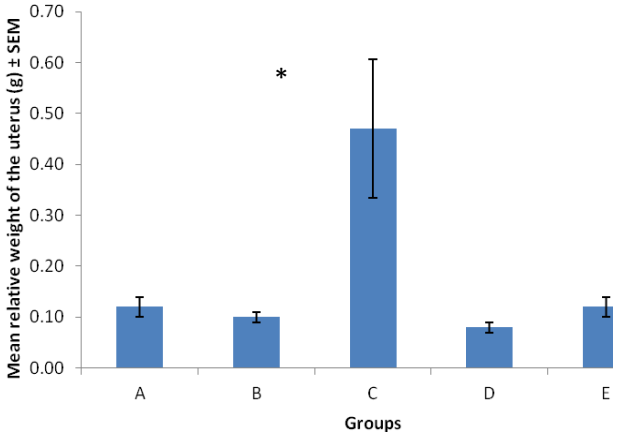

Figure 2: Oestrogenic activity of AMEUCP

***** Represents significant differences ($p < 0.05$) between groups

IV. Discussion

Evaluation of the estrous cycle in laboratory rodents can be a useful measure of the integrity of the hypothalamic-pituitary-ovarian reproductive axis and can also serve as a way of insuring that animals exhibiting abnormal cycling patterns are disincluded from a study prior to exposure to a test compound [26]. Assessment of vaginal cytology in regularly cycling animals also provides a means to establish a comparable endocrine milieu for animals even at necropsy. The results of this study (Table1) support the reports by other researchers of the ability of some plant extracts to alter the estrous cycle [27-29].

Administration of the aqueous methanolic extract of unripe *Carica papaya* (AMEUCP) disrupted the oestrous cycle and produced an irregular pattern of oestrous cycle in the treated rats. These rats showed a prolonged metestrus and diestrus pattern in each cycle thereby lowering the frequency of occurrence of the oestrus phase. Consequently the frequency of ovulation was reduced with a resultant impairment of fertility. The irregular pattern of oestrous observed in the treated rats may be attributed to the presence of phytoconstituents in the crude extract [16, 30] some of which have been incriminated to cause disorder in the oestrous cycle [31, 32]. The inhibitory effect of steroidal saponins (which are appreciably present in unripe *C. papaya*) on the oestrous cycle has also been reported [33] and these have been found to reduce fertility in animals upon continuous administration.

Similar reports indicates that plant extracts adversely affected oestrus cycle in rats during the diestrus phase by blocking the release of both follicle stimulating hormones (FSH) and leutenizing hormones (LH) [34, 35]. The findings of this study are also in agreement with the findings of Oluyemi [36] who reported that the methanolic extract of *Aspilia africana* resulted in a dose-dependent decrease in the duration of proestrous, estrous and metestrus, while it increased the duration of diestrus**.** Similarly**,** Uchendu [37] reported that ethanolic extract of *Dalbergia saxatilis* prolonged the diestrus phase of the cycle and thus reduced fertilization

in the affected experimental animals. In contrast, Marcondes [10] and Mustapha [38] independently reported a prolonged proestrus and estrus phases of the cycle and subsequent antifertility following oral administration of various plant extracts to rats.

Persistent irregularity in the oestrus cycle observed even after withdrawal of the extract administration (Table 1) suggests a possible alteration in the hormonal mechanisms that controls cyclicity. A similar effect has also been reported by Oluyemi [36]. This observation however differs from that of Gopalakrishnan [39] who reported in an earlier study that adult cycling females rats fed on different components of papaya showed continuous diestrous but reverted to normal cycle on discontinuing the treatment. There was no significant difference ($p > 0.05$) in the ovarian and uterine weights of the treated animals when compared to those of the control groups suggesting that the administration of the extract may not have produced any visible gross alteration on these organs. This however, is not conclusive as there are possibilities that histopathological alterations may have occurred which may not be evident grossly.

