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Research Article

EFFECT OF SEMI-RIPE CARICA PAPAYA FRUIT PULP AND SEED EXTRACTS ON FRUCTOSE CONTENT IN REPRODUCTIVE TISSUES OF MALE ALBINO RATS

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ABSTRACT

The aim of the present investigation is to analyze 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 fructose constituents in male albino rats. The present study was carried out to assess the effect of semi-ripe fruit pulp extract of *Carica papaya* (seeded and seedless fruits) on male albino Wistar rats.

Fructose content showed a considerable decline in testis and seminal vesicle of both experimental groups. The depletion of seminal vesicle and testicular fructose in rats administered with papaya pulp extract and seed from seeded fruits invariably affects the sperm motility and viability since fructose serve as the driving energy of the sperms which are androgen dependent. The quality and quantity of the sperm is altered by the *Carica papaya* fruit extract and seed which in turn may affect reproductive potential. This can be attributed to the anti-androgenic property of the extract.

INTRODUCTION

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern synthetic medicines. All over the world 80% of population use traditional medicine for primary medical problems. In spite of considerable development in contraceptive technology, search for male and female antifertility agent from plants continues to be a potential area of investigation (Kamboj, 1988; Heinrich, 2000). Recently efforts are being devoted to identity a plant based male contraceptive that is supposed by orally bioactive, non-toxic and more important cost effective based on ethano-medical information (Udoh, *et al* 2005a; 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 (Chopra, *et al* 1992; Lohiya *et al*, 2001; Heeshma khusbalani *et al 2006*; Changamma and Lakshman, 2010)

Sperm obtained directly from the epididymis contains hardly any fructose. During the passage through the male genital tract the semen acquires fructose from the accessory glands associated with reproduction, of which the seminal vesicle are the chief contribution of fructose. They provide the energy to the sperm for maintaining motility after ejaculation. The seminal vesicles produce a sugar-rich fluid (fructose) that provides sperm with a source of energy to help them move (Priyanka Raghuvanshi *et al* 2013). Their contents dependent on male sex hormone, which are chemicals that stimulate or regulate the activity of reproductive cells or organs.

MATERIAL AND METHODS

Semi-ripe seeded and seedless varieties of papaya, *Carica papaya* fruits were commercially obtained from wholesale fruit market in Chennai. 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 powered fruit pulp was macerated with cold water and passed through a fine

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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 4^0 C in a refrigerator for further used. 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 was 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.

Healthy male and female albino Wistar strain rats (*Rattus norvegicus*) were purchased from The King Institute of Preventive Medicine and Research, Guindy, Chennai. Rats weighing 155 ± 25g were used in the present study. The animals were maintained in polypropylene cages with metal grill top under standard environmental conditions of temperature 25 ± 2^0 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. Animals were treated humanely. Care and supervision was provided throughout the period 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 CPCSES guidelines. The rats were fasted for 3.- 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.

The fruit pulp power 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 for96 hr and LD_{50} was determined (Weis,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 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 determined and the combined preparation was administered orally for a period of 60 days to the experimental group-I of rats.

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, 2000 mg, 3000 mg, 4000 mg and 5000 mg/kg bw. 83%, 67%, 50% and17% mortality was observed in 5000 mg, 4000 mg, 3000 mg and 2000 mg doses, respectively. No mortality was observed in 1000 mg dosage. Fifty per cent 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. The animals were further observed for 48h post treatment for signs of toxicity and death before fixing the final dosage.

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 power and 500 mg of powdered seed from seeded semi-ripe papaya fruit (1000 mg in combination) were dissolved in water (1.0 gm dissolved in 1.0ml distilled water) for experimental group-I animals. 1000 mg fruit pulp power 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.

The extracts prepared were orally administered via gastric intonation using an orogastric tube comprising a 16-G polyethylene catheter fitted with a hypodermic syringe (volume of 10 ml). Administrations 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 equal weight. Each group consisted of six animals, maintained in separate cages.

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

Group II: Experimental group- I*:* Experimental male animals 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 a period of 60 days.

Group III: Experimental group-II*:* Experimental male animals which teceived normal feed and water, oral administration of seedless semi-ripe papaya fruit pulp extract alone (1000 mg / kg body weight / day) for a period of 60 days.

The animals were acclimatized to laboratory conditions for 15 days with normal feed and water before the start of the experiment. Initial body weights were recorded prior to and after treatment. 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 9.00A.M The animals were sacrificed by cervical dislocation on the 61^{st} day, 24 hour after administration of last dose .

