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Impact of the Chloroform Extract of *Carica papaya* Seed on Oestrous Cycle and Fertility in Female Albino Rats

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The impact of oral administration of 100 mg kg^{-1} b.w of chloroform extract of *Carica papaya* seed (CPE) on oestrous cycle, fertility and serum 17β-oestradiol levels, in female rats was investigated. Ten proestrous rats received 2.5% tween 80 in normal saline (vehicle for CPE) and served as the control in each part of the study. In the oestrous cycle study, ten proestrous rats were treated with CPE for 14 days. The phases and frequencies of the oestrous cycles of the rats were determined daily for another 14 days while CPE treatment continued. In the fertility study, ten proestrous rats were treated as in the oestrous cycle study for 14 days and were thereafter cohabited with fertile untreated male rats for another 14 days while CPE treatment lasted. CPE did not adversely affect body weight of the rats. However there was a significant decrease ($p<0.05$) in the weight of the ovary, but not in the uterus. There was a significant decrease ($p<0.01$) in serum 17β-oestradiol levels in CPE treated rats. The oestrous cycle became irregular, with prolonged diestrous phase from the 3rd week of CPE treatment. There were disorganization and degeneration in the ovary. The uterus showed signs of vacuolation and mild disorganization. The extract treated rats produced a significant decrease in litter number ($p<0.01$) but the fetal weight and morphology remain unchanged relative to the control. The results suggest that chloroform extract of *Carica papaya* seed has antifertility properties, possibly acting via inhibition of oestrogen secretion.

Key words: *Carica papaya* seed, oestrous cycle, fertility, 17β-oestradiol, rat

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INTRODUCTION

The role of medicinal plants in the induction of infertility in experimental animals and their possible development into contraceptive agents in male and female has continued to receive significant attention globally. Extracts of *Quassia amara*, *Azadirachta indica*, gossypol, a phenolic compound isolated from cottonseeds and glycosides extracted from xylem of *Tripterygium wilfordi*, appear to have been well-studied plant extracts in the induction of reversible infertility in male and female animals[1-4]. Castor bean extract has also been established to possess anti-ovulatory and anti-conceptive effects in cyclic female rats[5].

Carica papaya L. is a member of the small family Caricaceae allied to the Passifloraceae. As a dual- or multi-purpose, early-bearing, space-conserving, herbaceous crop, it is widely acclaimed, despite its susceptibility to natural enemies. In tropical folk medicine, the fresh latex of *Carica papaya* is smeared on boils, warts and freckles and often taken as an emmenagogue and given as a vermifuge. In India, latex and the seed are used as irritants to cause abortion. The root is ground to a paste with salt, diluted with water and given as an enema to induce abortion. A root decoction is claimed to expel roundworms[6].

Evidence from several studies suggests that *Carica papaya* fruits (epicarp, endocarp, seeds) and leaves have antifertility properties. According to Chinoy *et al.*[7], the crude aqueous extract of papaya seeds caused androgen deprivation effect on the target organs, alterations in the motility, morphology and metabolism of spermatozoa and thereby impaired fertility in male rodents. This study further revealed that the extract did not show anti-gonadotrophic nature, which was evidenced, by normal Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) levels in treated animals[7]. The influence of crude aqueous extract of *Carica papaya* seeds administered both orally and intramuscularly indicated that reversible sterility could be induced in male rats without adverse effects on libido and toxicological profile. There was a significant reduction in testicular sperm counts and motility associated with morphological defects. Oral administration of aqueous, methanol, ethyl acetate and chloroform extract of *Carica papaya* seeds in rats revealed reversible infertility efficacy but only the aqueous and chloroform extract were without significant toxicity and impairment to libido. In rats, treatments with chloroform extract, benzene chromatographic fraction of the chloroform extract and its methanol and ethyl acetate sub-fractions lead to azoospermia[8]. Owing to the proven contraceptive efficacy of chloroform extract of *Carica papaya* seeds in rats and rabbits, Lohiya and *et al.*[9] evaluated the antifertility activity of this extract by oral administration in a non-human primate, the Langue monkey. The results revealed gradual decrease in sperm concentration leading to azoospermia, total inhibition of sperm viability and increase in sperm abnormality, after 90 days of treatment. Treatment withdrawal resulted in a gradual recovery in these parameters to nearly pretreatment values at about 150 days.