The effect of estrogen on immature rat uterus has established the importance of this organ for the bioassay of estrogen-like substances [40, 41]. Estrogen is known to elicit uterine growth response in a non-genomic mechanism [40, 42] which involves inducing changes such as increase in vascular permeability, water imbibitions and cellular infiltration [43]. This sequence of events subsequently leads to an increase in uterine weight. The uterine ratios of the 'intact' treated and negative control (0.5 ml paraffin oil) groups were not significantly different indicating that the extract did not elicit estrogenic effect on the uterine tissues. However, the uterine ratios of the positive control (Group C – 0.2 mg/kg stilboesterol) differed significantly with those of the rest of the groups. The observed increase in the uterine ratio of the stilboesterol treated group could be attributed to estrogen related enlargement of endometrial cell size and alteration in the activity of the smooth muscles of the uterus which has been shown to exhibit signs of increased blood flow following the administration of estrogen in ovariectomised rats and rabbits [44]. Although phytochemical analysis of unripe *Carica papaya* revealed some phyoestrogenous components [16, 30] they may not be present in appreciable amount to elicit estrogenic observable effect.

Despite the fact that evidence of estrogenicity was not observed in this study, it is however interesting to know that strong anticonceptive and abortifacient activities were observed in another study when the aqueous methanolic extract of unripe *C. papaya* were administered to pregnant rats from days 1- 7 and 10 - 12 of pregnancy respectively (unpublished). The observed irregularities of the estous cycle may cause distortion of the endometrial function which may in turn lead to a failure of implantation and pregnancy with a resultant impairment of fertility.

V. Conclusion

The results of this research showed that although aqueous methanolic extract of *Carica papaya* did not elicit estrogenic activity, it however disrupted the estrous cycle of female rats by prolonging the metestrus and diestrus phases of the cycle; suggesting negative influences on the reproductive health or fertility of the animals.

References

[1]. S. L. Bodhankar, S. K. Garg and V. S. Mathur, Antifertility screening of plants, Part IX; Effect of five indigenous plants on early pregnancy in albino rats. *Indian Journal of Medical Research, 62 (6)*, 1974, 831 – 837.

[2]. N. R. Farnsworth, A. S. Bingel, G. A. Cordell, F. A. Cranea and H. H. Fong, Potential value of plants as source of new antifertility agent. *Indian Journal of Pharmaceutical science, 64 (4)*, 1974, 535 - 598.

[3]. A. O. Prakash, V. Saxena, S. Shukla, R. K. Tewari, S. Mathur, A. Gupta et al. *Anti-implantation activity of some indigenous plants in rats. Acto Eur Fertil. 16 (6)*, 1974, 441-448.

[4]. C. N. Uchendu and T. Isek , *Antifertility activity of aqueous ethanolic leaf extract of Spondias mombin (Anacardiaceae) in rats. African Health Science, 8*, 2008, 163–167.

[5]. N.K. Lohiya, B. Manivannan, S.S. Bhande, S. Panneerdoss and S. Garg, *Perspective of contraceptive choices for men. Indian Journal of Experimental Biology, 43*, 2005, 1042-1047.

[6]. E. C. Mbegbu, I. S. Ochiogu, E. O. Odirichukwu, C. E. Onyia, E. C. Nweze, O. A. Agbonu, and C. N. Uchendu, *In vitro Uterotonic Effects of Ethanolic Fruit Extract of Picralima nitida (Stapf) on Isolated Uterine Smooth Muscles of Rats. IOSR Journal of Agriculture and Veterinary Science, 7*, 2014 37-43.

[7]. A. Raj, A. Singh, A. Sharma, N. Singh, P. Kumar and V. Bhatia, Antifertility activity of medicinal plants on reproductive system of female rat, International Journal of Bio-Engineering Sciences & Technology, 2(3), 2011.

[8]. B. Hafez and E. S. E. Hafez, Reproduction in farm animals. 7th edition Lippincott Williams and Wilkins publication 2000; 03-95.

[9]. A. M. Mandl, The phases of the oestrus cycle in the adult white rat. *Journal of Experimental Biology, 28*, 1951, 576-584.

[10]. F. K. Marcondes, F. I. Bianchi and A.P Tanno, *Determination of the oestrous cycle phases of rats: some helpful considerations, Brazilian Journal of Biology, 62*, 2002, 609.614.

