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Research Article

ANTISPERMATOGENIC EFFECT OF CARICA PAPAYA SEED EXTRACT ON STEROIDOGENESIS IN ALBINO RATS

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ABSTRACT

Steroidogenesis plays a key role in the development and maintenance of male reproductive function and fertility. The objective of the present study was to investigate the effect of Carica papaya seed extract on steroidogenesis. The reduced cholesterol levels indicate decreased mobilization towards androgenesis which leads to decreased steroidogenesis and thereby inhibition of spermatogenesis in testes. The lowering of the 3β-HSD and 17β-HSD activity levels in the testes suggest the antifertility agents interfere with steroid hormone biosynthesis, which ultimately result in impaired spermatogenesis and infertility. The oleanolic glycoside, sinigrin, present in papaya seeds is acting on spermatogenesis by inhibiting the steroidogenesis which leads to antispermatogenesis. Thus the carica papaya seed extraction shows its infertility effect on spermatogenesis.

Keywords: 3β- HSD, 17β-HSD, Carica papaya, cholesterol, spermatogenesis.

INTRODUCTION

Medicinal plants have successfully been used to induce sterility in laboratory animals[1-3]. Pawpaw seed (*Carica papaya*)[4,5] reported high success in using. Pawpaw (Carica papaya) seeds had been used as fertility control agents in some animal models and even on human beings[6,7] respectively. Chloroform extract of papaya seeds tested in langen monkeys for one year, caused a steady decrease in sperm production with no sign of toxicity[8,9]. Crude extract fed to male rats deteriorated quantity and quality of the sperm[10,11]. At higher dose, it provided 100% contraception, but resulted in weight loss, possibly due to toxicity[7,12,9]. Suppression of spermatogenesis was observed in rats following the administration of papaya seed extract[13]. The Oral administrations of extract induced reversible male infertility[14-17]. The biochemical studies on carbohydrate metabolism reveals the decreased oxidative metabolism[18]. Male reproduction is a multifaceted process that involves the testes, epididymis, accessory sex glands and associated hormones. Testes perform two highly organized and intricate events, called spermatogenesis and steroidogenesis, which are vital for the perpetuation of life. Spermatogenesis, a highly dynamic and synchronized process, takes place within the seminiferous tubules of the testis with the support of somatic Sertoli cells, leading to the formation of mature spermatozoa from undifferentiated stem cells[19.] Pawpaw (*Carica papaya*) seeds contain antifertility properties, particularly of the seeds[20]. A complete loss of fertility has been reported in male rabbits, rats and monkeys fed an extract of papaya seeds[20,8,21]. Thus the steroidogenesis is important for spermatogenesis. Hence in the present study it is important to know how the steroid enzymes are modulating during spermatogenesis and antispermatogenesis.

MATERIALS AND METHODS

Healthy adult male Wistar strain albino rats (90days old, weight 160±10g) were administered with 100mg/kg body wt/day of alcoholic extract of papaya seed orally for 15days. The alcoholic extract was prepared according to WHO 1983[22] protocol CG-04. Seeds were shed-dried, powdered and extracted with 95%ethanol (v/v) at 55-60ºC for 3h. The

solvent was distilled off 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 in standard air conditioned animal house at a temperature of 25±2ºC, exposed to 12-14h day light and fed on standard rat feed obtained from Hindustan Lever Ltd., Bombay, India. The usage of animals was approved by the Institutional Animal Ethics committee (Regd.No. 438/01/a/CPCSEA/dt.17/02/2001) in its resolution number 9/IAEC/SVU/Zool/dt.4-3-2002.

Twenty four h after the last dose, the animals were autopsied. The tissues like testes, epididymis, seminal vesicle, prostate gland and liver were isolated, chilled immediately and blood was collected, used for biochemical analysis. The cholesterol estimated by Natelson 1971[23]. The enzymes like 3β-HSD&17β-hydroxy steroid dehydrogenase by Bergmeyer 1974[24] were estimated in control and experimental rat tissues.

