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EFFECT OF *CARICA PAPAYA* FRUIT PULP EXTRACT ON BIOCHEMICAL CONSTITUENTS OF TESTIS IN MALE ALBINO RATS

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ABSTRACT

The present study was carried out to investigate the effect of semi-ripe fruit pulp extract of *Carica papaya* (seeded and seedless fruits) on male albino Wistar rats. The experimental animals were divided into three groups, One group served as control, Group- II received papaya pulp extract from seeded fruits and Group - III received papaya pulp extract from seedless fruits. Both groups were orally administered with pulp extract (10ml/kg/body weight) for 60 days. Biochemical analysis of testis after the completion of experimental period on the 20st, 40st and 60st day shows a significant increase in contents of glycogen and cholesterol while a decrease was observed in protein contents in both experimental groups. These results may indicate that *Carica papaya* semi-ripe seeded and seedless fruit pulp extract possess antifertility effects.

INTRODUCTION

The development of new fertility regulating drug from medicinal plants is an attractive preposition. A wide variety of synthetic contraceptive agents are available but these are not without side effects. Today different chemical barrier, hormonal combined oral pills, injectables, implants, microspheres, microcapsules, gels, LHRH antagonists are available to women (Fpa, 2006). Many plants are herbs have been reported to have potential antifertility properties (Hamman *et al* 2011). A number of studies show the antifertility effect of *Carica papaya* seeds which has shown great promise in male contraception in animal models (Lohiya *et al* 2005, 2006). Recently efforts are being devoted to identify a plant based contraceptive that is supposedly orally bioactive, nontoxic and more important cost effective based on ethnomedical information. With a view to elucidate the possible mode of action of the antifertility effect, the present study attempts to analyze the possible modulatory influence of aqueous extract of semi- ripe papaya fruit pulp on the biochemical constituents which may play an important role in testicular functioning.

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MATERIALS AND METHODS

Plant Materials used for the study

Semi- ripe seeded and seedless *Carica papaya* fruits were commercially obtained from local market. The fruits were washed, outer skin and the inner seeds were removed. The aqueous extract of the pulp was prepared with required amount of distilled water with and seeds a blender for evaluation of its antifertility effects in male rats.

Experimental animals

Healthy male Wistar strain rats (*Rattus norvegicus*) weighing about 125-150 g were used in the present study. The animals were bred and housed in polypropylene cages (8" x 12" x 8") with metal grill top under standard environmental conditions at temperature (25 ± 2°C) and ventilation. The animals were fed with standard balanced pelleted diet (Gold Mohur brand, Hindustan Lever Ltd) They were maintained in layers mesh, exposed to a 12h light: 12h dark cycle and water *ad libitum*. Animals were treated humanely. Care and supervision were provided throughout the period of study. The study protocols were duly approved by the ethical committee Regn. No.

Table 1. Effect of *Carica papaya* fruit extract on glycogen content in testis of male albino rats

Experiment Period	Control Normal feed + water	Experimental group I Normal feed + Seed Papaya fruit juice	Experimental group II Normal feed + Seedless Papaya fruit juice
20th day	4.10 ± 0.27	4.23±0.31	4.20±0.37
40th day	4.82 ± 0.18	5.40±0.28**	5.38±0.38**
60th day	4. 99 ± 0.21	7.04±0.25***	6.60±0.25***

Values are expressed as mg/gm wet tissue
Values are Mean ± SD (n=6) observations
Statistical significance denoted as **p < 0.01; ***p < 0.001 (Compared between Control Vs Experimental Group I ; Control Vs Experimental Group II)

Table 2. Effect of *Carica papaya* fruit extract on total protein content in testis of male albino rats

Experiment Period	Control Normal feed + water	Experimental group I Normal feed + Seed Papaya fruit juice	Experimental group II Normal feed + Seedless Papaya fruit juice
20th day	40.01 ± 4.4	29.02 ± 2.8***	30.12 ± 2.2***
40 th day	47.72 ± 2.4	32.22 ± 3.0***	31.92 ± 3.6***
60th day	48.10 ± 3.2	37.92 ± 2.68***	36.22 ± 2.4***

Values are expressed as mg/gm wet tissue
Values are Mean ± SD (n=6) observations
Statistical significance denoted as ***p < 0.001 (Compared between Control Vs Experimental Group I ; Control Vs Experimental Group II)

Table 3. Effect of *Carica papaya* fruit extract on cholesterol content in testis of male albino rats

Experiment Period	Control Normal feed + water	Experimental group I Normal feed + Seed Papaya fruit juice	Experimental group II Normal feed + Seedless Papaya fruit juice
20th day	3.32 ± 0.30	3.92 ± 0.32	3.99 ± 0.33
40th day	3.79 ± 0.21	5.38 ± 0.19**	5.85± 0.38***
60th day	4.23 ± 0.31	6.60 ± 0.21***	5.66 ± 0.29***

Values are expressed as mg/gm wet tissue
Values are Mean ± SD (n=6) observations
Statistical significance denoted as **p < 0.01, ***p < 0.001 (Compared between Control Vs Experimental Group I ; Control Vs Experimental Group II)

CPCSCA/CA/ORG/2006/65/4. Studies were performed in accordance with the CPCSEA guidelines.