The studies on *Carica papaya* seeds extract on reproduction seem to be concentrated in male. This is evidenced by the gamut of literature on *Carica papaya* extract and antifertility activities in several models of male experimental animals. The action of *Carica payaya* seed in female animals is largely a product of folk medicine and there is a dearth of information in the literature in this direction. In view of this, the present study was undertaken to explore the impact of chloroform extract of *Carica papaya* seeds on estrous cycle and fertility in female albino rats.

MATERIALS AND METHODS

Animal model: The study was carried out on young adult female rats of Wistar strain weighing between 130-190 g initially. The animals were kept in cages in the animal house of the department of Physiology, University of Ibadan under standard environmental conditions (12 h light and 12 h dark cycles). The animals were fed with pelleted rat diet (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. The oestrous cycles of the animals were assessed by daily vaginal smears and those with three consecutive regular oestrous cycles were selected for the experiment.

Collection and preparation of extract of *Carica papaya* seed: The seeds of *Carica papaya* Linn (Caricaceae) from ripe fruits were collected at the Botanical garden, University of Ibadan, Ibadan, Nigeria. The plant material was identified and authenticated at the Forestry Research Institute of Nigeria where a voucher specimen with herbarium number F.H.I 106879 was deposited. The seeds of *Carica papaya* were air-dried and ground into powder. Extraction was carried out with chloroform using Soxhlet extractor at 58°C for 12 x 3 h. The filtrate obtained was concentrated under reduced pressure and yielded a dark brown semi-solid mass (w/w, 6%), which was used in this study.

Experimental design: A total of forty proestrous female albino rats were used. The study was made up of two experimental parts; oestrous cycle study and fertility

study. Each part had 20 rats. The oestrous cycle of each rat was established and only those with three consecutive regular oestrous cycles were included in the study.

Experiment 1: Oestrous cycle study: Twenty proestrous female rats were divided into two equal groups of 10 rats each. Group I served as the control and received 2.5% tween 80 (vehicle for *Carica papaya* seed extract, CPE) orally. Group II rats received 100 mg kg^{-1} b.w of CPE orally. The dose of CPE was based on the study of Lohiya *et al*[8]. Treatment with the vehicle or extract was done daily for 2 weeks. The phases of the oestrous cycle of these rats were determined during and 2 weeks after treatments by vaginal smear method. A cycle was designated as a 4-day period extending from the first day of leukocytes appearance (diestrous) and preceded by a cornified vaginal smear, characteristic of estrous phase, till the end of the next sequence of cornified vaginal smear. Proestrous corresponded to nucleated epithelial cells while metestrous corresponded to a short phase between estrous and diestrous.

Experiment 2: Fertility study: Twenty proestrous female rats were divided into two groups of 10 rats each. Group I served as the control and received vehicle for CPE. Group II received 100 mg kg^{-1} b.w of CPE. Vehicle and CPE administrations were done orally for 14 days before cohabitation with untreated male rats of proven fertility in the ratio of 3 CPE treated females to 1 untreated male. Vehicle and CPE administrations were continued during and after mating for another 14 days. Successful mating in each case was confirmed by the presence of spermatozoa in the vaginal smear and/or presence of vaginal plug. Day 1 of pregnancy was taken for the day of successful mating. The gestation period of each treated rat, the number and body weight of litters delivered and physical morphology of the litters were assessed.