RESULTS AND DISCUSSION

The data represented in tables 1-3and figures 1-3 shows the effect of Carica papaya seed extraction on cholesterol in reproductive and non reproductive tissues and 3β- HSD & 17β-HSD in testes of albino rats.

The cholesterol levels were significantly decreased in testes as it is necessary for steroidogenesis (table-1, fig.-1). This result indicates the decreased steroidogenesis which leads to decreased spermatogenesis. The impact of treatment on cholesterol is more in liver (+59.39), the central organ in cholesterol metabolism than in blood (table-2). In sex accessories there were no significant changes in epididymis while in seminal vesicle and prostate cholesterol levels were slightly(P<0.01) increased.

Cholesterol is the precursor of the steroid hormones [25], providing the backbone of the steroid molecule. The biosynthesis of testosterone directly from cholesterol can only occur in the Leydig cells[26].

Cholesterol is one of the most important sterols and is a structural component of membranes as well as the precursor for bile acids and steroid hormones. Cholesterol is a sterol with special functions in various tissues and organs. First of all, it is a structural component of all cell membranes. Furthermore, it is the precursor molecule of steroid hormones, such as progesterone, testosterone and cortisol. The unsaponifiable fraction of the neutral lipid fraction of the rat testis represents primarily cholesterol and steroids [27]. Cholesterol is an important precursor for the steroid hormones. The testis and its metabolism are dependent on the plasma and endogenously synthesized cholesterol. Hence the cholesterol levels were estimated and found to be significantly decreased in the experimental rat testis. This observation indicates either its decreased uptake from the plasma or increased synthesis or decreased mobilization towards androgenesis or decreased catabolism. This was supported by enhanced blood cholesterol levels (Table-2) by the treatment in the present study.

Table: 1. The Levels of Cholesterol in reproductive tissues of control and papaya seed extraction treated rats. Means ± SD of six individual observations. + and – indicates percent increase and decrease respectively over control. a- indicates P<0.001 the level of significance, b- indicates P<0.01, e- indicates Non significant changes.

S.No	Name of the tissue	Control	Experime-ntal	% change
1.	Testis (mg/g wet wt.)	0.685 ±0.054	0.548 ±0.039	-20.01[a]
2.	Epididymis (mg/g wet wt.)	1.345 ±0.101	1.307 ±0.099	-3.47[e]
3.	Seminal Vesicle (mg/g wet wt.)	0.265 ±0.014	0.291 ±0.017	+9.81[b]
4.	Prostate gland (mg/g wet wt.)	0.279 ±0.015	0.319 ±0.019	+14.34[b]

Table: 2. The Levels of Cholesterol in Liver and Blood of control and papaya seed extraction treated rats. Means ± SD of six individual observations. + and – indicates percent increase and decrease respectively over control. a-indicates P<0.001 the level of significance.

S.No	Name of the Tissue	Control	Experimental	% Change
1.	Liver (mg/g wet wt.)	5.32 ±0.421	8.48 ±0.736	+59.39[a]
2.	Blood (mg/100ml)	194.85 ±10.42	160.29 ±12.04	-17.74[a]

The spermatogenesis is a complex process which is strictly regulated by the hypothalamo–pituitary–

testicular axis, which involves the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Apart from LH, FSH and androgens, various growth factors, hormones and estrogens are involved in regulating the testicular functions[28]. Thus the reduced levels of cholesterol in testes by the treatment in the present study indicate the antispermatogenic effect of Carica papaya seed extraction through decreased steroidogenesis[29].

Table 3: The levels of 3β-HSD&17β-HSD in control and Papaya seed extraction treated rat testes. Mean ± SD of six individual observations. + and – indicates percent increase and decrease respectively over control. a-indicates P<0.001 the level of significance.

