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Screening for the Hormonal Properties of the Chloroform Extract of *Carica papaya* Linn. Seeds for Antifertility Investigation

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Abstract – The chloroform extract of the seeds of *Carica papaya* has been screened for the hormonal properties using ovariectomized female rats for estrogenicity, estrogen primed immature rats for progestogenicity and castrated adult male rats for androgenicity. The results revealed that the extract lacks progestogenicity and androgenicity as evident from the failure of the extract treated animals to mimic progestogen and androgen related changes in the target tissues. The increased weight of vagina and uterus, open status of vagina, cornified and epithelial cells in the vaginal smears and hypertrophy in the uterine epithelium, endometrium and stroma with increased glycogen and sialic acid content in the uterus of the chloroform extract treated animals, which are comparable to those of the ovariectomized estrogen treated animals, suggest that the chloroform extract possesses mild estrogenic activity.
Key words – *Carica papaya* seeds, Chloroform extract, Estrogenicity, Progestogenicity, Androgenicity.

Introduction

The seeds of *Carica papaya* are proven to possess reversible contraceptive efficacy in male mice, rats and rabbits (Chinoy *et al.*, 1984; Vyas and Jacob, 1984; Lohiya and Goyal, 1992; Lohiya *et al.*, 1998). It is reported that the contraceptive efficacy of the seeds is post testicular by altering the epididymal milieu which adversely affects the motility of the spermatozoa in rats and mice (Lohiya and Goyal, 1992; Chinoy *et al.*, 1995). Lohiya *et al.* (1998) reported that the effects are mediated through the testis in rabbits by arrest of spermatogenesis resulting into severe oligospermia and subsequent azoospermia. It is suggested that these effects are possibly due to mild estrogenic activity of the seeds (Vyas and Jacob, 1984; Lohiya and Goyal, 1992; Lohiya *et al.*, 1992). Chinoy *et al.* (1994) reported that the aqueous extract of the seeds of *Carica papaya* is non estrogenic. In the plant contraceptive research, the inhibition of sperm function is attributed to the estrogenic or antiandrogenic activity of the plant (Akbersha *et al.*, 1990; Chinoy *et al.*, 1994, 1995). As regard to the hormonal properties of the crude extracts of the seeds of *Carica papaya*, the available reports are conflicting.

Hence the present study is aimed to determine the possible hormonal properties, i.e., estrogenic, antiestrogenic, androgenic, antiandrogenic or progestogenic, of the chloroform extract of the seeds of *Carica papaya*, using rats as animal model.

Materials and Methods

Animals – Colony bred 20-21 days old immature female Sprague Dawley rats, weighing 130-150 g (for estrogen and progestogen screening) and 4 to 5 months old adult male Sprague Dawley rats weighing 200-230 g (for androgen testing) were used in the present investigation. The animals were maintained in the Department Animal Facility at 26±2°C with 12:12 hour light dark schedule. The animals were fed with rat pellet diet and water was provided *ad libitum*. The animals were maintained under veterinary supervision and the guidelines for care & use of animals in scientific research (Indian National Science Academy, 1992) were strictly followed throughout the course of the investigation.

Test Material – The commercially obtained ripe honey dew variety seeds of *Carica papaya* were used. The seeds were dried in shade, powdered, and soxhleted with chloroform (LR) at 58°C for 12×3 hrs. The extract was concentrated under reduced

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pressure, dried and used with double distilled water by gavage (Lohiya and Goyal, 1992).

Test for Estrogenicity – Estrogen activity of the seeds of *Carica papaya* was tested according to WHO Protocol MB-70/71 (1983) in the ovariectomized 20-21 day old immature female rats. Twenty-one animals were equally divided into following three groups with 7 animals in each group:

Group I – Control (ovariectomized; vehicle treated)

Group II – Treated with Estradiol dipropionate twice daily (s.c.), @ 100 μg/kg body weight from day 8 onwards following ovariectomy, for 3 days.

Group III – Treated with chloroform extract of the seeds of *Carica papaya* twice daily (gavage) @ 5 mg/kg body weight from day 8 onwards following ovariectomy, for 3 days.

