

The contraceptive potential of *Carica papaya* seed on oestrus cycle, progesterone, and histomorphology of the Utero-ovarian tissue of adult wistar rats

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ABSTRACT

Objective: The study's goal was to ascertain the contraceptive effects of Aqueous extract of *Carica papaya* on female rats by assessing changes in the body weight, estrous cycle, serum progesterone level and the cyto-architecture of the Utero-ovarian tissue.

Methods: We used twenty (20) healthy young Adult Female Albino rats. The study ran for 7 and 21 days, respectively. Each study group has their Experimental (treated 200mg/kg aqueous extract of *Carica papaya* seed extract) and Control group (n=5). We determined daily the phases and frequencies of the estrous cycles of the rats during the administration of the extract. We processed the utero-ovarian tissue for histological analysis, and we assessed serum progesterone level and the oestrus cycle pattern.

Results: There was a significant increase in body and Ovarian weights after 21 days of treatment when compared to controls and those treated for 7 days. However, uterine weight reduced significantly ($p < 0.05$), serum progesterone level decreased ($p < 0.05$) in the treated rats, mostly in those submitted to 21 day-treatments; the ovary showed marked degeneration of the theca cells, granulosa and corpus luteum, and loss of mucin granules in the uterine tissues. *Carica papaya* administered for 7 and 21 days caused the animals to have more proestrus and diestrus phases as compared to the control animals. The estrous cycle became irregular, with prolonged diestrus and proestrus phase.

Conclusion: The aqueous extract of *Carica papaya* seeds caused antifertility, anti-implantation, by a reduction in progesterone level, disruption of oestrus pattern and histological alteration of utero-ovarian tissue.

Keywords: *Carica papaya*, progesterone, utero-ovarian tissue, oestrus cycle

INTRODUCTION

Over the years, the whole *Carica papaya* plant parts, leaves, seeds, ripe and unripe fruits, and their juice have been used as traditional medicine due to their high antioxidant activities (Arvind *et al.*, 2013; Sindhu *et al.*, 2019). Papaya juice or the fruit is generally served either green or ripe; while its leaves and young stems are sometimes steamed and served as a vegetable (Arvind *et al.*, 2013). Nowadays, papaya (*Carica papaya*, Linn) is considered as a nutraceutical fruit, because of its exceptional nutritional and multifaceted medicinal properties (Parle & Guditta, 2011; Sindhu *et al.*, 2019) which include antibacterial, anti-inflammatory, anti-aging, anti-proliferative, diuretic, anti-hypertensive, hypolipidemic, anti-helminthic, wound healing, anti-fungal and anti-tumor (Sarker *et al.*, 2010; Kaushal *et al.*, 2015; Gunde & Amnerkar, 2016; Sindhu *et*

al., 2019) and free radical scavenging activities that helps to reduce atherosclerosis, strokes, diabetes, and heart attacks (Arvind *et al.*, 2013). Phytochemically, the whole plant contains enzymes, lycopene, carotenoids, alkaloids, monoterpenoids, flavonoids, minerals and vitamins (Sindhu *et al.*, 2019). The seeds have fatty acids, protein, fiber, papaya oil, sinigrin, Carpaine, benzyl isothiocyanate, benzyl glucosinolate, glucotropacolin, benzylthiourea, hentriacontane, β -sitosterol, caricin and an enzyme called myrosin (Azarkan *et al.*, 2003; Adebisi *et al.*, 2002), while the *Carica papaya* leaves has alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones and cardenolides (Arvind *et al.*, 2013; Sindhu *et al.*, 2019). *Carica papaya* black seeds are edible, having sharp and spicy taste; hence, its ground form is used as an alternative for black pepper (Sindhu *et al.*, 2019). Studies have reported that these seeds have more potent medicinal properties as compared to other parts of the papaya plant due to their strong antioxidant properties (Zhou *et al.*, 2011; Fatima & Shahid, 2018; Otsuki *et al.*, 2010; Sindhu *et al.*, 2019); for example we have antibacterial (*E.coli*, Salmonella and Staphylococcus infections), liver protection, anthelmintic, typhoid treatments (Sindhu *et al.*, 2019), nephroprotective effects (Otsuki *et al.*, 2010) and in folk medicine it is an intestinal worm expellant in humans and ruminants.

Medicinal plants have played an important role in new drug developments. Birth control, also known as contraception and fertility control, is a method or device used to prevent pregnancy (Taliaferro *et al.*, 2011). According to Westwood (2008) and Yuan & Foley (2002) drugs and chemical agents can interfere with the normal functions of the female reproductive cycle as well as interfere in the normal morphology of the reproductive organs. Studies have documented the role of medicinal plants in inducing infertility in experimental animals, and their possible development into contraceptive agents in males and females has gained continuous global attention by the pharmaceutical industry. Extracts of *Quassia amara*, *Azadirachta indica*, gossypol, a phenolic compound isolated from cottonseeds and glycosides extracted from xylem of *Tripterygium wilfordii*, appears to have been well-studied plant extracts for the induction of reversible infertility in male and female animals (Cherian, 2000).