S.No	Parameter	Control	Experimental	%change significance
1.	3β-HSD (μmol NAD^+reduced /mg protein/min)	0.475 ±0.011	0.330 ±0.012	-30.53[a]
2.	17β-HSD (μmol NADPH oxidized/mg protein/min)	0.592 ±0.023	0.492 ±0.031	-16.89[a]

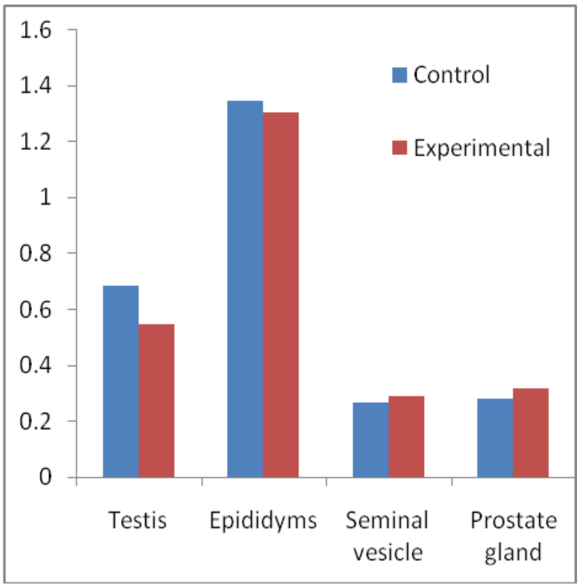

Fig: 1a Histograms showing the changes in Cholesterol of Reproductive tissues in Control and Papaya Seed extraction treated rats.

The activity levels of 3β- HSD and 17β-HSD the key enzymes of androgenesis were decreased significantly in the treated rat testis (table-3, fig-3).

The decreased cholesterol content reveals that there is no dearth of substrate for steroidogenesis. Since the 3β- HSD and 17β-HSD activity levels were decreased. Carica papaya seed extraction inhibits testicular steroidogenesis. Suggesting the impaired steroidogenesis[29-30]. There is decreased activity levels of 3β- HSD and 17β-HSD in the testes suggest the antifertility agents interfere with steroid hormone biosynthesis, which ultimately result in impaired spermatogenesis and infertility. The kinetic characteristics of 17β-HSD were determined in the cell system which would reflect the native kinetic properties of the enzyme under the influence of native intracellular milieu[30].

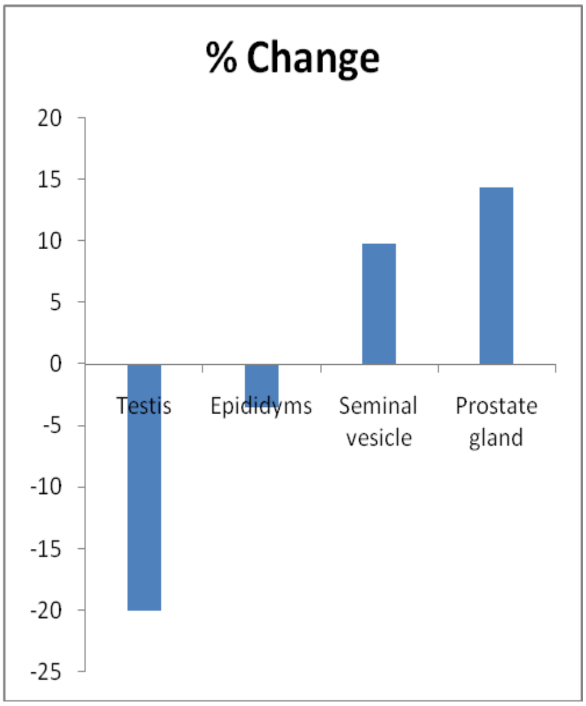

Fig: 1b Histograms showing the % changes in Cholesterol of Reproductive tissues in Control and Papaya seed extraction treated rat.

