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Effect of Semi-Ripe *Carica papaya* Fruit Extracts on the Reproductive Structures in Female Albino Rats - An Histological Study

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ABSTRACT

The aim of the present investigation is to analyse the mechanism of action of aqueous extract of seeded papaya fruit pulp and seed extract administered as a combined dose and seedless variety papaya fruit pulp extract on ovarian and uterine function in female albino rats. The experimental animals were divided into three groups, Group - I served as control, experimental Group - II received combined papaya pulp and seed extract from seeded fruits and experimental Group - III received papaya pulp extract from seedless fruits. Experimental groups were orally administered with the extract (1gm / kg / body weight) for 60 days. These results indicate that *Carica papaya* semi-ripe seeded and seedless fruit pulp extract possess antifertility effects.

Key words *Carica papaya* (seeded and seedless fruits), Antifertility effect, Female albino rats, Ovary, Uterus.

INTRODUCTION

Recently, efforts have been devoted to identity a plant based contraceptive formulation which are supposed to be orally bioactive, non-toxic and more important cost effective based on ethano- medical information (Uchendu *et al.,* 2000; Heeshma Khushalani *et al.,* 2006 ; Manisha *et al.,* 2012; Varsha *et al.,* 2014). Evidences from several studies suggest that the fruits of *Carica papaya* (epicarp, endocarp and seeds) and the leaves have antifertility properties. Normal consumption of ripe papaya during pregnancy may not be dangerous, however un-ripe or semi-ripe papaya contain high amount of latex that produced marked uterine contraction that could be unsafe for consumption during pregnancy (Adebiyi *et al.,* 2004; Krishna *et al.,* 2008). Benzyl isothiocyanate (BITC) the main bioactive compound in *Carica papaya* seed has been shown to be responsible for antifertility effect (Kermanshai *et al.,* 2001). BITC is capable of damaging the endometrium, making the uterus non-receptive and thus adversely affect implantation (Adebiyi *et al.,* 2003). Pregnant women are strictly forbidden from eating the unripe or semi-ripe fruit for its fear of teratogenic and abortifacient effects (Adebiyi *et al.,* 2002).

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Several studies have been carried out on the effect of leaves, seeds and bark of papaya plant on the female reproductive system of rat and mice, proving the antifertility, anti-implantation and abortifacient properties (Oderinde *et al.,* 2002; Chinoy *et al.,* 2006). Papaya latex extract was tested on rat uterine preparation *in vitro* at various stages of the estrous cycle and gestation periods (Dosumu *et al.,* 2008). The latex of *Carica papaya* contains many biologically active compounds responsible for the reported abortificient effects (Schmidt, 1995).

The levels of these compounds varies in the fruit, latex, leaves and roots (Brocklehurst and Salih, 1985). Rat uterine contractile activity was remarkably increased by different doses of papaya latex extract in proestrous and estrous stages compared to metaestrous and diestrus stages of the estrous cycle. The crude papaya latex contains a uterotonic principle comprising of a combination of enzymes, alkaloids and other substance, which evoked sustained contractions of the uterus by acting mainly on the alpha adrenergic receptor population of the uterus at different stages (Cherian, 2000). Papain and chymopapain, the constituents of the latex of papaya increase the chance of uterine contractions as papain act like prostaglandins and oxytocin, also cause marked oedema and haemmorrhagic placentas which cause severe complication in pregnancy and an early delivery (Adebiyi *et al*., 2003). The present study was to assess the effect of *Carica papaya* (seeded and seedless) semi-ripe fruit pulp extract on structural alteration in reproductive organs of female albino rats.

MATERIALS

Papaya fruits: Semi- ripe seeded and seedless varieties of *Carica papaya* fruits were commercially obtained from wholesale fruit market in Chennai. The fruit specimen was identified and authenticated by Dr P. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, India. A voucher specimen with number (PARC / 2013 / 2319) has been deposited in the herbarium of the same department.

Preparation of aqueous extracts from fruit pulp and seeds of papaya fruits

The fruits were washed with double distilled water, and the outer skin and the inner seeds were removed. The fruit pulp was sliced. The seeds and sliced fruit pulp were separately air dried in shade. The dried pieces of fruit pulp and seeds of seeded variety and the fruit pulp of seedless variety were pulverized separately into a coarse texture form using an electrical blender. The powdered fruit pulp was macerated with cold water and passed through a fine muslin cloth. The filtrate was collected and dried. The dried material was stocked in an airtight plastic container. The dried fractions of the fruit pulp and seeds were preserved at 4^{o}C in a refrigerator for further use. Two different types of aqueous extracts were prepared. The fruit pulp and seed of seeded variety were combined and the fruit pulp of seedless variety were prepared with required amount of distilled water. A fresh sample of required amounts was prepared from the stock prior to administration of extracts to the animals.