Carica papaya seed extract has been associated with antifertility, documented by various studies in a quest to discover male contraceptive agents (Lohiya & Goyal, 1992; Lohiya *et al.*, 1994; 1999; 2002; 2005; Chinoy *et al.*, 1995; Pathak *et al.*, 2000; Kusemiju *et al.*, 2002; Udoh *et al.*, 2005). Studies have reported antifertility potentials of plant parts of *Carica papaya* fruits and leaves in isolates without actually explaining the key mechanistic processes of antifertility. Studies have documented the antifertility effects of pawpaw seeds on the male reproductive system

(Udoh & Kehinde, 1999; Chinoy *et al.*, 1995; Lohiya *et al.*, 1999; Udoh *et al.*, 2005). Adebisi *et al.* (2002) reported the unsafe ingestion of these seeds during pregnancy. The action of *Carica papaya* seeds in female animals is largely a product of folk medicine and there are varying reports in the literature on its mechanism of action in causing infertility in females (Karunamoorthi *et al.*, 2014).

Assessment of the reproductive (oestrus) cycle is a valid way to study animals exhibiting abnormal oestrus patterns due to exposure of any compound and that caused changes to normal patterns (Goldman *et al.*, 2007; McLean *et al.*, 2012; Cora *et al.*, 2015). This study was undertaken to explore the impact and mechanism of action of aqueous extract of *Carica papaya* seeds on estrous cycle and fertility in female albino rats.

MATERIALS AND METHODS

Experimental Animals

There were twenty (20) healthy young Adult Female Albino rats, of average weight of 120g, used for this study. We procured them from the Ahmadu Bello University, Zaria, Kaduna state, Nigeria. They were kept in well-aerated cages in the Animal House of Anatomy Department, Bingham University, Karu Nigeria. They were cared for according to guidelines from the National Research Council (US) Institute for Laboratory Animal Research (2011) and the Canadian Council on Animal Care (CCAC, 1984). They were allowed two (2) weeks acclimatization in standard laboratory conditions [12 hrs dark: 12 hrs light cycle, room temperature and humidity 52%]. They had free access to water and pelleted rat feed (Ladokun Feeds, Ibadan). Approval from the departmental research review ethical committee was obtained before the experiments. Rats have a short oestrus cycle, of 4 to 5 days (Long & Evans, 1922; Freeman, 1988; Mandl, 1951) and this makes it a perfect animal to investigate the effects of *Carica papaya* on the female reproductive system (Marcondes *et al.*, 2001; McLean *et al.*, 2012).

Oestrus Cycle study

The rats' oestrus cycles were studied daily according to Marcondes *et al.*, (2001; 2002) and Hubscher *et al.* (2005)'s method. We selected rats with consecutive similar oestrus cycles for the experiment. The estrous cycle in a female rat is characterized by four different phases, which are diestrus, proestrus, oestrus, and metestrus. This cyclical pattern is completed within 4 to 5 days and they are microscopically evaluated via vaginal smear (Nelson *et al.*, 1982; Goldman *et al.*, 2007; McLean *et al.*, 2012; Gonzalez, 2016).

Preparation of the Vaginal Smear

The vaginal smear was done between 8:00 - 9:00 a.m. and 4:00 - 6 p.m. We collected the vaginal fluid using a plastic pipette filled with 0.5 ml of normal saline (NaCl 0.9%). The tip of the pipette was gently inserted into the rat vaginal opening. We then placed the aspirated vaginal fluid on a microscopic glass slide. We used a different microscopic glass slide for each experimental animal examined. We deposited one drop of the vaginal fluid on a slide and examined the unstained vaginal smear histology using an Olympus Light Microscope at 10 and 40 x objective lenses (Naik *et al.*, 2015). We examined the four phases, described by Goldman *et al.* (2007) and Byers *et al.* (2012) they were: diestrus (D), when the cells are predominantly leucocytes; proestrus (P), with numerous nucleated cells; the oestrus (E) phase is marked by the presence of large numbers of cornified epithelial cells and, the last phase, metestrus (M), characterized by scattered squamous epithelial cells and several neutrophils. We used

this procedure daily to assess the effects of *Carica papaya* seed extract on the oestrus cycle, for 21 days.

Plant Material and Aqueous extract preparation

Collection of *Carica papaya* seed: Twelve (12) mature ripe *Carica papaya* (Paw-Paw) fruits were bought at the Mararaba market, Karu, Nasarawa State, Nigeria. The collected samples were identified and authenticated by a botanist from the Forestry Research Institute of Nigeria, where the Voucher specimen were documented in the Herbarium (106879).