Twentyfour hours after the last dose, i.e., day 11 after ovariectomy, the animals were weighed and sacrificed. The status of vagina (open or close), types of cells in the vaginal smear (if open) and the weight of the uterus were recorded. A small piece of uterus was fixed in Bouins fluid for histology and the remaining pieces were used for the quantitative estimation of glycogen (Montgomery, 1957) and sialic acid (Svennerholm, 1960).

Test for Progestogenicity – Twentyone immature female rats, 20-21 days old, were primed with Estradiol dipropionate, 100 μg/kg body wt./day for 7 days. Then the animals were divided into following three groups with 7 animals in each group:

Group IV – Vehicle treated control

Group V – Treated with progesterone (i.m.), @ 100 μg/kg body wt. for 5 days

Group VI – Treated with chloroform extract of the seeds of *Carica papaya* twice daily (gavage), @ 10 mg/kg body wt. for 5 days

Animals were autopsied 24 hours after the last dose. The body weight and the weights of the vagina, uterus, ovary and adrenal gland were recorded. A small piece of uterus was fixed in Bouins fluid for histology and the remaining pieces were used for the quantitative estimation of glycogen and sialic acid as described previously (Montgomery, 1957 and Svennerholm, 1960).

Test for Androgenicity – Adult male rats, 4-5 months old, were bilaterally castrated and used for the test for androgenicity. The animals were divided into the following three groups with 7 animals in each group:

Group VII – Control (castrated; vehicle treated)

Group VIII – Treated with 150 μg testosterone propionate (s.c.)/kg body wt./day from day 8 onwards following castration, for 7 days.

Group IX – Treated with chloroform extract of the seeds of *Carica papaya* twice daily (gavage) @ 10 mg/kg body wt/day from day 8 onwards following castration, for 7 days.

Animals were sacrificed 24 hours after the last dose. The body weight and the weights of epididymis (caput, corpus and cauda), seminal vesicle and ventral prostate were recorded. The epididymis, seminal vesicle and ventral prostate were also used for the quantitative estimation of sialic acid (Svennerholm, 1960), fructose and acid phosphatase (WHO manual, 1992), respectively.

Statistical Analysis – Students t test was used for statistical comparison and $P<0.05$ level was considered significant.

Results

Estrogenicity – The final body weight of the animal showed a significant increase ($P<0.001$ and $P<0.01$, respectively) in the estrogen and chloroform extract treated animals, when compared to their initial body weight. The vagina was open and the vaginal smear showed cornified and epithelial cells in the estrogen and chloroform extract treated animals, while closed vagina was observed in the control animals. The weights of the uterus and the vagina of the estrogen and extract treated animals showed a significant increase ($P<0.001$ and $P<0.01$, respectively), when compared to the control animals. Similarly, quantitative estimations of glycogen and sialic acid of the uterine tissue, showed a significant increase ($P<0.001$ and $P<0.01$, respectively) in the estrogen and extract treated animals (Table 1).

The uterus of the immature ovariectomized rats (control) composed of subdued endometrium, few uterine glands in the stroma and a narrow lumen lined with short stratified epithelium. The epithelial cells were ovoid and the nuclei appeared pyknotic (Figs.1 and 2). Estrogen treated animals showed an increase in the height of the uterine epithelium. The epithelium appeared cryptic and the cells showed hypertrophy. The endometrium was well developed and the stroma appeared thicker, when compared to

Table 1. Screening for estrogenicity of the chloroform extract of the seeds of *Carica papaya* in immature ovariectomized albino rats (Values are SEM of 7 animals).