Hormonal assay: Blood samples were collected from the orbital sinus of each rat into sterile sample bottle without anticoagulant during proestrous phase at 0900 h. An aliquot sample of blood supernatant was used for determination of 17β-oestradiol levels. The serum concentration of 17β-oestradiol was estimated using the tube-based enzyme immuno-assay (EIA) method. The EIA kit produced by Immunometrics (UK) was used in the estimation of the hormone. The intra and inter-assay coefficients of variation were 10.1 and 10.4%, respectively.

Autopsy: The animals were sacrificed 24 h after the experiment by exsanguinations under ether anaesthesia. The oestrous cycle rats were sacrificed at proestrous phase. The ovary and the uterus of each rat were dissected out, freed of any adherent tissues and weighed immediately.

Histology: Immediately after weighing, the ovary and uterus were fixed in 10% formal saline for histological analyses. The preserved ovary and uterus were then passed through routine histological procedures. Slides were stained with haematoxylin and eosin.

Statistical analysis: Paired t-test was used for dependent variables while Student t-test was used for independent variables. Values were recorded as mean±SEM. Confidence interval of 95% was accepted as the least statistically significant level.

RESULTS

Effects of CPE on body and organ weights: Oral administration of *Carica papaya* seed extract to rats for four weeks had no negative effect on body weight (Fig. 1). The control rats exhibited a 26.2% weight gain. The percentage body weight gain in the CPE treated rats was 27.7%. There was a statistically significant increase ($p<0.01$) in the final body weight in each group when compared with the respective initial body weight. A significant decrease ($p<0.01$) in the mean ovarian weight of the CPE treated rats was recorded when compared with the respective control. However, the mean weight of the uterus in CPE treated rats remained unchanged when compared with their control counterparts.

Effects of CPE on length and phases of oestrous cycle in albino rats: The control rats exhibited normal regular 4 days oestrous cycle with normal alternating diestrous, proestrous, oestrous and metestrous phases. However, CPE treated rats showed an extended oestrous cycle. The cycle was longer by about 3-6 days; 60% had a 9-12 days cycle and 40% had a 7-8 days cycle. The normal 4-5 days cycle was totally abolished in this group. The mean lengths of diestrous and proestrous phases were significantly increased ($p<0.01$) in the CPE treated rats when compared with the control. However, the mean length of the oestrous phase showed a significant decrease ($p<0.01$). The oestrous phase was almost abolished; only about 2.5% of the whole cycle remained in the CPE treated rats. The diestrous phase was dominant and accounted for 68.5% of the oestrous cycle while proestrous phase was about 29% of the cycle (Fig. 2).

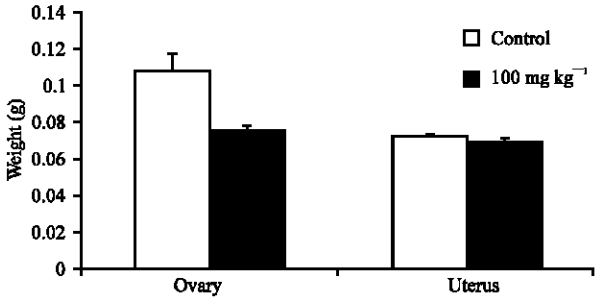

Fig. 1: Effect of *Carica papaya* seed extract on mean relative weight of reproductive organs