Preparation of *Carica papaya* seed extract: The pawpaw fruits were cut open and the wet seeds brought out. They were gently rinsed in running tap water to clean it, and then they were air-dried for two weeks at room temperature. The dried *Carica papaya* seeds were then pulverized into a fine powder using a blender. 240g of powdered *Carica papaya* seed were macerated in 1000 ml of hot water for thirty (30) minutes and stirred every 5 minutes to ensure it was well soaked. Thereafter, the *Carica papaya* seed solution was filtered using a clean filter paper (Whatman Filter Paper) and we collected the filtrate into a conical flask according to the method described by Adebisi *et al.* (2002), Dosumu *et al.* (2008) and Naggayi *et al.* (2015). We refrigerated the aqueous solution in an airtight container. The final filtrate was completely oven-dried at a preset temperature of 60°C for a week, producing a fine aromatic and chocolate color solid residue (the dry aqueous seed extract) according to Naggayi *et al.* (2015).

Aqueous *Carica papaya* seed extract dosage and mode of administration

Each experimental animal was given 1ml of 200mg/kg aqueous extract of *Carica papaya* seed Extract daily for 7 and 21 days respectively, according to Ruiz-Luna *et al.* (2005) and Naik *et al.* (2015). Olagunju *et al.* (2009) reported that LD50 of Aqueous *Carica papaya* seed extract is greater than 200mg/kg when taken orally.

Experimental Design

Twenty (20) proestrus rats were used for this study, according to recommendations from Raji *et al.* (2005). They were studied for 7 and 21 days, respectively; each with their control groups for the study period (n=5). The phases and frequencies of the estrous cycle of the rats were determined daily for 21 days during the extract administration.

Experimental Animal Euthanasia

Twenty-four hours after the last drug administration, we measured their body weights and the Animals were euthanized through neck dislocation.

Blood collection and Preservation for Serum Hormone Analysis

The blood samples were collected using a 5ml syringe via cardiac puncture into the left ventricle, before neck dislocation. The collected samples were transferred into blood specimen bottles, they were then centrifuge at 2,500 rpm for 5 minutes to obtain the sera, which were aliquoted into plain specimen cuvettes and kept in the freezer ready for Progesterone ELISA kit quantitative spectrophotometric analysis.

Serum Progesterone Analysis

Their serum progesterone activity was quantified using the Progesterone Enzyme-Linked immunosorbent assay (ELISA) kit (BXE0661A), Fortress Diagnostic Ltd. UK). We performed the assay according to the instructional manual from the kit manufacturer.

Ovary and Uterine Tissues Collection

We made an incision on the pelvic region using a scalpel and carefully traced and excised the uterus and ovaries using a fine forceps. We measured their wet weights and preserved the tissues in a specimen bottle containing 10% formaldehyde saline ready for histological processing, according to Bancroft & Gamble's (2008) method.

Histological Tissue Processing and Staining

We fixed the ovary and uterus tissues in 10% formol saline and processed it using a Leica automated tissue processor. The processed tissues were embedded using paraffin wax in a Leica Embedding Machine. The tissue blocks were then serially sectioned using Leica Rotatory Microtome set at 5 μ m. We then mounted the sectioned tissues using DPX on glass slides, ready for Hematoxylin and Eosin, and Periodic acid Schiff stain (PAS) for Mucin granules, according to Bancroft & Gamble's (2008) method.

Tissue Photomicrography and Analysis

We examined the tissue slides using an Olympus Light Microscopy, with an attached digital camera with x10 objectives. We viewed the tissues at x10, x40 but the digital picture was taken at x10.

Statistical Analysis

We analyzed the data using the SPSS version 17.0, and expressed it as mean \pm Standard error of the mean (SEM). We used the Student t-test to compare the data from both groups. The level of statistical significance (*) was set at $p < 0.05$.

RESULTS

Effects of Aqueous *Carica papaya* seed extract on body and organ weights

Oral administration of *Carica papaya* aqueous seed extract for 7 days had no significant effect on body weight gain when compared to the control animals ($p < 0.05$) (Fig. 1). There was no significant difference between the initial and final weights of *Carica papaya* treated animals ($p < 0.05$). However, we found that there was a significant increase in final body weight gain in those treated for 21 days when compared to their initial weights. There was a significant weight change between

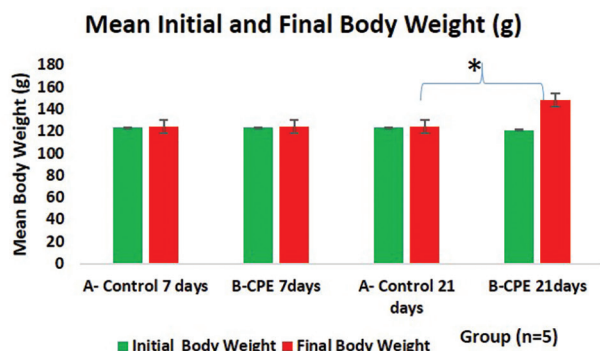


Figure 1. Graphical representation of the mean initial and final body weights of the experimental animals treated with 200mg/kg of aqueous *Carica papaya* seed extract. The asterisk (*) shows statistical significance ($p < 0.05$).