Group†	BodyWeight (g)		Reproductive Organs Weight (mg)		Uterus Biochemistry (mg/g)	
	Initial	Final	Uterus	Vagina	Glycogen	Sialic acid
Group I	141.9±0.06	142.0±0.04	30.75±2.17	65.61±7.6	10.03±0.83	0.73±0.14
Group II	141.8±0.06	160.1±0.09***	51.53±1.55***	103.22±6.7***	14.31±0.47***	1.32±0.05***
Group III	141.7±0.06	145.1±1.24**	38.75±1.88**	88.11±6.3**	12.72±0.28**	1.06±0.02**

†Group I Immature ovariectomized vehicle treated control animals.
Group II - Immature ovariectomized estrogen treated animals.
Group III - Immature ovariectomized chloroform extract of *Carica papaya* seeds treated animals.
** = P<0.01
*** = P<0.001

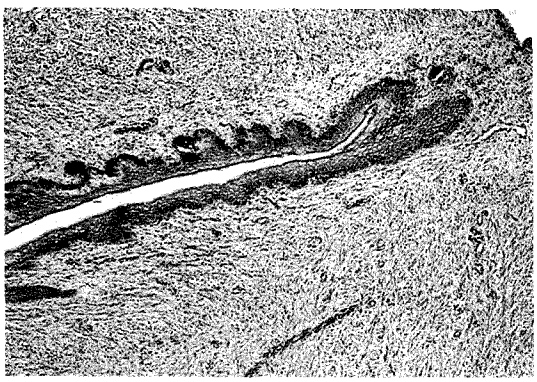

Fig. 1. The uterus of the immature ovariectomized vehicle treated control rats. ×100.

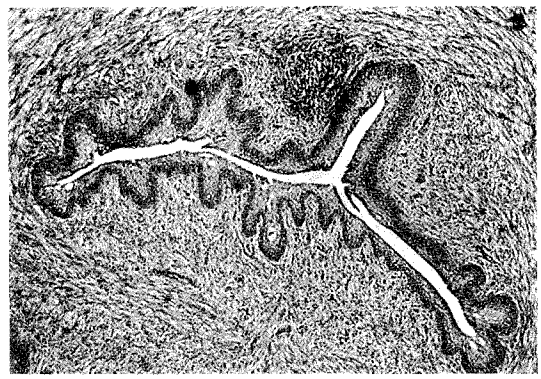

Fig. 3. The uterus of the immature, ovariectomized estrogen treated rats. Note the epithelial proliferation. ×100.

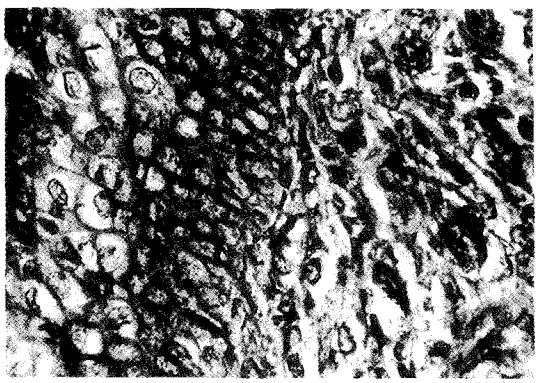

Fig. 2. A portion of the epithelium of the endometrium magnified to show the cell types. The cells showed shrinkage and the nuclei appeared pycnotic. ×400.

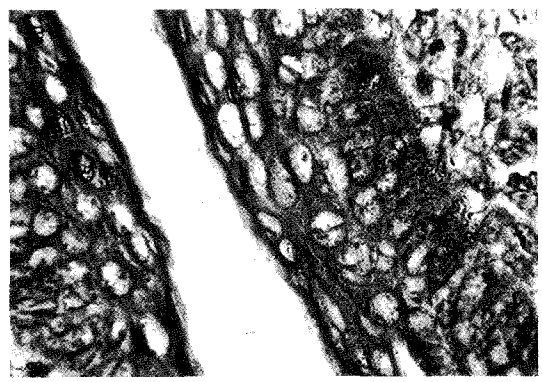

Fig. 4. A portion of the epithelium of the endometrium magnified to show the cell types. Note the enlargement of the cells and appearance of granules in the cytoplasm. ×400.

control animals (Figs. 3 and 4). The chloroform extract treated animals showed the similar features as those of estrogen treated animals. The lumen was widened and the epithelium showed proliferation. However, the height of the epithelium (qualitative) was slightly lower than that of estrogen treated animals. The cells showed mild hypertrophy when compared to control animals (Figs. 5 and 6).