[11]. L. S. Gill, *Ethnomedical Uses of Plants in Nigeria*. University of Benin press, Nigeria. 1992, pp. 276

[12]. A. E. Eno, O. I. Owo, E. H. Itam, R. S. Konya, *Blood pressure depression by the fruit juice of Carica papaya (L) and DOCA-induced hypertension in rat. Phytotherapy Research, 14*, 2000, 235-239.

[13]. A. P. Adebiyi, G. Adaikan and R. N. Prasad, Papaya (*Carica papaya*) consumption is unsafe in pregnancy: fact or fable? Scientific evaluation of a common belief in some parts of Asia using a rat model. *British Journal of Nutrition, 88*, 2002, 199–203.

[14]. A. Adebiyi, P. G. Adaikan and R. N. Prasad (2003). *Tocolytic and toxic activity of papaya seed extract on isolated rat uterus. Life Sciences, 74*, 581-592.

[15]. P. A. Akah, A. N. Oli, N. M. Enwerem and K. Gamaniel. Preliminary studies on purgative effect of *Carica papaya* root extract. *Fitoterapia, 68,* 1997, 327-331.

[16]. A. C. Ezike, P. A. Aka , C. O. Okolie, N. A. Ezeuchenne, and S. Ezeugwu, *Carica papaya unripe fruits may be beneficial in ulcer. Journal of Medicinal Food, 12,* 2009, 1268-1273.

[17]. N. Pathak, P. K. Mishra, B. Manivannan and N. K. Lohiya, Sterility due to inhibition of sperm motility by oral administration of benzene chromatographic fraction of the chloroform extract of the seeds of Carica papaya in rats. *Phytomedicine*, **3,** 2000, 325-333.

[18]. O. Kusemiju, C. Noronha and A. Okanlawon, The effect of crude extract of the bark of Carica papaya on the seminiferous tubules of male Sprague-Dawley rats. *Nigerian Postgraduate Medical Journal, 9,* 2002, 205-209

[19]. R. J. Verma, and N. J. Chinoy, Effect of papaya seed extract on contractile response of cauda epididymal tubules. *Asian Journal of Andrology, 4,* 2002, 77-78.

[20]. P. Udoh, I. Essien and F. Udoh, Effects of *Carica papaya* seed extract on the morphology of pituitary - gonadal axis in male Wister rats. *Phytotherapy Research,* 19, 2005, 1065 - 1068

[21]. J. C. Saha, E. C. Savini, and S. Kasinathan (1961). Ecbolic properties of Indian medicinal plants. Part I. *Indian Journal of Medical Research, 49,* 130.

[22]. Schmidt, H. Effect of Papain on different phases of prenatal ontogenesis in rats. *Reproductive Toxicology, 9,* 1995, 49-55.

[23]. National Institute of Health (NIH) Guide for the care and use of laboratory animals, DHEW Publication. Office of Science and Health Reports, Bethesda, U.S.A., 1985)

[24]. F. K. Marcondes , Influence of the estrous cycle on the hormonal responses from female rats submitted to stress. Ph. D. Thesis, State University of Campinas, *Campinas, São Paulo,* Brazil, 1998.

[25]. J. V. Kamath, and A. C. Rana, Preliminary study on antifertility activity of *Calotropis procera* roots in female rats. *Fitoterapia, 73,* 2002, 111–115.

[26]. J. M. Goldman, A. S. Murr and R. L. Cooper, The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. Birth Defects Research Part B: *Developmental and Reproductive Toxicology, 80,* 2007, 84–97.

[27]. W. Shibeshi, E. Makonnen, A. Debella and L. Zerihu. Phytochemical, contraceptive efficacy and safety evaluations of the methanolic leaves extract of Achyranthes aspera in rats. *Pharmacologyonline 3,* 2006, 217–224.

[28]. D. N. Kage, V. B. Malashetty, Y. N. Seetharam, P. Suresh, S. B. Patil. Effect of ethanol extract of whole plant of Trichosanthes cucumerina var. cucumerina L.on gonadotropins, ovarian follicular kinetics and oestrous cycle for screening of antifertility activity in albino rats. *International Journal Morphology 27(1),* 2009 173 – 182.