Legend: CPE = *Carica papaya* extract.

control and *Carica papaya*-treated groups during the 21 day-treatment study (Fig. 1).

Aqueous *Carica papaya* seed extract reduced changes in ovary and uterus weights

This study shows that aqueous extract of *Carica papaya* caused a statistically significant decrease in ovary weight following 7 days of treatment, as compared with its control group (Fig. 2). However, those treated for 21 days had a statistically significant increase in ovarian weight when compared with the Control animals for 21 days, and ACPSE treated for 7 days. As shown in Fig. 3, there was a significant reduction in the uterus's wet weight in 7 and 21 day-treatments as compared with the control group ($p < 0.05$).

Aqueous *Carica papaya* seed extract decreases serum progesterone level

This study shows that the *Carica papaya* aqueous extract caused a statistically significant decrease in the serum progesterone level ($p < 0.05$) in treated rats as compared with their control rats in their proestrus phase of the cycle (Fig. 4). Those treated for 21 days had a

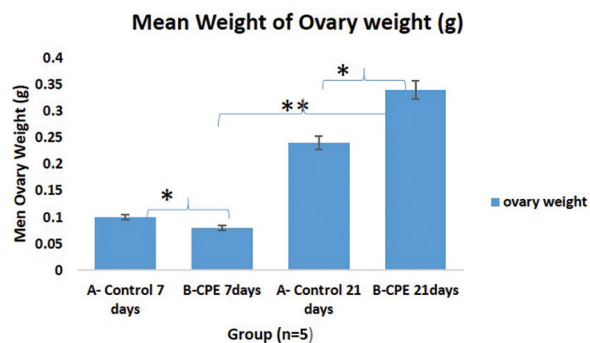


Figure 2. Graphical representation of the Mean Ovary Weight of the Experimental Animals treated with 200mg/kg of aqueous *Carica papaya* seed extract. The asterisk (*) shows statistical significance ($p < 0.05$). *: A vs. B; 7 days and A vs. B for 21 days; **: B 7 days treated vs. B 21-day treatment.

Legend: CPE = *Carica papaya* extract

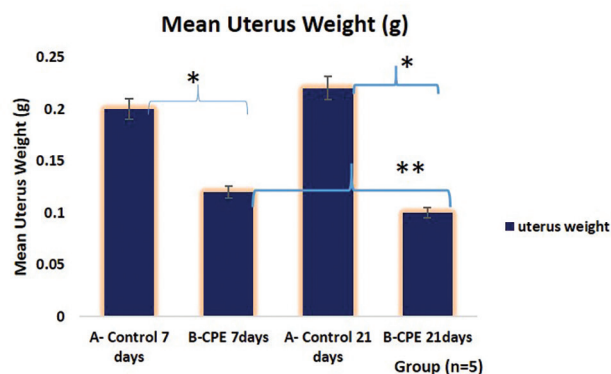


Figure 3. Graphical representation of the Mean Weight of the Uterus of Experimental Animals treated with 200mg/kg of aqueous *Carica papaya* seed extract. The asterisk (*) shows statistical significance ($p < 0.05$). *: A vs. B; 7 days and A vs. B for 21 days; **: B 7 days treated vs. B 21 days treatment.

Legend: CPE = *Carica papaya* extract

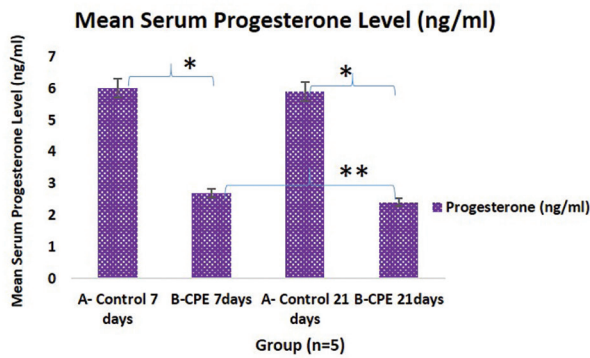


Figure 4. Graphical representation of the Mean Serum Progesterone level of Experimental Animals treated with 200mg/kg of aqueous *Carica papaya* seed extract. The asterisk (*) shows statistical significance ($p < 0.05$). *: A vs. B; 7 days and A vs. B for 21 days; **: B 7 days treated vs. B 21 days treatment. Legend: CPE = *Carica papaya* extract

significant (**) reduction in progesterone levels when compared to the 7-day treatment group.