Experimental animals

Healthy female albino Wistar strain rats (*Rattus norvegicus*) were purchased from The King Institute of Preventive Medicine and Research, Guindy, Chennai. Healthy rats weighing 155 ± 25 g were used in the present study. The animals were maintained in polypropylene cages with metal grill top under standard environmental conditions of temperature 25 ± 2^{o}C and proper ventilation. They were exposed to a 12h light: 12h dark cycle and provided with water *ad libitum*. The animals were fed with standard balanced pelleted diet (Sai Durga Foods, Bengaluru). Animals were treated humanely. Care and supervision was provided throughout the period of study. The study protocols were duly approved by the Animal Ethical Committee Regn.No. CPCSEA/CA/ORG/2006/65/4. Studies were performed in accordance with the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

METHODS

Acute oral toxicity study

In order to assess the toxic effects and tolerance limit and to determine a safe dose, acute oral toxicity study was carried out as per the CPCSEA guidelines. The rats were fasted for 3-4 hours before administration of extracts. The extracts were administered in a single dose by using gastric intubation. Three groups of six rats each, were used in each group.

Testing of tolerance limit for seeded variety (fruit pulp and seed powder combined)

The fruit pulp powder and powered seed of seeded variety were taken in equal proportion, combined and mixed thoroughly in distilled water for oral administration. The combination of the extract was treated with a dose of 1000 mg , 2000 mg, 3000 mg, 4000 mg and 5000 mg / kg bw and mortality was observed for 96 hr and the LD_{50} was determined (Miller and Tainter, 1944; Weils,1952). The test dose was given at 9.00 AM. Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24h. 100%, 67%, 50% and 33% mortality was observed in 5000 mg, 4000 mg, 3000 mg and 2000 mg doses respectively. No mortality was observed in 1000mg dosage. 50% mortality was observed in 3000 mg / kg bw. From this one- third of the dose which would be safe for the animals were determined and the combined preparation was administered orally for a period of 60 days to the experimental group- I of rats.

Testing of tolerance limit for seedless variety fruit pulp

Toxicity and tolerance limit were also carried out for rats administered with seedless variety papaya fruit pulp. The pulp extract was treated with at a dose of 1000 mg , 2000mg, 3000 mg, 4000 mg and 5000 mg / kg bw. 83%, 67%, 50% and 17% mortality was observed in 5000 mg, 4000 mg, 3000 mg and 2000 mg doses respectively. No mortality was observed in 1000 mg dosage. 50% mortality was observed in 3000 mg / kg bw. From this one- third of the dose which would be safe for the animals were fixed and the aqueous preparation was administered orally for a period of 60 days for experimental group- II rats. Conditions of tremors and convulsions were also observed. The animals were further observed for 48h post treatment for signs of toxicity and death before fixing the final dosage.

Dose Determination of various extract

Final doses were determined for the experiment after testing the tolerance limit for both the types of extracts. 500 mg of the dried fruit pulp powder and 500 mg of powdered seed from seeded semi-ripe papaya fruit (1000 mg in combination) was dissolved in water (1.0 gm dissolved in 1.0 ml distilled water) for experimental group – I animals. 1000 mg fruit pulp powder from seedless papaya was dissolved in water (1.0 gm dissolved in 1.0 ml distilled water) for experimental group - II animals. Animals in each group received the same dose throughout the treatment period.

Route of administration of extracts

The extracts prepared were orally administered via gastric intubation using an orogastric tube comprising a 16-G polyethylene catheter fitted with a hypodermic syringe (volume of 10 ml). Administration of extracts were carried out every morning after a 24 hour interval for 60 consecutive days.

Experimental design

The animals were weighed and divided into three groups of similar weight. Each group consisted of six animals, maintained in separate cages.

Group I: Control: Female albino rats which received normal feed and water.

Group II: **Experimental Group- I** : Female albino rats which received normal feed and water, oral administration of seeded semi- ripe papaya fruit pulp and seed extract combined (1000 mg/kg body weight/day) for 60 days.