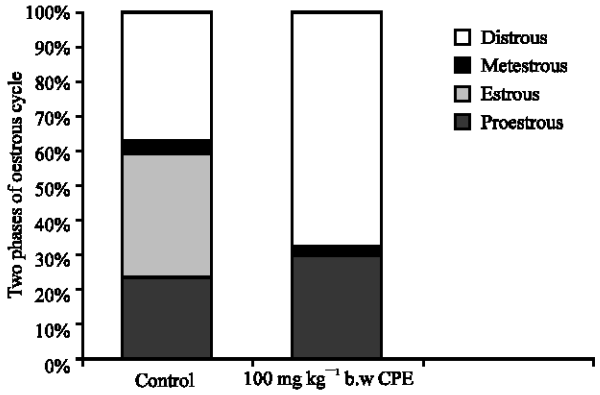

Fig. 2: Effect of *Carica papaya* seed extract on oestrous cycle phases

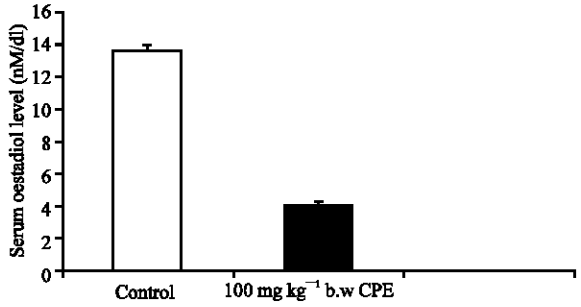

Fig. 3: Effect of *Carica papaya* seed extract on serum 17β-oestradiol levels

Serum 17β-oestradiol levels: The mean 17β-oestradiol levels were significantly reduced ($p<0.01$) in all CPE treated rats when compared with the respective control groups (Fig. 3).

Fertility test: While all control rats got pregnant within one week of cohabitation with untreated male rat, only 60% of the CPE treated rats were pregnant during this period. The mean gestation period and morphology of all treated rats were not significantly different when compared with the control ($p>0.05$). However the CPE

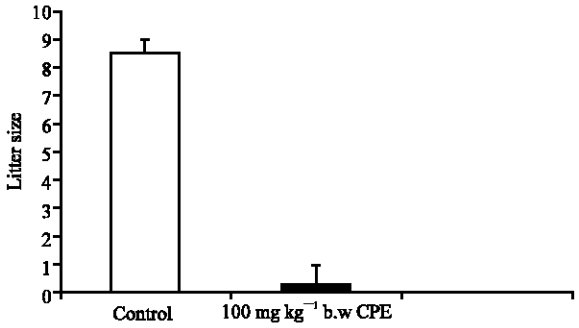

Fig. 4: Effect of *Carica papaya* seed extract on litter size

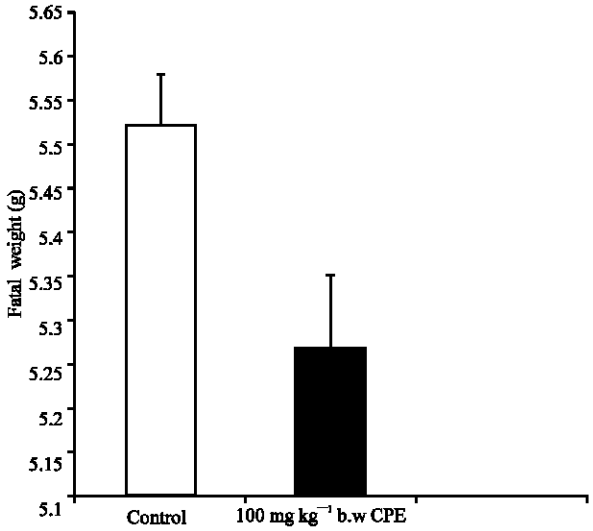

Fig. 5: Effect of *Carica papaya* seed extract on fetal weight

treated rats exhibited a statistically significant decrease ($p<0.01$) in the litter number relative to the control (Fig. 4). There was however no significant difference in the mean fetal weight of the CPE treated rats when compared with the control (Fig. 5).

Histology of the ovary and uterus: There are visible lesions in the histological section of the ovary of CPE treated rats when compared with the control (Fig 6a and b). There was a gross reduction in the size of the follicular antrum. Follicular maturation was adversely affected with the appearance of atretic follicles. The atresia was characterized by gross thickening of the basement membrane between the granulosa and theca layers. The uteri of control rats appeared normal histologically, while those of CPE treated rats had histological alterations ranging from mild degeneration to massive vacuolation in the basal region (Fig. 7a and b).