Effects of aqueous *Carica papaya* seed extract on of oestrus cycle’s phases:

The control rats showed a regular and normal alternating cycle of diestrus, proestrus, estrus and metestrus phases. However, the *Carica papaya* seed extract-treated animals showed an extended pattern in the estrous cycle. The normal 4-5 day-cycles described by the study from Marcondes *et al.* (2002) was truncated in the *Carica papaya* study groups. *Carica papaya*-treated animals for 7 and 21 days had more Proestrus and diestrus phases as compared to their control counterparts. In this study, we found that the diestrus phase was dominant in the *Carica papaya* treatment as compared to the proestrus phase (Table 1).

Effects of aqueous *Carica papaya* seed extract on ovarian tissue histology

The *Carica papaya* study group had a distinctive lesion or degeneration of ovarian primordial and primary follicles as compared to the control group (Fig. 5). Follicular cells proliferation was grossly affected. Theca and granulosa cell

arrangements were distorted as compared to the control group. The Corpus luteum appears degenerated and we noticed numerous striations/vacuolations in the ovarian stroma (Fig. 5).

Effects of aqueous *Carica papaya* seed extract on uterine histoarchitecture:

There was a gross loss of uterine tissue histological integrity in the *Carica papaya*-treated groups (Fig. 6). The uterine glands were poorly stained as compared to the control group with visible tubular uterine gland and well-pigmented endometrial lining. However, the severity of distortion or loss of cellular integrity was more often seen in the 21-day treated groups displaying severe loss of cellular pigmentation. Periodic Acid Stained Uterus demonstrated mucin granules, showing that the control groups were positive for PAS-stained structures, being positive for the presence of Mucin granules while the *Carica papaya*-treated groups (Fig. 7) were not positive for the mucin granules, indicating that the *Carica papaya* extract caused severe loss of mucin granules in the epithelial cells cytoplasm.

DISCUSSION

The results obtained from this study showed that the aqueous extract of *Carica papaya* seed has a severe adverse effect on the oestrus cycles, progesterone level and the uterus and ovary histology. These aforementioned results justify its reported use as an abortifacient agent (Dosumu *et al.*, 2008; Raji *et al.*, 2005; Ekhaton & Shelu, 2015).

Carica papaya prolonged intake caused an increase in body weight gain. However, the organ weight had varying effects; the ovary recorded an increase in weight while the uterus had a decrease in weight gain. *Carica papaya* caused reduction in testicular weight with no significant change in body weight (Lohiya *et al.*, 1994). The aqueous extract of *Carica papaya* had a significant effect on body weight in this study. Body weight plays an important role in the regulation of gonadotropin secretion, and its crucial role in menstrual cycle regulation is well established. Microscopic evaluation of the estrous cycle in laboratory rats and mice is an index to estimate the functional status of the hypothalamic-pituitary-ovarian axis; it serves as a medium to evaluate changes in the estrous pattern when studying a compound to evaluate the effects on female reproductive function (Goldman *et al.*, 2007; Cora *et al.*, 2015).

Table 1. Changes in the Oestrus Cycles of the Experimental Animals treated with 200mg/kg of aqueous <i>Carica papaya</i> seed extract. Legend: CPE: <i>Carica papaya</i> extract; D: Diestrus phase; P: Proestrus phase; E: Estrus phase; M: Metestrus phase							
A week’s Oestrus Cycle during the administration of 200mg/kg of <i>Carica papaya</i> aqueous seed extract							
Groups (n=5)/days	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
A-Control 7 days	D	P	E	M	D	D	P
B-CPE 7 days	P	P	P	D	D	D	P
Last week of administration - Oestrus Cycle - 200mg/kg of <i>Carica papaya</i> aqueous seed extract							
Groups (n=5)/days	Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
A-Control 21 days	M	E	D	D	P	E	P
B-CPE 21 days	P	P	D	D	D	D	D