Group III: **Experimental Group- II** : Female albino rats which received normal feed and water, oral administration of seedless semi- ripe papaya fruit pulp extract alone (1000 mg/kg body weight/day) for 60 days.

The animals were acclimatized to laboratory conditions for 15 days with normal feed and water before the start of the experiment. Initial and final body weights were recorded prior to and after treatment. Administration of the extract began when the animals were on diestrous, while the animals were sacrificied on proestrous after the last dose since they denote the beginning and end of each estrous cycle and our experiment also coincide with it. The seeded semi- ripe papaya fruit pulp and seed extract and the seedless variety semi-ripe papaya fruit pulp extract was given orally through a gastric intubation daily at 9.00 A.M. The animals were sacrificied by cervical dislocation on the 61st day, 24 hour after administration of last dose.

Histological Studies

For histological studies of ovary and uterus the tissues were fixed in 10% formalin. They were processed routinely for paraffin embedding and sectioned to 5µ thickness for staining by haematoxylin and eosin (H&E) method (Luna, 1968) for histological examination.

RESULTS

Histology of the ovary

Normal ovary of control rats (Fig. 1) reflected matured graffian follicle, normal development of oocyte surrounded by zona pellucida with multiple layers of granulosa cells, healthy follicular cells at various stages of development, absence of fragmented granulosa cells and cell debris in the antral cavity. Female rats treated with seeded *Carica papaya* fruit pulp and seed extract combination for 60 days showed degeneration in the ovarian cortex, thining of cell lining, degeneration of follicular cells and mature follicles undergoing follicular atresia. Disorganized, fragmented and atrophied granulosa cells and ruptured thecal cells. Separation of thecal layers, theca externa and interna from the follicles (Fig, 2). Female rats treated with seedless *Carica papaya* fruit pulp extract, how regressing follicles, large preantral, small antral and graffian follicles were less frequently observed (Fig, 3).

Histology of the uterus

Normal uterine tissues of control rats (Fig, 4) exhibited endometrium having large epithelial cells with nuclei, showing pits and folds in the uterine epithelium, spongy layer filled with blood vessels, presence of large number of irregular shaped uterine glands, uterine lumen highly distended and stroma with normal vascularity.

Transverse section of ovary of control rats

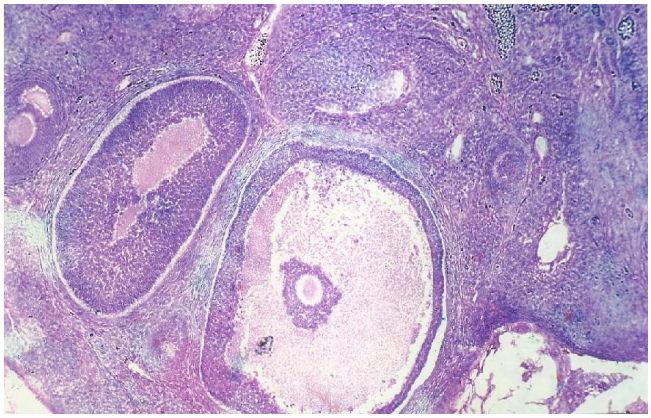

Fig.1. Normal structure of ovary showing mature graffian follicles and various follicular stages of development (x 100) Granulosa cell; Oocyte, Thecal Layer

Transverse section of ovary experimental female rats treated with seeded semi-ripe papaya fruit pulp and seed extract combined for 60 days

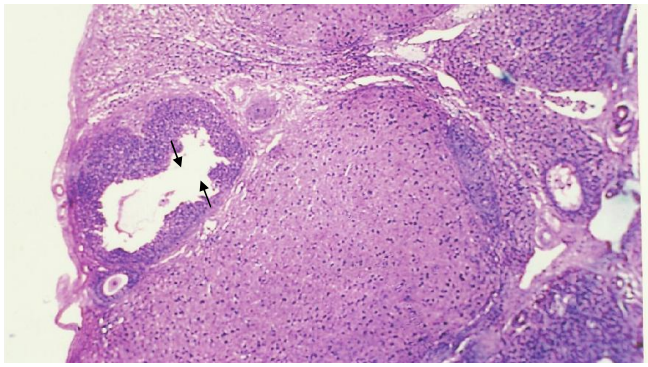

Fig.2. Atretic follicles (arrow), graffian follicles less frequently observed, granulosa cells less in number (x100)