[29]. A. H. Abu and C.N. Uchendu, Effects of ethanolic extract of Hymenocardia acida stem bark on estous cycle of albino rats, *Journal of medicinal plant research , 5(8),* 2011, 1280-1283.

[30]. O. I. Oloyede Chemical Profile of Unripe Pulp of Carica papaya. *Pakistan Journal of Nutrition, 4,* 2005**.** 379-381

[31]. N. R. Farnsworth, A. S Bingel, G. A Cordell, F. A. Crane and H.H.S. Fong, Potential value of plants as source of new antifertility agents. II. *Journal of Pharmaceutical Sciences,* 64, 1975, 717–754.

[32]. O. A. Olabiyi, A. A, Oremosu, C. E Noronha and A. A. Okanlanwa. Effects of cotton seed oil (gossypol spp) and cotton seed meal on the estrous cycle, ovulation and histoarchitechture of the female reproductive organs of adult Sprague –Dawley rats. *Nigerian Journal of Health and Biomedical sciences*, **5**, 2006, 21-26

[33]. K. Tamura, H. Honda, Y. Mimaki, Y. Sashida and H. Kogo Inhibitory effect of a new steroidal saponin, OSW- 1, on ovarian functions in rats. *British Journal of Pharmacolology, 121,* 1997**,** 1796-1802,

[34]. T. Benie, J. Duval and M. L. Thieulant, Effects of some traditional plant extracts on rat oestrus cycle compared with clomid, *Phytotherapy research, 17,* 2003, 748 -755.

[35]. M. Ganguly, N. Devi, R. Mahanta and M. K. Borthakur, Effect of *Mimosa pudica* root extracts on vaginal oestrous and serum hormones for screening of antifertility activity in albino mice, *Contraception, 76,* 2007, 482 – 485.

[36]. K. A. Oluyemi, I. O Omotuyi, O. R .Jimoh, O. A . Adesanya, C. L. Saalu and S. J. Josiah, Erythropoietic and anti-obesity effects of *Garcinia cambogia* in Wistar rats, *Biotechnology and Applied. Biochemistry, 46,* 2007, 69-72.

[37]. C. N. Uchendu, T. N. Kamalu, and I. U. Asuzu. A preliminary evaluation of antifertility activity of a triterpenoid glycoside from *Dalbergia saxatilis* in female Wistar rats. Pharmacological Research, *41(5),* 2000, 521 -525.

[38]. A. R Mustapha,., Bawa E. K., Ogwu, D., Abdullahi, U. S., Kaikabo, A.A and Diarra, S.S. (2011). Effects of ethanolic extract of *Rhynchosia subolata* (schumach) Meikle on estrous cycle in Wistar rats. *International Journal of Medicinal and Aromatic plants,* **1**, 122-127

[39]. M. Gopalakrishnan and M. R. Riasekharasetty (Effect of papaya (Carica papaya linn) on-pregnancy and estrous cycle in albino rats of Wistar strain. *Indian Journal of Physiology Pharmacology, 22,* 1978, 66-70.

[40]. C. Sonnenschein and A. M. Soto. An updated review of environmental estrogen and androgen mimics and antagonists, J Steroid Biochem Mol Biol *65,* 1998, 143-150.

[41]. K .Yamasaki, M. Sawaki ,S. Noda and M. Takatuki M. Effects of age and weaning on the immature rat uterotrophic assay using ehtynylestradiol, *Exp Animal 50,* 2001, 87-89.

[42]. G. Grunert, G. Neumann, M. Porcia and A. N. Tchernitchin, The estrogenic responses to Clomiphene in the different cell types of the rat uterus: Morphometrical evaluation. *Biol Reproduct 37,* 1987, 527-538.

[43]. L.C. Rockwell, S. Pillai, C. E. Olson, and R. D Koos. Inhibition of vascular endothelial growth factor/vascular permeability factor action blocks estrogen induced uterine edema and implantation in rodents, *Biol Reprod 67, 2002,*1 804-1810

[44]. R. J. Miksicek, Estrogenic flavonoids: structural requirements for biological activity. *Proceedings of the Society for Experimental Biology and Medicine, 208,* 1995, 44–50.