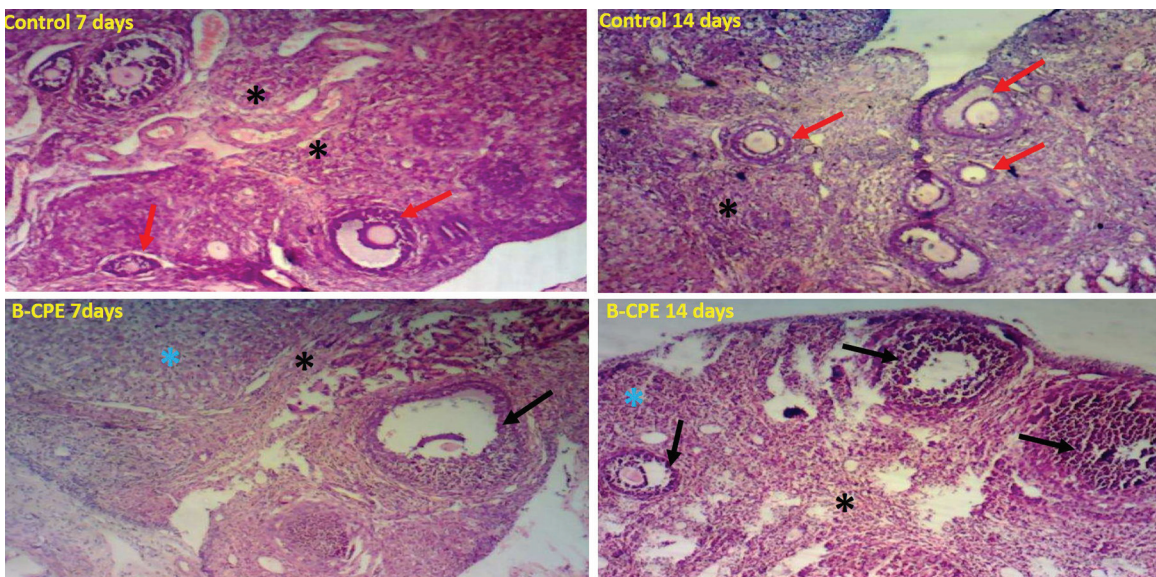


Figure 5. Micrograph of a cross-section of the Ovary of Experimental Animals treated with 200mg/kg of aqueous *Carica papaya* seed extract. Stained with Hematoxylin and Eosin (H and E stain). Mag x 100. The control group (A) at 7 and 14 days shows many healthy ovarian follicles in various stages of development. The theca and granulosa cells around the developing oocytes are well arranged, as compared to the *Carica papaya*-treated groups having few ovarian follicles, degeneration of the theca and granulosa cells of the ovarian follicles and corpus luteum (B- 7day-treatment) though mild as compared to 21-day treated *Carica papaya*.

Legend:

- CPE = *Carica papaya* extract
- Ovarian stroma = black asterisk
- Corpus Luteum = blue asterisk
- Degenerated Follicles = black arrows
- Healthy follicle= red arrows

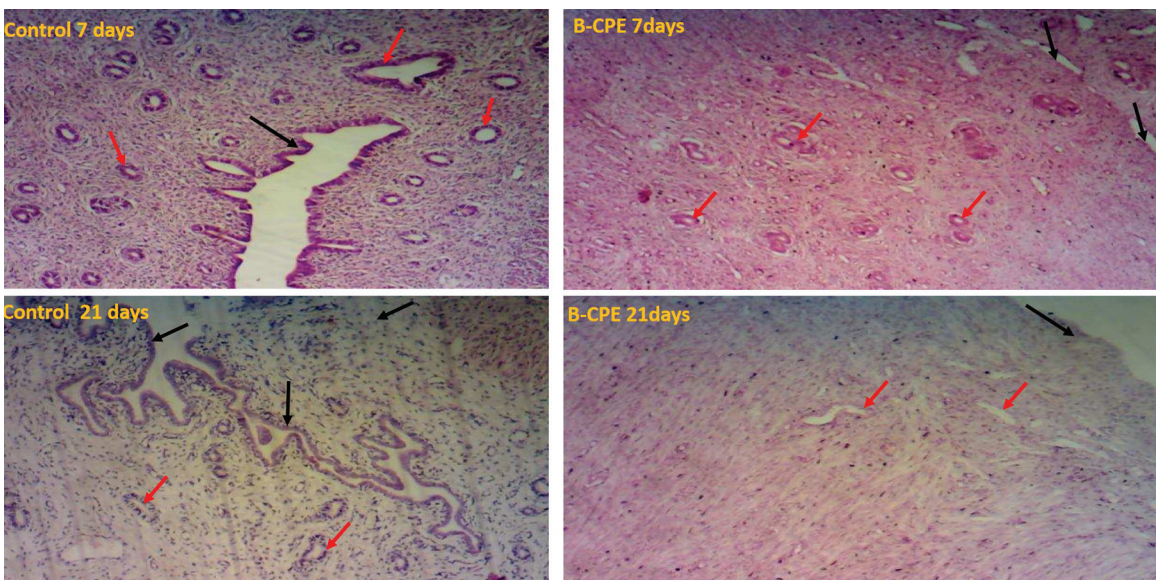


Figure 6. Micrograph of a cross-section of the Uterus of Experimental Animals treated with 200mg/kg of aqueous *Carica papaya* seed extract. Stained with Hematoxylin and Eosin (H and E stain). Mag x 100. The control group (A) at 7 and 21 days shows normal simple cuboidal epithelial cells lining the uterine gland, and well-pigmented endometrial cells as compared to *Carica papaya*-treated groups. The severity of uterine cellular distortion has higher in those treated for 21 days.