Transverse section of ovary of experimental female rats treated with seedless semi-ripe papaya fruit pulp extract for 60 days

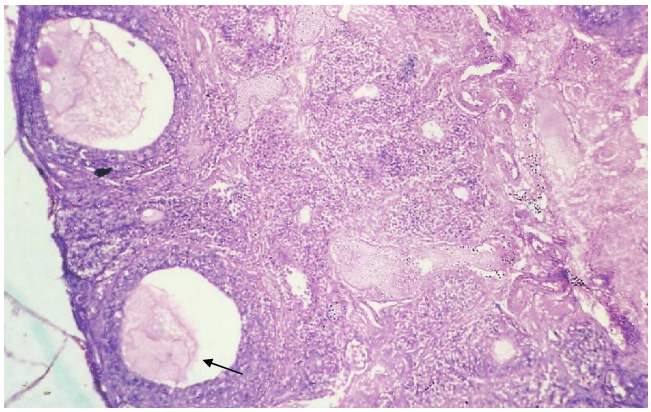

Fig.3. Thining of cell lining, fewer graffian follicles, granulosa cells disorganized (arrow) characteristics of follicular atresia (arrow) (x 100)

Transverse section of uterus of control rats

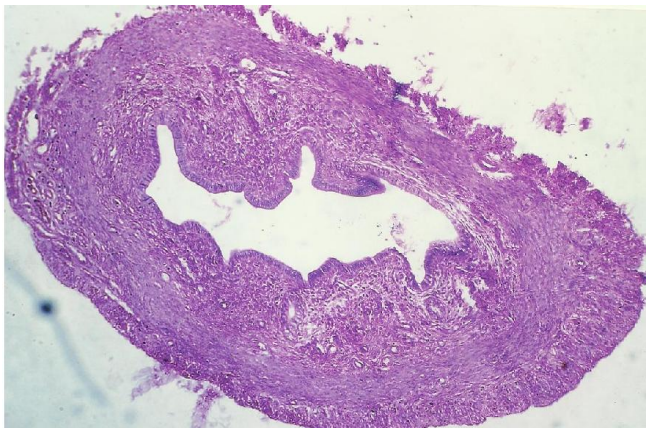

Fig.4. Normal structure of uterus with endometrium showing uterine lumen, Myometrium, Pits and folds of uterine epithelium (x 100)

Transverse section of uterus of experimental female rats treated with seeded semi-ripe papaya fruit pulp and seed extract combination for 60 days

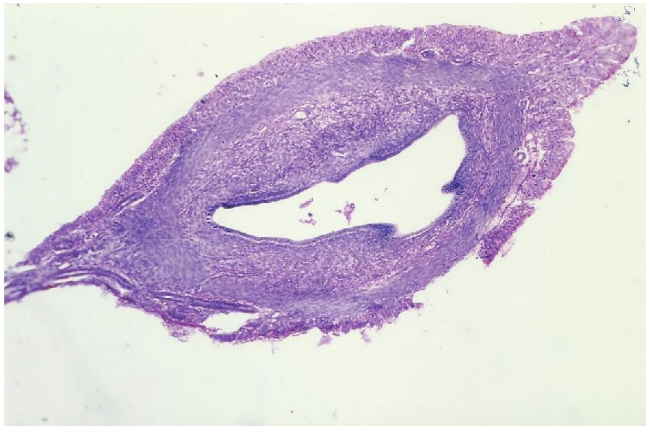

Fig.5. Distortion and irregular endometrial epithelium, uterine lumen altered in shape sparse distribution of columnar cells, reduction in luminal epithelium (x 100)

Transverse section of uterus of experimental female rats treated with seedless semi-ripe papaya fruit pulp extract for 60 days

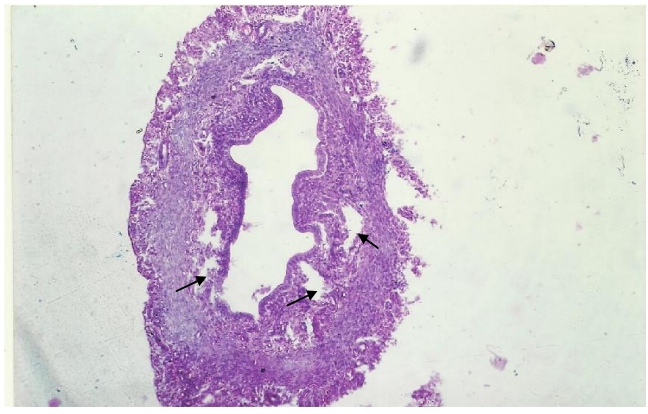

Fig.6. Alteration in endometrial stroma. Degenerating epithelial cells with cavities (arrow) folds of luminal epithelium altered (x 100)

