Impact of *Carica papaya* Linn. seed extract on reproductive metabolic profiles in male albino rat

S. Hashim Basha, J. Lakshman, A. Govardan Naik and C. Changamma
Department of Zoology, S.V. University PG Centre, Kavali-524 201, A.P., India

Summary

The effect of *Carica papaya* Linn. (Paw-paw) seed extract on protein and carbohydrate metabolism of albino rat was studied. Administration of papaya seed extract (50mg/kg body wt/day for 20 days) to Wistar male rat through oral route affected total proteins, free amino acids and nucleic acids. The protein content of testis and sex accessory glands decreased, accompanied by accumulation of proteins in epididymis and no significant changes in liver, indicating reduced protein metabolism, particularly in testes. Due to accumulation of proteins in the epididymis, the sperm arriving at the epididymis did not undergo proper maturational changes, which led to infertility. The increased amino acids caused decrease in alkalinity of the semen in prostate gland, which led to decrease in the motility and survival of sperm and protection of their genetic material. The decrease in RNA/DNA ratio in liver reflected loss of ribosomes and shrinkage of cytoplasm in liver cells. Carbohydrate profiles suggest either increased glycogenolysis or decreased glycogenesis in testes. The decreased lactic acid content, in spite of elevated glycolysis, suggests the possible stepped-up mobilization of lactic acid into citric acid cycle. Thus, the results suggest that *C. papaya* seed extract at low doses may be used as an antispermatogenic agent for bringing about temporary infertility.

Key words: Carica papaya, testis, sperm, male infertility, carbohydrate metabolism, protein metabolism, nucleic acids.

Introduction

Plants, since ancient times, have been used globally across varied cultures throughout the known civilizations as a valuable and safe natural source of medicine. The Indian and Chinese systems of traditional medicines are well established, with written records dating back to thousand of years (Patwardhan et al., 2005). A majority of plants possess pharmacological principles. According to the World Health Organization (WHO) reports, 70%–80% of the world population practice traditional medicines for primary healthcare (WHO, 2002). The medical historians have recorded plants that could be used as contraceptives, emmenagogues and abortifacients (Kritiker and Basu, 1975).

Carica papaya is recognized from ancient times for its medicinal properties, and the contraceptive efficacy of papaya seed extracts has been reported during the 1970s (Chinoy and Geetha Ranga, 1984). Degeneration of germ cells and germinal epithelium, reduction in the number of Leydig cells and presence of vacuoles in the seminiferous tubules were observed when crude ripe seeds of papaya were administered through oral route to male Wistar rats at a dose of 100 mg per kg bw (Udoh and Kehinde, 1999). The crude chloroform extract of papaya seeds at a dose of 5 mg per animal per day for 40–60 days reduced the fertility potential to 0%, with the suppression of cauda epididymidal sperm motility (Lohiya and Goyal, 1992). Administration of chloroform extract of papaya seed to male rabbits for 150 days caused decline in sperm concentration (oligospermia) on the 75th day and azoospermia after 120 days. Membrane damage in the acrosome, bent midpiece, coiled tail, detached head and arrest of spermatogenesis beyond the level of spermatocytes were also observed (Lohiya et al., 1999). However, most of these studies were confined to germinal cells and sperm metabolism. There is lack of information on the metabolic status. Hence, the present study was undertaken.

Materials and Methods

Healthy adult male Wistar strain albino rats (90 day old, weight 160±10g) were administered with 50 mg/kg body wt/day of alcoholic extract of papaya seed through oral route for 20 days. The alcoholic extract was prepared according to WHO (1983) protocol CG-04. The seeds were shed-dried, powdered and extracted with 95% ethanol (v/v) at 55-60ºC for 3 h. The solvent was distilled under reduced pressure; the resulting mass was dried under vacuum and kept at 24ºC until use. The control animals were given normal saline or sterile distilled water. Both control and experimental rats were maintained under standard animal house facilities, with a temperature of 25±2ºC, and 12-14 h day light, and fed on standard rat feed obtained from Hindustan Lever Ltd., Mumbai, India.

Correspondence to be addressed to: Dr. Challa Changamma, Ph.D., E-mail: challa_changamma@yahoo.co.in

Twenty four hour after the last dose, the animals were autopsied and testes, epididymes, seminal vesicles, prostate glands and liver were isolated, chilled immediately and used for biochemical analysis. The total proteins (Lowry et al., 1951), nucleic acids (Munro and Fleck, 1966) and free amino acids (Moore and Stein, 1954) were determined as described by Colowick and Kaplan (1957). The carbohydrate profiles, viz., total carbohydrates (Carrol et al., 1956), glucose (Mendal et al., 1954), glycogen (Kemp and Van Heijningen, 1954) and lactic acid (Barker and Summerson, 1941) were also determined.