Legend:

- CPE = *Carica papaya* extract
- Uterine glands = red arrows
- Endometrial lining = black arrows

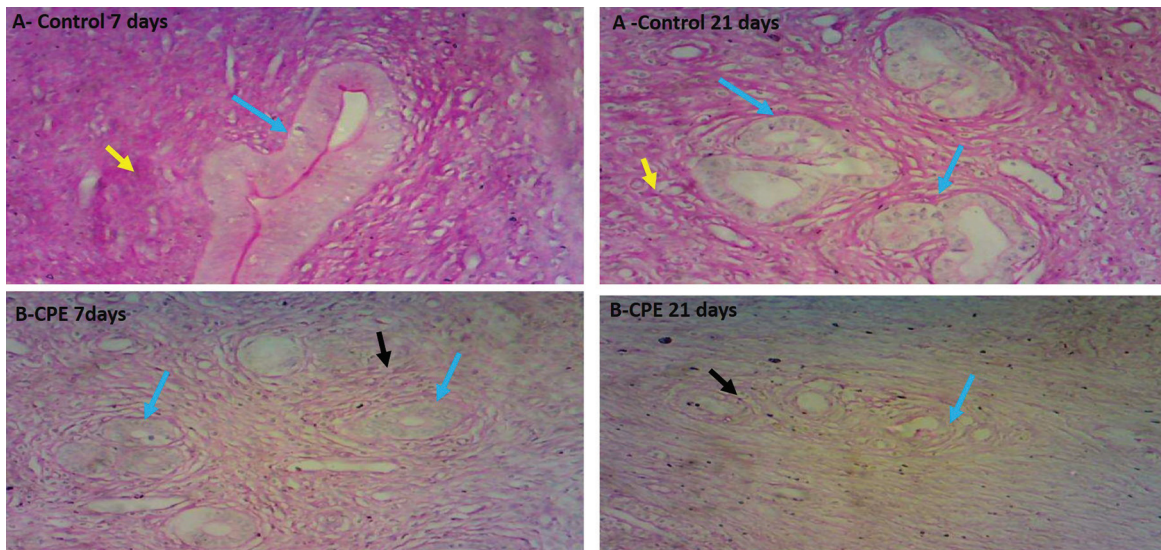


Figure 7. Micrograph of a cross-section of the Ovary of Experimental Animals treated with 200mg/kg of aqueous *Carica papaya* seed extract. Stained with Hematoxylin and Eosin (H and E stain). Mag x 100. The control group (A) at 7 and 14 days shows many healthy ovarian follicles at various stages of development. The theca and granulosa cells around the developing oocytes are well arranged, as compared to *Carica papaya*-treated groups, having few ovarian follicles, degeneration of the theca and granulosa cells of the ovarian follicles and corpus luteum (B- 7days treatment), though mild, as compared to 21 day-treated *Carica papaya*.

Legend:

CPE = *Carica papaya* extract

Uterine glands = blue arrows

Well stained mucin granules = yellow arrows

Poorly stained mucin granules = black arrows

Carica papaya increased the length of oestrus cycles and the frequency of diestrus. The diestrus phase persistence indicates that the extract interfered with the hypothalamic-pituitary-ovarian axis function, as seen in our study (Goldman *et al.*, 2007). Through the gonadotropin hormones (FSH and LH), the pituitary-gonadal axis controls the pattern and frequency of the oestrus cycling (Cora *et al.*, 2015). This abnormal cycling caused by *Carica papaya* seed was also reported by Raji *et al.* (2005), Dosumu *et al.* (2008), Odirichukwu *et al.* (2016) and Ganguly *et al.* (2007) studies on the *Mimosa pudica* root extract, and the Taid *et al.* (2016) study on *Plumeria acuminata*; thereby herbal plants can change oestrus cycles, indicating a potent anti-fertility effect. The root cause is linked to phytochemicals such as alkaloids, flavonoids, saponins, glycosides and phenolic compounds, having a chemical activity similar to reproductive hormones; hence mimicking hormone-like functions (Verma *et al.*, 2016; Soni *et al.*, 2016). Studies on alkaloids, flavonoids, saponins, and phenolic isolates reported they disrupt the oestrus patterns in animals (Ifeanyi *et al.*, 2011; Verma *et al.*, 2016; Soni *et al.*, 2016; Fatima & Shahid, 2018).

The female estrous cycle is the time between periods of sexual receptivity. In mice and rats, this cycle is completed in 4/5-day intervals and reoccurs after that. The results obtained from this study on the effect of *Carica papaya* on the estrous cycle of female albino rats showed that the extract caused a significant alteration in the duration of the different phases of the estrous cycle (Taid *et al.*, 2016). The normal process of the cycle as displayed by the control group was replaced by more presentation of the diestrus phase, which was prolonged. In addition, the numbers of cycles was reduced. Estrous is the heat period or mating phase, its duration was altered, as shown in our study, which suggests some antifertility effects of *Carica papaya*.