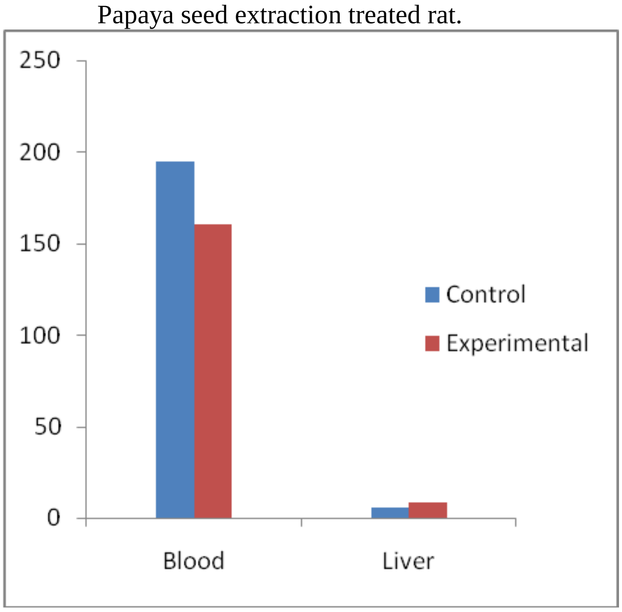

Fig: 2a Histograms showing the changes in Cholesterol levels in Blood &Liver in Control and Papaya Seed extraction treated rats.

Two different pathways of androgen biosynthesis have been reported in testis, (1) progesterone pathway or Δ^4-pathway and (2) dehydroepiandrostenedione pathway or Δ^5-Pathway. 3β-HSD acts on C-19 and C-21 steroids by specifically acting on 3β-hydroxy groups. This enzyme has NAD^+ as the preferred cofactor and is localized in the microsomes. It converts the pregnenolone to progesterone. This enzyme is almost irreversible reaching equilibrium towards the progesterone formation.

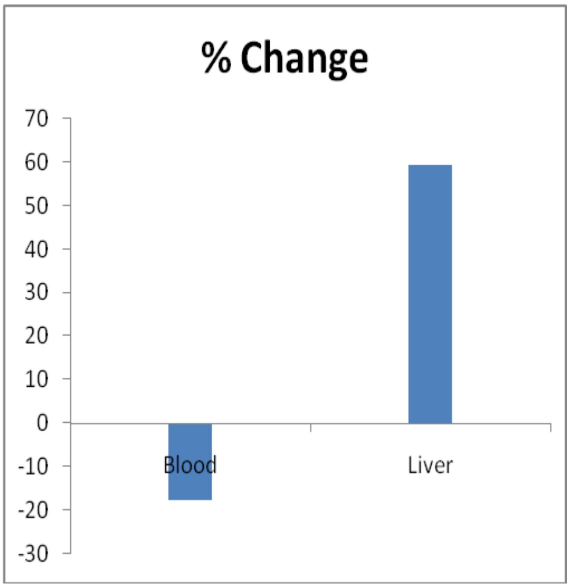

Fig: 2b Histograms showing the % changes in Cholesterol of Control and Papaya seed extraction treated rat non reproductive tissues.

The 17β-hydroxysteroid dehydrogenases (17β-HSD enzymes) are a group of alcohol oxidoreductases which catalyse the dehydrogenation of 17-hydroxysteroids in steroidogenesis. The major reactions catalysed by 17β-HSD the conversion of androstenedione to testosterone are in fact hydrogenation (reduction) rather than dehydrogenation (oxidation) reactions that can affect the primary and/or secondary sex characteristics of both males and females. In sex steroid metabolism 17 β -hydroxysteroid dehydrogenases (17 β -HSDs) catalyze the final steps in androgen and estrogen biosynthesis, thus playing a crucial role in the biosynthesis and inactivation of sex steroid hormones[31].

The Carica papaya seeds contain active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor, and oleanolic glycoside[32], which caused sterility in male rats[33]. Pawpaw (*Carica papaya*) seeds yield 660-760mg (bactericidal a glycone of glucotropaeolin benzyl isothiocyanate), a glycoside, sinigrin, the enzyme myrosin, and carpasemine. Hence oleanolic glycoside, sinigrin is acting on spermatogenesis by inhibiting the steroidogenesis which leads to antispermatogenesis in the present study.

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