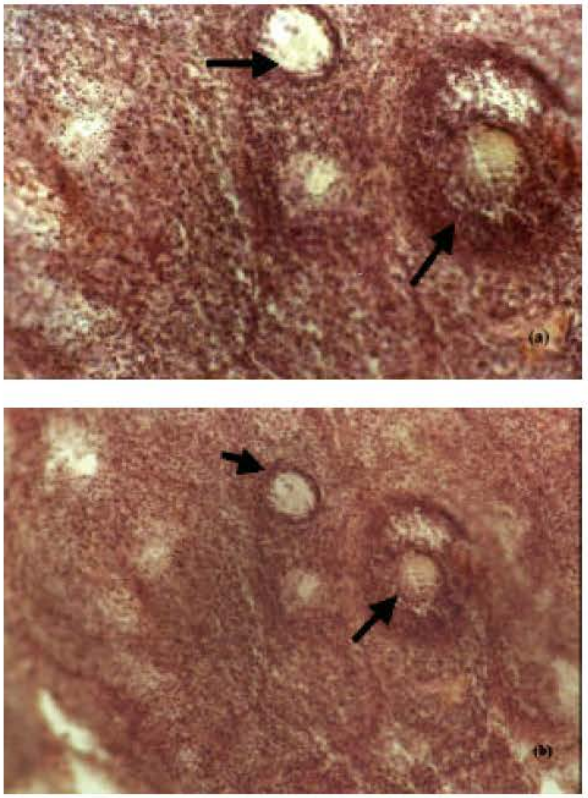

Fig. 6a: Light micrograph of the ovary of a control rat at proestrous. There were no histological changes. Arrows show the various developing follicles and follicular antrum. b: Light micrograph of the ovary of a CPE treated rat at proestrous. Arrows show gross reduction in the follicular antrum

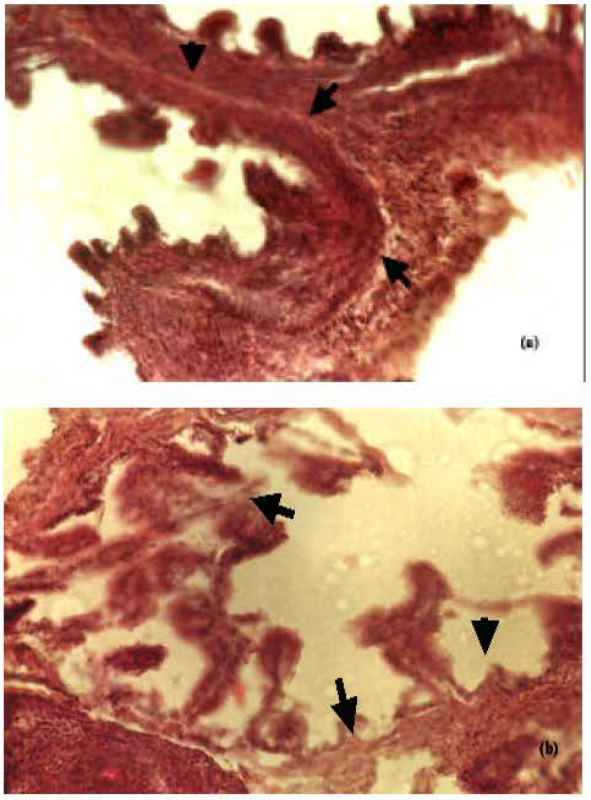

Fig. 7a: Light micrograph of the uterus of a control rat at proestrous. Arrows show normal endometrium without vacuoles. b: Light micrograph of the uterus of a CPE treated rat. Arrows show massive vacuolation and disruption of the endometrium