Hence, a reduction in the frequency of oestrous cycles from this study corresponds to a decrease in the rate of ovulation, which occurs from the beginning of pro-estrous to the end of oestrus (Young *et al.*, 1941; Schwartz, 1964), resulting in decreased fertility (Nayaka *et al.*, 2014). Progesterone plays an important role in influencing cycle length in rodents. It works in synergy with estrogen (Morali & Beyer, 1979). vom Saal *et al.* (1994) mentioned that the elevation of progesterone occurs before ovulation in young rats, but its decrease is linked to prolonging oestrus cycle length. Fluctuations in circulating levels of the ovarian steroids estradiol and progesterone, follicle-stimulating hormone and luteinizing hormone affects the estrous cycle and histological appearance of female reproductive organs (Lerner, 1969; McLean *et al.*, 2012; Aritonang *et al.*, 2017).

This present study shows that *Carica papaya* caused a significant decrease ($p < 0.05$) in the serum level of progesterone. This indicates a normal function of progesterone which is the endometrial stromal fibroblast differentiation, uterine gland secretion and proliferation of uterine myometrium (Aritonang *et al.*, 2017) is disrupted. A similar report was documented in male rats, where *Carica papaya* reduced in serum FSH and testosterone (Ansah *et al.*, 2016). According to Biswal (2014), the level of estrogen and progesterone reaches its peak during proestrus, and a decline results in unbalanced ovarian functions, which invariably affect the estrus cycle and fertility index. The lowered level of progesterone seen is possibly linked to disruption in follicular development and absence of corpus luteum (a postovulatory follicle), that secretes progesterone, responsible for preparing the endometrium for the implantation of the fertilized ovum (Hadley, 1975; Pessina *et al.*, 2006; Gonzalez, 2016). The loss of mucin granules and uterine gland proliferation seen in PAS stained uterus (Fig. 7) correlates with reports from

Shapiro *et al.* (1980), a study in endometrium culture reporting that progesterone is linked with glycogen buildup in endometrial tissue, hence its reduction will reduce glycogen. Our study shows that *Carica papaya* induced a decrease in progesterone, resulting in the loss of glycogen/mucin granules in the endometrium. There were mild distortions, degeneration of follicular cells in the ovary, and the uterine cells were lightly stained, correlating with reports from Raji *et al.* (2005).

The significant structural morphological alterations seen in the histological sections of the *Carica papaya* seed extract-treated rats showed that the histological variation seen is associated with hormonal changes that also effect the oestrus cycle, explaining its antifertility effects. This extract induced severe degeneration of the follicular wall of the ovarian follicles, which explains the possible decrease in progesterone secretion (Fig. 4), and this resulted in anovulatory cycles. The hormonal and histological changes could lead to a significant reduction in ovarian and uterine weight (Figs. 2 and 3). Chinoy *et al.* (1994) showed that *Carica papaya* seed aqueous extract from 7 and 15 days caused more effects on the reproductive physiology in the ovary, as compared to the uterus, in addition to irregular oestrus cycle, characterized by a predominance of dioestrus. In this present study, we had severity in the uterus, contrary to the report from Chinoy *et al.* (1994).

The present study reports on the effect of Aqueous extract of *Carica papaya* on progesterone mechanistic secretion and influence on oestrus cycle and utero-ovarian histological appearance. The exact principles that caused the irregularity in the estrous cycle and infertility in *Carica papaya*-treated rats has not been fully documented. The fertility index is linked to the rate of ovulation (Young *et al.*, 1941; Schwartz, 1964). *Carica papaya* decreases the rate of ovulation due to a prolonged diestrus phase; thereby, resulting in decreased fertility (Poharkar *et al.*, 2010; Nayaka *et al.*, 2014; Naik *et al.*, 2015). It has been scientifically demonstrated that *C. papaya* seeds possess contraceptive and abortifacient activities. Our studies on the aqueous seed extract also showed reduced fertility, evidenced by irregular estrous cycle, reduction of progesterone activity, loss of mucin granules and an indication of uterine tubular glands secretory activity. The proestrus phase of the estrous cycle corresponds to the human follicular phase of the menstrual cycle with elevated FSH concentrations. This mechanism of action, illustrated from results obtained from this present study, supports abortifacient, anti-implantation linked to female infertility (Poharkar *et al.*, 2010; Ansah *et al.*, 2016). This gives an indication that the *Carica papaya* seed extract may act on the ovary through altered endocrine pathways, associated with decreased progesterone levels. In addition to decreasing infertility, papain might cause abortions shortly after conception. Papain dissolves proteins responsible for the newly fertilized egg adhering to the uterine wall. Goyal *et al.* (2010) carried out a study to authenticate scientific documentation of the papaya seeds being traditionally used for contraception, and establishing the safety of the papaya seeds methanol sub-fraction (MSF) as a male contraceptive following long term oral intake.

CONCLUSION

The *Carica papaya* seed extract shows antifertility, anti-implantation and abortifacient effects in the treated animals associated with its ability to alternate hormonal activity that regulates the estrous cycle. Hence, it can be used as a contraceptive agent.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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