Fructose was assayed by standard procedure of Monsigny (1988). Data expressed as Mean ± SD and subjected to statistical analysis by one way analysis of variance (ANOVA) followed by Duncan multiple range tests and the statistical significance was tested at 1% and 5% levels using SPSS version 6.0.

RESULTS AND DISCUSSION

The testis and seminal vesicle fructose content showed a significant decrease ($p< 0.001$) in both the experimental groups administered with *Carica papaya* fruit pulp and seed extracts of seeded variety and fruit pulp extracts of seedless variety. The seminal vesicle exhibited a varying pattern in both the groups, while testis in experimental group administered with *Carica papaya* fruit pulp and seed extracts combination, a considerable decline in fructose content was observed while an insignificant change was reported in animals treated with fruit pulp extract of seedless papaya (Table-I; Fig -1).

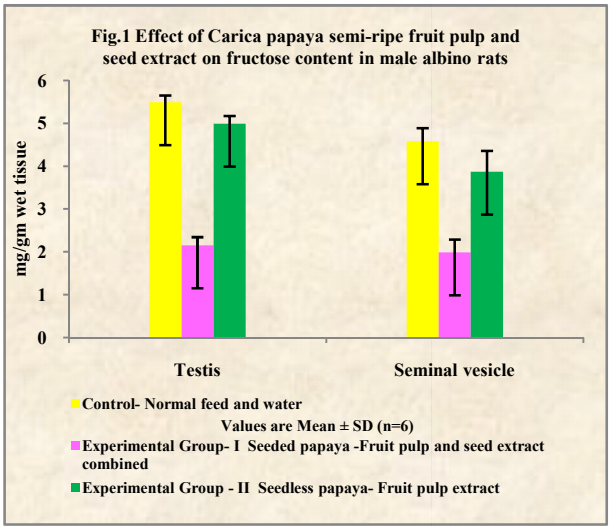
Sperm obtained directly from the epididymis contains hardly any fructose. During the passage through the male genital tract the semen acquires fructose from the accessory glands associated with reproduction, of which the seminal vesicle are

the chief contribution of fructose (Priyanka Raghuvanshi *et al* 2013). They provide the energy to the sperm for maintaining motility after ejaculation (Spring Mills and Hafez, 1979).

Table 1 Effect of *Carica papaya* semi-ripe fruit pulp and seed extracts on fructose content in male albino rats

Tissues	Control	Experimental Group-I	Experimental Group- II	F- value	P -value
Testis	$3.49^{b} \pm 0.16$	$2.15^{a} \pm 0.19$	$2.91^{a} \pm 0.18$	527.840	< 0.001**
Seminal vesicle	$4.58^{c} \pm 0.31$	$2.99^{a} \pm 0.30$	$3.87^{b} \pm 0.49$	73.302	< 0.001**

Values are expressed as mg/gm wet tissue Values are Mean ± SD (n=6) observations.** denotes significance at 1% level Different alphabets between groups denotes significance at 5% level using Duncan multiple range test (DMRT) Means carrying atleast one common superscript between groups do not differ significantly



The significant depletion of fructose level in the seminal vesicle and testis show that the extract has the potential to penetrate the blood test is barrier. Baddessarini (1980) reported that effects of chemical agents on sperm composition is attributed to their ability to penetrate this barrier. The depletion of seminal vesicle and testicular fructose in rats administered with papaya pulp extract and seed from seeded fruits invariably affects the sperm motility and viability since fructose serve as the driving energy of the sperms which are androgen dependent. The reduction is proportional to the circulating levels of testosterone. Similarly decrease in sperm qualities points to reduction in the circulating androgen level (Zhou *et al* 2008; Gonzales, 1997). This could also be an indication in the decreased levels of sperms in the epididymal region. Similar results of antifertility efficacy has been implicated in reproductive endocrine malfunction in rats which received extracts of *Azadirachta indica* leaves (Raji *et al* 2003). Low sperm concentration, reduced percentage of spermatozoa with progressive motility, reduced fructose concentration in the seminal plasma, prostatic and seminal vesicular fluid alterations may lead to sperm anomalies (Bajpai *et al*, 1998) The quality and quantity of the sperm is altered by the *Carica papaya* fruit extract and seed which in turn may affect reproductive potential. This can be attributed to the antiandrogenic property of the extract. The reduction is proportional to the circulating levels of testosterone. Similarly decrease in sperm qualities points to reduction in the circulating androgen level.

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