DISCUSSION

The results obtained from this study showed that chloroform extract of *Carica papaya* seed possess adverse effects on oestrous cycle and fertility in female rats and justify the folkloric use of papaya seeds as an abortifacient agent. We had recently reported the anti-implantation and abortifacient properties of the chloroform extract of *Carica papaya* seed in female rats[10]. The normal oestrous cycle and ovulation are functionally regulated by appropriate oestrogen level, which usually peaks during the oestrous phase of the cycle. This gives an indication that the extract of *Carica papaya* seed may act on the ovary through altered endocrine functions associated with decreased oestrogen level. The serum oestrogen levels in all treated rats showed a significant decrease relative to the control. The present study reports for the first time the effect of CPE on oestrogen secretion. Previous studies have focused on the effect of CPE in the male. Evidence in support of the present findings could therefore be inferred only from the male studies. Chinoy *et al.*[11] reported that *Carica papaya* bark extract manifested androgen deprivation effect on target organs thereby causing infertility in male rats. The work of Kusemiju *et al.*[12] also corroborate this finding, with the suggestion that *Carica papaya* extract eliminated testosterone and other Leydig cell factors required for steroidogenesis in male rats. There is a growing body of evidence[13,14] to show that *Carica papaya* causes the release of catecholamines, epinephrine and norepinephrine, from alpha-adrenergic receptors. Since catecholamines are known to induce gonadal inhibition[15,16], it has been suggested that they may interfere with the production of testosterone[17] via this mechanism. Thus, impairment in the production of testosterone that is a pre-hormone for oestrogen is probably an indirect impairment on oestrogen production. Moreover, Van Denmark and Boyd[18] speculated that a combination of enzymes, alkaloids and other substances

in *Carica papaya*, might themselves inhibit testosterone production and ultimately oestrogen production.

The significant structural alterations in the histological sections of the ovary and uterus in CPE treated rats compared with the control further support the possible deleterious impact of CPE on female reproduction. This extract induced degeneration of the follicular wall, which may be responsible in part for the significant decrease in serum oestrogen level. This probably led to the anovulatory cycles and the consequent decrease in litter number. The fetal weights and morphology were not adversely affected which was indicative of non-toxic profile of the chloroform extract of *Carica papaya* seed on fetal development if administered before conception. Lucidi *et al.*[19] suggested that steroidogenesis could be influenced by active development of the oocyte. It then follows that the atretic follicles in the histological sections could be due to a decrease in oestrogen level. The hormonal and histological changes could lead to the significant reduction in ovarian weight. The uterine histological section showed prominent vacuolation and mild degeneration.

There was no treatment related disorders in body weight throughout the period of treatment. Aqueous extract of *Carica papaya* had earlier been reported not to show any untoward effect on body weight despite its antifertility effects in male rats[9]. Body weight is known to play an important role in the regulation of gonadotrophin secretion and its crucial role for regular cyclic function is well established[20,21]. Thus, the adverse response of *Carica papaya* extract on endocrine functions and oestrous cycle in this study was not primarily a function of impact on general body weight. The exact principles that caused the irregularity in the oestrous cycle and infertility in CPE are not known. Phytochemical characterization of this extract with investigation into the biological activity of the resultant products is expected to unravel the compounds in the extract with these female reproductive activities.