Female rats treated with seeded *Carica papaya* fruit pulp and seed extracts combination for 60 days (Fig, 5), showing distortion of endometrial epithelium, endometrial folding disrupted, shrunken appearance and reduction in the number of uterine glands, endometrial stroma disaggregated with poor vascularity and musculature was highly affected. Treatment of female rats with seedless *Carica papaya* fruit pulp extracts shows the endometrial epithelial cell appearance slightly altered, uterine lumen and myometrium appears to be normal (Fig, 6).

DISCUSSION

The ovary and uterus are important organs in female reproductive system. Ovary consists of aggregation of three endocrine tissues such as stroma, follicles and corpus luteum. The graffian follicle of the control ovary showing normal development of the oocyte surrounded by zona pellucida with multiple layers of granulosa cells. Histological examination of follicle of various classes in ovaries of treatment groups showed that there was arrest in the development of follicles. In rats treated with seeded papaya fruit pulp and seed extract granulosa cells are disorganized and the graffian follicle shows the characteristic of follicular atresia and granulosa cells have started undergoing degenerative changes. Atretic follicles or degeneration of preovulatory follicles takes place due to non-availability of steroid hormones essential for maturation and differentiation. The presence of increased atretic follicles in rats treated with fruit pulp and seed extract promotes degeneration of pre-ovulatory follicles. This could be attributed to the decline in pituitary gonadotropin as FSH is the critical regulator of growth and development of follicles (Wassarman and Albertini, 1994). Similar histological changes have been reported in female mice administered with extract of *Butea monosperma* powder (Neelam Gupta *et al.,* 2010). The result of the present findings are in support with other studies on *Piper betel* antifertility effect on female albino rats (Sharma *et al.,* 2007).

Control groups show wide uterine lumen with presence of pits and folds in the uterine epithelium, uterine glands and myometrium, Treated rats show reduced uterine lumen, decrease in pit and folds in the epithelium, reduced number of uterine glands and decreased myometrial thickness. The myometrial structures along with smooth muscles of blood vessels may have been acted upon by the phytochemical constituents of the extract leading to their atrophy. The results of the present study are in correlation with studies of Shukla *et al.,* 1989 reported complete obliteration of lumen of uterus in rats treated with neem extract whereas Kholkute *et al.* (1976) observed atrophic uteri and uterine epithelium devoid of mitotic figures in rats after administration of seeded extract of *Hibiscus rosasinensis* for 30 days. Subcutaneous administration of neem seed oil has been reported to cause significant damage to luminal epithelium and uterine glands (Tewari *et al.,* 1989) and female albino rats treated with seeds of *Melia azedarachta* showed similar reports (Mandal and Dhaliwal, 2007). Dixit *et al.,* 1976 and Patil *et al.,* 1999 have reported decreased myometrial volume in proportion to uterine weight and marked regression of uterine gland in female gerbils treated intraperitonially with *Cannabis* extract and decreased thickness of myometrium and height of luminal epithelium in uterus of rats administered nictoine at 2mg and

4mg/kg body weight for 20 days respectively. Flavonoids and alkaloids are reported to have contraceptive activity. They have been shown to reduce plasma concentrations of LH, FSH and estradiol (Ankush Raj *et al.,* 2011). It is reported that the high concentration of these substances interfere with the secretion of pituitary gonadotropins and releasing hormones from the hypothalamus which are necessary for ovulation. The anti-fertility activities observed in the present study might be attributed to these active principles and it can be presumed that these may individually or synergistically affect the ovary and uterus leading to decrease in reproductive function. The fruit extract may have brought about its effect through pituitary - gonadal axis which resulted in diminished gonadotropins release in turn reducing reproductive organ weights and estrogen levels affecting cyclicity. Change in estrogen level alter the structural and functional activity of the reproductive organs.

In this direction the study has been aimed to identify a potent antifertility agent with minimum side effects from an herbal source that could serve as an alternative remedy for available synthetic medicine and to formulate it into a suitable contraceptive formulation.

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