Results and Discussion

The data presented in the table-1 reveal the pattern of changes in protein profiles of testes, accessory glands and liver of male rats treated with *Carica papaya* seed extract. The total proteins were reduced significantly in testes, seminal vesicles and prostate gland, with marked increase in epididymis and no significant changes in liver. It indicates the reduced protein metabolism (Prasad and Vijayan 1986; Gupta et al., 2002; Chaturvedi et al., 2003). In epididymis there was increased protein metabolism. The spermatozoa formed in the testes arrive at the caput epididymidis, then corpus and finally reach the cauda region, where they are stored. The sperm entering the caput epididymidis are physiologically immature; they lack the ability to make forward progression and to fertilize the egg. During their transit through epididymis, sperm undergo maturational changes necessary for them to acquire these attributes (Frandson et al., 2009). Due to the *Carica papaya* seed extract administration an accumulation of proteins occurs in epididymis, where upon the sperm entering epididymis fail to undergo adequate maturation which would lead to infertility (Changamma and Lakshman, 2010a).

In the testes and prostate glands the free amino acids increased, in the seminal vesicles and liver they decreased and there was no change in the epididymis. Due to the decreased proteins in testes the free amino acids were accumulated as they are not utilized for spermatogenesis. The prostate gland showed marginal increase (12.7%; $P<0.01$). The function of prostate is to secrete, store and contribute a slightly alkaline (pH 7.29) fluid, which constitutes 25-30% of the volume of the semen. The alkalinity of semen helps neutralize the activity of the vaginal tract, prolonging the lifespan of sperm. The prostatic fluid provides for better sperm motility, longer survival and better protection of the genetic material (DNA) (Frandson et al., 2009). The decreased amino acids decrease the alkalinity of the semen. So, it affects the motility and survival of the sperm and protection of the sperm genetic material which would lead to infertility. The decreased amino acids in seminal vesicles, and to a greater

Table: 1 Protein profiles in testes, epididymis, seminal vesicles, prostate gland and liver of control and papaya seed extract treated rats. Mean+ SD of six individual observations. + and – , percent increase or decrease, respectively, over control. [a]-$P<0.001$, [b]-$P<0.01$, [c]- $P<0.05$, [d]- $P<0.02$ the level of significance & [e]- not significant changes.

Parameter	Control, seed extract, % change and significance				
	Testis	Epididymis	Seminal vesicles	Prostate gland	Liver
Total Proteins (mg/g wet wt.)	154.26 ±10.01 112.46 ±9.92 -27.10[a]	108.71 ±9.01 148.05 ±9.92 +36.19[a]	319.96 ±9.01 199.31 ±7.92 -37.70[a]	188.07 ±9.01 137.50 ±10.92 -26.89[a]	196.56 ±11.01 185.32 ±14.92 -5.72[e]
Amino acids (mg/g wet wt.)	1.353 ±0.067 1.824 ±0.072 +34.812[a]	1.311 ±0.068 1.296 ±0.094 -1.144[e]	0.964 ±0.0089 0.780 ±0.0069 -19.087[a]	1.078 ±0.091 1.215 ±0.089 +12.709[b]	9.473 ±0.851 2.970 ±0.198 -68.648[a]
DNA (mg/g wet wt.)	13.210 ±1.11 10.260 ±1.01 -22.33[a]	16.040 ±1.42 16.440 ±1.62 +2.49[e]	6.150 ±0.54 5.770 ±0.49 -6.18[e]	6.010 ±0.61 5.525 ±0.53 -8.07[a]	16.086 ±1.49 17.935 ±1.73 +11.49[c]
RNA (mg/g wet wt.)	11.63 ±1.09 10.50 ±0.99 -9.72[c]	18.76 ±1.28 19.76 ±1.89 +5.33[e]	9.17 ±0.87 10.54 ±1.01 +14.94[a]	8.70 ±0.79 8.29 ±0.76 -4.71[e]	18.77 ±1.56 15.51 ±1.47 -17.37[a]
RNA/DNA ratio	0.880 1.023 +16.25	1.169 1.202 +2.82	1.491 1.827 +22.54	1.448 1.499 +3.522	1.167 0.865 -25.878

Table: 2 Carbohydrate profiles in testes, epididymis, seminal vesicles, prostate gland and liver of control and papaya seed extract treated rats. Mean+ SD of six individual observations. + and – percent increase and decrease respectively over control. [a]- P<0.001, [b]- P<0.01, [c]- P<0.05, [d]- P<0.02 the level of significance & [e]- not significant changes.