Experimental design

The animals were divided into three groups with 6 animals in each

Group I: Control animals which received normal feed and water.

Group II: Experimental animals which received normal feed and oral administration of seeded semi- ripe papaya fruit juice (10ml/kg body weight/day) for a period of 60 days.

Group III: Experimental animals which received normal feed and oral administration of seedless semi- ripe papaya fruit juice (10ml/kg body weight/day) for a period of 60 days.

The animals were acclimatized to laboratory conditions for a week with normal feed and water before the start of the experiment. Initial and final body weights were recorded before and at the end of experimental period. The seed and seeded semi- ripe papaya fruit pulp extract was given orally through a gastric intubation tube daily at 9.00 A.M. The animals were sacrificed by cervical decapitation subsequently on the 21st, 41st and 61st day after the completion of experimental period. The testis was dissected out freed from adhering tissues, washed with 0.9 N saline solution, blotted on a filter paper, weighed. The weighed tissues were homogenized in 1 ml of 0.1 M Tris buffer (pH 7.2) for estimation of glycogen and proteins. The homogenate of the tissues were centrifuged at 5000 rpm for 15 minutes and the clear supernatant were used for biochemical analysis. For Estimation of cholesterol, lipids were extracted by using chloroform- methanol mixture.

Biochemical Estimations

The quantitative determination of biochemical constituents were performed by UV spectrophotometer using the following methods. Total glycogen (Caroll *et al* 1956), Total protein (Lowry *et al* 1951), Extraction of lipids (Folch *et al* 1957) and Cholesterol (Parekh and Jung, 1970)

Statistical Analysis

Data was expressed as Mean ± SD. Student's t test was used for statistical comparison.

RESULTS

Male albino rats were orally administered with extracts of semi-ripe seed papaya and seedless papaya fruit pulp extract for a period of 60 days (10ml/kg body weight/day). Biochemical analysis was carried out in testicular tissues twenty four hours after the administration of the last dose after 20, 40 and 60 days. The results show a significant increase in the content of glycogen in the test is, after 40 and 60 days of administration of semi-ripe papaya fruit pulp in both the experimental groups, when compared with the control animals. No significant changes were observed on the 20th day (Table 1). Protein contents was decreased significantly throughout the experimental period in the testicular tissues, after 20, 40 and 60 days of fruit pulp administration in both the experimental groups (Table 2). The content of cholesterol was elevated significantly during the 40th and 60th day of administration of the extract in both experimental groups. No significant changes were noticed on the 20th day (Table 3).

DISCUSSION

In recent years relatively more emphasis is being laid on the examination of natural products including susbtances of plant

origin for their antifertility activity (Kusemiju, *et al*., 2012). The mechanism of action of these substances can be realized by the study of the biochemical parameters of the reproductive tract. Studies on the effects of the plant products specifically with reference to unripe or semi-ripe fruit pulp on male reproductive system and fertility are comparatively scarce. The present paper will provide a number of observation regarding the effects of aqueous extract of *Carica papaya* semi- ripe fruit pulp of seed and seedless fruits on testicular function in male albino rats. The present study showed for the first time that the *Carica papaya*, semi-ripe fruit pulp extract impair reproductive activities in male rats possibly by inhibiting spermatogenesis and steroidogenesis. In our investigation based on the results of acute oral toxicty study 30% of the LD_{50} cutoff value were selected as dose and used for pharmacological screening.

The increase in glycogen content of the testis after the 40^{th} and 60^{th} day suggest that the increase in carbohydrates reserve might be due to arrested spermatogenesis leading to reduction of spermatids and spermatozoa population as the germ cell rely more on carbohydrates for energy sources which have not been utilized (Changamma *et al* ., 2010). It has been reported that protein level is directly correlated with the secretory activity of the testis which in turn depends on androgen levels (Hasim Basha *at al*., 2010). The protein biosynthetic machinery was inhibited in the testis as evidenced from our results. In past researchers have shown that the testicular volume correlate positively with testicular function (Yama *et al*., 2011c , Kusemiji, 2012) as well as testosterone level (Mahmoud *et al*., 2003). In our findings, the volume of testicular tissues form rats administred with *Carica papaya* fruit pulp were reduced compared to control group, which could be due testicular degeneration or lesions reducing protein contents or inhibiting protein synthesis. Similar results have been observed in the testis of *Momordica charantia* seed extract (Yama *et al*., 2011c).

Mammalian cells require cholesterol which play an important role in acting as precursor molecule in the synthesis of steroid and its level in related to fertility (Thejaswini *et al*., 2012). The increased testicular cholesterol level in the present study after 40 and 60 days of treatment indicate decreased steroidogenesis as it is the precursor molecule for synthesis of testosterone, the androgens predominant in male fertility regulation (Gupta *et al*., 2005). Non-utility of cholesterol substrates could also be due to the decrease in the activity of steroidogenic key enzymes 3β-HSD and 17β-HSD as reported from earlier studies (Lakshman and Changamma, 2013). The impaired cholesterol metabolism in the testis could be due to effect of papain an enzyme, carpasemine and oleanolic glycoside present in *Carica papaya* semi -ripe fruits (Barret and Buttle, 1985). The present study indicates the antiandrogenic and antispermatogenic effects of the fruit pulp of *Carica papaya* altering the carbohydrate, protein and lipid contents of the testis.

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