Progestogenicity – There were no appreciable changes in the body weight, the weights of ovary, uterus and vagina and the glycogen and sialic acid contents of the uterus and ovary observed between

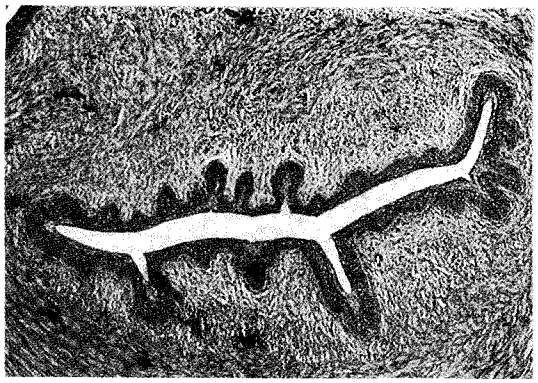

Fig. 5. The uterus of the immature, ovarictomized chloroform extract of the *Carica papaya* seeds treated rats. Note the epithelial proliferations comparable to ovariectomized estrogen treated animals. ×100.

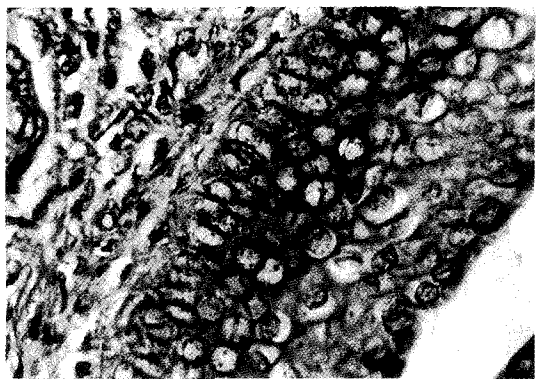

Fig. 6. A portion of the epithelium of the endometrium magnified to show the cell types. Note the enlargement of cells comparable to ovariectomized estrogen treated animals. ×400.

control and the chloroform extract treated animals. However, a statistically significant elevation ($P<0.05$) in the weights of ovary, uterus and vagina and a significant decrease ($P<0.05$) in the levels of glycogen and sialic acid in the ovary and uterus were observed in the progesterone treated animals (Table 2).

Androgenicity – The body weight of the animals did not show appreciable changes in the control and the chloroform extract treated animals. Similarly, the weights of the epididymis, seminal vesicle and ven-

Table 2. Screening for progestogenicity of the chloroform extract of the seeds of *Carica papaya* in immature female albino rats (Values are SEM of 7 animals).

Group†	Body Weight (g)		Reproductive Organs Weight (mg)			Ovary/Uterus Biochemistry (mg/g)			
	Initial	Final	Ovary	Uterus	Vagina	Glycogen		Sialic acid	
						Ovary	Uterus	Ovary	Uterus
Group IV	151±1.0	153±2.3	38.1±2.1	80.6±1.6	60.4±2.3	11.53±0.71	12.21±0.35	0.48±0.03	0.43±0.06
Group V	150±2.5	160±3.2*	45.0±2.5*	86.4±2.1*	66.1±1.8*	9.41±0.88*	11.05±0.48*	0.42±0.03*	0.31±0.02*
Group VI	150±1.2	152±1.1	35.6±1.8	83.1±1.9	58.3±2.0	12.32±0.65	12.34±0.35	0.47±0.05	0.42±0.05

† = Group IV Vehicle treated control animals.
Group V Treated with progesterone.
Group VI Treated with choroform extract of *Carica papaya* seeds.
* = $P<0.05$

Table 3. Screening for androgenicity of the chloroform extract of the seeds of *Carica papaya* in adult castrated male rats (Values are SEM of 7 animals)