Parameter	Control, seed extract, % change and significance				
	Testis	Epididymis	Seminal vesicles	Prostate gland	Liver
Total Carbohydrates (mg/g wet wt.)	35.78 ±2.078 25.75 ±1.08 -28.03[a]	22.50 ±0.978 20.34±1.08 -9.6[c]	9.92 ±0.578 14.95 ±0.08 +50.71[a]	27.41±0.178 20.80 ±0.98 -24.12[a]	181.21±12.078 149.89 ±10.08 -17.28[a]
Glucose (mg/g wet wt.)	1.425 ±0.092 1.546 ±0.101 +8.419[c]	1.035 ±0.089 0.886 ±0.071 - 14.396[b]	3.034 ±0.125 2.814 ±0.211 -6.361[c]	2.854 ±0.198 2.823 ±0.200 -1.086[e]	5.627 ±0.429 4.967 ±0.396 -11.729[d]
Glycogen (mg/g wet wt.)	1.778 ±0.111 1.675 ±0.121 - 5.793[c]	1.138 ±0.092 1.052 ±0.089 - 7.557[c]	3.164 ±0.268 2.823 ±0.211 - 10.777[d]	2.716 ±0.201 2.496 ±0.192 - 8.100 [c]	5.449 ±0.421 5.178 ±0.498 -4.973[e]
Lactic acid (mg/g wet wt.)	9.900 ±0.872 9.004 ±0.745 -9.051[c]	7.363 ±0.653 5.224 ±0.478 -29.051[a]	2.935 ±0.198 3.085 ±0.268 +5.111[e]	2.985 ±0.199 4.577 ±0.389 +53.333 [a]	12.289 ±1.005 9.751 ±0.821 -20.653 [a]

extent in the liver, indicates that the secretion from the seminal vesicles containing proteins, enzymes, fructose, mucus, vitamin C, flavins, phosphoryl choline and prostaglandins is affected. The decrease of amino acids indicates decrease of proteins and enzymes. In the liver the total proteins were not disturbed by the treatment but free amino acids were affected greatly which would lead to some disturbances in liver protein metabolism through the changes in liver enzymes (von Eckardstein et al., 2003). The nucleic acids content was not altered to any great extent except in testes (Prasad and Vijayan, 1986). RNA/ DNA ratio was decreased significantly in liver reflecting ribosomal loss and cytoplasmic shrinkage (Prasad and Vijayan, 1986). The status of RNA/DNA ratio in testes and seminal vesicles indicates that there was no such ribosomal loss and cytoplasmic shrinkage. Thus, it is presumed that these changes are mediated by blocking the androgen biosynthesis and/or by infertility, with normal function of hypothalamus-pituitary-gonadal axis maintained.

The data presented in the table-2 reveal the pattern of changes in some carbohydrate profiles. The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms. Glucose and other carbohydrates are part of a wide variety of metabolic pathways across species. Generally, the reproductive tissues like testes depend largely on carbohydrates for spermatogenesis to occur (Ewing et al., 1966). Glycogen has been localized in the testicular spermatogonia and spermatocytes (Udoh and Kehinde, 1999; Sharma and Jacob, 2001). Therefore, the study on carbohydrate metabolism was undertaken to understand any impairment in the carbohydrate metabolism. The glycogen content was drastically decreased due to the treatment. It could be due to either increased glycogenolysis or decreased glycogenesis.

The increased level of glucose in testes and its decrease in the accessory organs indicate accumulation of glucose due to the treatment suggest a negative implication on spermatogenesis. However, the lactic acid content decreased in the testes of treated rats. The decreased lactic acid content, in spite of elevated glycolysis, suggests the possible stepped-up mobilization of lactic acid into citric acid cycle. NAD-LDH, which mobilizes lactic acid into oxidative metabolism, when elevated, leads to decreased lactic acid content (Changamma and Lakshman 2010 b&c). Lactic acid may also efflux into the blood from the testes (Changamma, 2008).

Thus, the results suggest that *Carica papaya* seed extract could be used as an antispermatogenic agent at lower doses for bringing about temporary infertility.

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