REFERENCES

1. Raji, Y. and A.F. Bolarinwa, 1997. Antifertility activity of *Quassia amara* in male rats *in vivo* study. Life Sci., 64: 1067-1074.
2. Parveen, S., S. Das, C.P. Kundra and B.M.J. Pereira, 2003. A comprehensive evaluation of the reproductive toxicity of *Quassia amara* in male rats Reproductive Toxicol., 17: 45-50.
3. Raji, Y., U.S. Udoh, O.O. Mewoyeka, F.C. Ononye and A.F. Bolarinwa, 2003. Implication of reproductive endocrine malfunction in male antifertility efficacy of *Azadirachta indica* extract in rats. African J. Med. Med. Sci., 32: 159-165.
4. Qian, S.Z., 1987. In reply to: Fertility Regulation Today and Tomorrow; (Diczfalusy, E. and M. Bygdeman Eds.) Ravan Press, New York, pp: 217-232.
5. McNeil, R.T., C.C. Noronha, T.O. Kusemiju and A.O. Okanlawon, 2003. The anti ovulatory effects of *Ricinus communis* Linn. Nig. J. Health Biomed. Sci., 2: 31-34.
6. Morton, J., 1987. Papaya. In: Fruits of warm climates. Julia F. Morton, Miami, F.L., pp: 336-346.
7. Chinoy, N.J., J.M.D. Sonza and P. Padman, 1984. Effect of crude aqueous extract of *Carica papaya* seed in male albino rat. Reproductive Toxicol., 8: 75-79.
8. Lohiya, N.K., B. Manivannam, P.K. Mishra and N. Pathak, 2001. Prospects of Developing a Plant Based Male Contraceptive Pill. In: Chowdhury, S.R., C.M. Gupta and V.P. Kamboj (Eds.), Current Status in Fertility and Modern Approaches. Cental Drug Research Institute, pp: 99-119.
9. Lohiya, N.K., B. Manivannam, P.K. Mishra, N. Pathak, S. Sririam, S.S. Bhande and S. Pann, 2002. Chloroform extract of *Carica papaya* seeds induces long term reversible azoospermia in langue monkey. Asian J. Androl., 4: 17-26.
10. Raji, Y., A.O. Morakinyo, O.S. Akinsomisoye, A.K. Oloyo, P.R.C. Esegbue-Peters and O.T. Kunle-Alabi, 2005. An evaluation of the abortifacient properties of chloroform extract of *Carica papaya* l. Seed in female albino rats. Intl. J. Pharmacol., (In Press)
11. Chinoy, N.J. and S.M. George, 1983. Induction of functional sterility in male rats by low dose of *Carica papaya* seed extract treatment. Acta European Fertility, 14: 425-432.
12. Kusemiju, O., C. Noronha and O.A. Okanlawon, 2002. The effect of crude extract of the bark of *Carica papaya* on the seminiferous tubules of male Sprague-Dawley rats. Nigerian Postgraduate Med. J., 9: 205-209.
13. Cherian, T., 2000. Effect of *Papaya latex* extract on grand and non-gravid rat uterus preparations *in vitro*. J. Ethnopharmacol., 70: 205-212.
14. Eno, A.E., O.I. Owo, E. Itam and R.S. Konya, 2000. Blood pressure depression by fruit juice of *Carica papaya* (I) in renal and DOCA-induced hypertension in the rat. Pytother. Res., 14: 235-239.

15. Biswas, N.M., P.B. Patra, S. Sanyal and C. Deb, 1979. Adrenogonadal interactions in nicotine induced alteration of steroidogenesis in male rat. Adrologia, II: 227-233.
16. Favaretto, A.L., M.M. Valenca, M. Hattori, K. Wakabayashi and J. Autunes-Rodrigues, 1998. Characterization adrenergic control of the laying cell steroidogenesis: Identification of both stimulatory and inhibitory components. Brazilian J. Med. Biol. Res., 21: 539-43.
17. Mayerhofer, A., A.G. Amador, R.W. Stager, J.G.M. Shire and A. Batke, 1993. Catecholamines and testicular function: Study in BALB/e J mice, a substrain with high levels of adrenomedullary catecholamine synthesizing enzymes. Neuroendocrin. Lett., 15: 417-424.
18. Van Denmark, N.L. and L.J. Boyd, 1996. The effect of epinephrine upon testicular functions in rabbits. Luterm J. Fertility, 81: 245-247.
19. Lucidi, P., N. Bernabo, M. Turriani, Mattioli and B. Barboni, 2003. *Cumulus steroidogenesis* is influenced by the degree of oocyte maturation. Reprod. Biol. Endocrinolol., 1: 45-55.
20. Knuth, U.A., M.G. Hull and H.S. Jacobs, 1977. Amenorrhea and loss of weight. Br. J. Obstetrics Gynecol., 84: 801-807.
21. Schwartz, N.B., 1969. A model for the regulation of ovulation in the rat. Recent Progress in Hormone Research, 25: 1-55.