Group†	Body Weight (g)		Reproductive Organs Weight (mg)			Tissue Biochemistry		
	Initial	Final	Epididymis	Seminal Vesicle	Ventral Prostate	Sialic Acid (mg/g) (Epididymis)	Fructose (mg/g) (Seminal Vesicle)	Acid Phosphatase (B.U.) (Ventral Prostate)
Group VII	225±1.15	228±2.20	213.16±1.08	203.11±5.10	93.61±1.64	4.28±0.37	3.75±0.01	1.68±0.07
Group VIII	229±1.23	250±1.19***	295.18±2.01***	241.19±3.81***	190.31±1.73***	6.68±0.29***	4.81±0.02***	2.51±0.08***
Group IX	228±1.08	230±1.18	215.31±1.61	205.20±4.61	95.43±1.81	4.31±0.24	3.76±0.03	1.85±0.08

† = Group VII- Vehicle treated control animals.
Group VIII- Treated with testosterone dipropionate.
Group IX- Treated with chloroform extract of the seeds of *Carica papaya.*
*** = $P<0.001$

tral prostate and the biochemical composition, viz., sialic acid in epididymis, fructose in seminal vesicle and acid phosphatase in the ventral prostate did not show appreciable changes compared to those of control levels. However, all these parameters showed a significant elevation ($P<0.001$) in the testosterone treated animals (Table 3).

Discussion

The plant products affecting the male reproduction bring about the effects through either of two mechanisms namely estrogenic effect or antiandrogenic effect (Kasinathan *et al.*, 1972). The leaves, flowers, fruits or seeds of several plants are known to possess estrogen or antiandrogen like substances which act on the reproductive system of male and female, thus inhibiting fertility (Akbersha *et al.*, 1990; Lohiya and Goyal, 1992; Shaikh *et al.*, 1993 and Chinoy *et al.*, 1995).

The antiandrogenic activity of the aqueous extract of the seeds of *Carica papaya* has been reported in rat (Chinoy and George, 1983). The non-estrogenic activity of the aqueous extract of the seeds of *Carica papaya* has also been reported in male mice (Chinoy *et al.*, 1994). It is reported that the effects are post-testicular in nature and are mediated by altering the epididymal milieu. Spermatogenesis was reported to be normal (Chinoy *et al.*, 1995). Lohiya *et al.* (1994) reported that the aqueous extract of the seeds of *Carica papaya* affects the testicular as well as epididymal functions and attributed these effects to mild estrogenic activity of the extract. Lohiya and Goyal (1992), reported a similar effect in the chloroform extract treated rats. Recently, we have observed that the chloroform extract of the seeds of *Carica papaya*, treated to male rabbits, affects the spermatogenesis, resulted into severe oligospermia and subsequently azoospermia, which suggested that the effects are mediated through the testis (Lohiya *et al.*, 1998). Spermatogenic arrest is attributed to the estrogenic (Bartke, 1977; Chinoy *et al.*, 1994), antiandrogenic (Lohiya and Sharma, 1983) and androgenic (Jayaprakash *et al.*, 1994) activity of the given drug.

The female accessory sex organs, namely the uterus and vagina are estrogen dependent. Glycogen and sialic acid are the major biochemical constituents of the uterus, which are directly controlled by the estrogen (Coppola and Ball, 1966; Gregoire *et al.*, 1971). Changes in the uterine glycogen and the sialic acid levels are dependent on the ovarian hormones (Carlborg, 1969). Ovariectomy resulted into the suppression of the metabolic activity of these organs and the exogenous administration of estrogen restores these effects. Similarly, in the male the accessory sex organs, namely the seminal vesicle and prostate are androgen dependent. Castration diminishes the metabolic activity of these organs and exogenous administration of the testosterone to castrated animals restores these effects to normalcy (Turner and Bagnara, 1976).

In the present study, an attempt has been made to test the estrogenic, antiandrogenic or androgenic nature of the chloroform extract of the seeds of *Carica papaya*. The chloroform extract restores the ovariectomy related changes in the uterus and vagina to normalcy, comparable to ovariectomized animals treated with estrogen, suggesting mild estrogenic activity of the plant. While, the other hormonal screening, viz., androgenicity and progestogenicity did not induce the desired effects akin to those induced by androgen/progestogen in the castrated/estrogen primed immature female rats suggesting that this plant lack androgenic/antiandrogenic and progestogenic property.

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