Antifertility effects of aqueous seed extract of *Carica papaya* Linn. on seminal electrophoretic proteins of mice (*Mus musculus*).

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ABSTRACT

Objective: To evaluate the effects of *Carica papaya* seed extract on seminal protein concentration in male mice. **Methods:** 60 adult male Swiss albino mice were divided into two groups: group 1st (treated group) was fed 0.1 ml of *Carica papaya* seed extract (50 mg/kg body wt.) while group 2nd (control group) was fed same amount of distilled water with similar exposure. After 10 to 50 days of treatment mice were sacrificed. Semen samples were collected from cauda epididymis of both groups of the mice. These samples were filtered, centrifuged and processed for electrophoresis. **Results:** Results showed that papaya seed extract mainly induced the anodic protein concentration in semen of treated group of mice than the control. However, cathodic protein concentration increased insignificantly than the control. Due to increase in anodic protein concentration, total electrophoretic protein concentration of treated group of mice also increased significantly ($P<0.001$) after 10 to 50 days of treatment than the control. **Conclusion:** Increase in anodic protein concentration in semen of treated group of mice may add more negative charge on the sperm surface membrane which may inhibit capacitation as well as fertilizing ability of the spermatozoa.

Key words: Anodic protein, Cathodic protein, Semen, Antifertility, Relative mobility.

INTRODUCTION

Several medicinal plant extracts show antifertility activities including *Carica papaya*. It is a common fruit plant in India and easily available throughout the year. Different parts of plant contain various pharmacological and antifertility properties. For this reason *Carica papaya* Linn. is included in the investigation to observe the antifertility effects in semen of male mice.

Carica papaya belongs to family Caricaceace and originated from Central America. The fruit, leaves, seed and latex of this plant have medicinal values. *Carica papaya* possesses activities like anti-cancer [1], anti-bacterial [2,3], anti-inflammatory [4], anti-ulcer [5], anti-oxidant [6], immunomodulatory [7], antidiabetic [8], anti-dengue [9] and antifertility activity [10, 11].

Thus, the present study was carried out to evaluate the antifertility effects of *Carica papaya* seed on seminal electrophoretic protein concentration in male albino mice.

MATERIALS AND METHODS

Test animals: 60 adults, healthy male Swiss albino mice (*Mus musculus*) weighing between 25 to 30gm were selected in the present study. These mice were procured from the University Department of zoology, T.M. Bhagalpur University Bhagalpur. All mice were maintained under normal husbandry condition at temperature of 25±2˚ with proper ventilation. They were maintained 12 hours light: 12 hours dark photoperiod and fed bread, milk, seasonal vegetable, germinated seed, and water ad libitum.

Test materials: Ripe *Carica papaya* fruit were obtained from local market. The ripen seed were removed, washed, shed dried and coarsely powered. The powder was filtered and weighed which was mixed into measured amount of distilled water for 24 hours at room temperature. Then the content was filtered and filtrate was stored in glass container into refrigerator.

Experimental design: All mice were divided into 2 groups. 1st group served as treated group and another is control group. Treated mice were fed 0.1ml *Carica papaya* seed extract at a dose of 50mg/kg body weight while control group received same amount of distilled water with similar exposure. Six mice of both groups were sacrificed by cervical dislocation after the exposure of 10, 20, 30, 40 and 50 days. After killing, semen samples were collected from cauda epididymis of each mice.

Electrophoretic protein concentration: Semen samples were filtered, centrifuged and processed for electrophoretic studies. Electrophoresis was performed using polyacrylamide disc gel after the method of Smith [12]. After separation of protein, gels were processed through staining solution for localisation of protein bands. The gels were stained with Commassie brilliant blue R-250 including methanol and acetic acid for about 30 minutes. Then the gels were destained with destaining solution. The quantification of band was done by using Gel Documentation and analysis system. Gels were stored in fixative solution. Protein showing Rm<0.5 were considered cathodic protein while RM>0.5 were known as anodic protein. Concentration of different protein bands were calculated against the known concentration of bovine serum albumin (BPB) in the terms of mg/ml of semen.

Statistical analysis: The results presented as mean ± SEM. Student t-test was applied for statistical comparison between control and treated group.

RESULTS

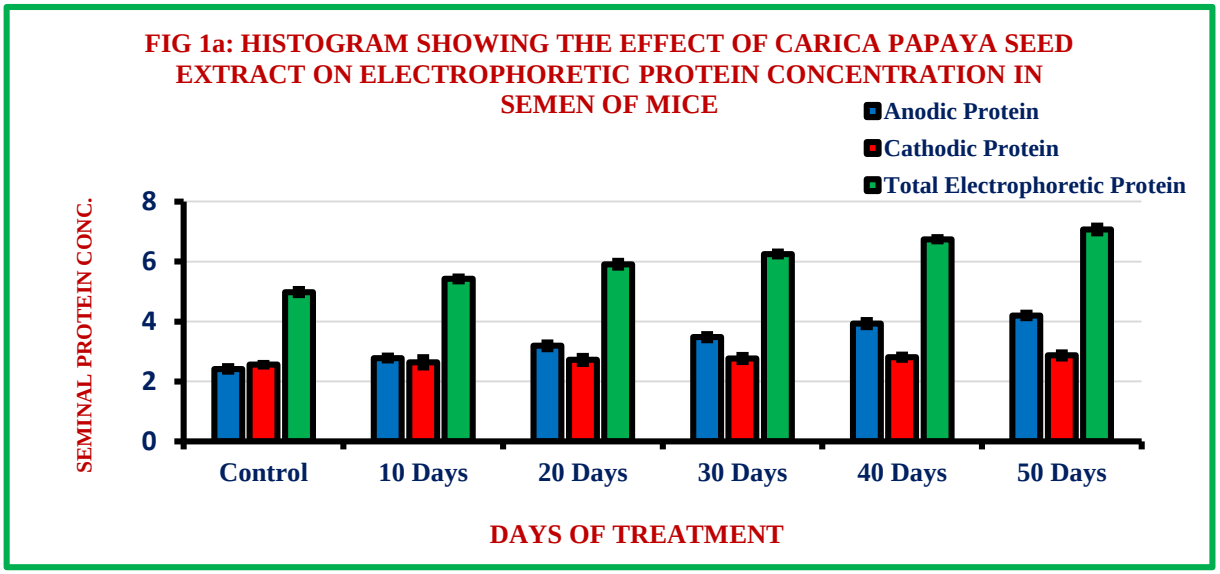
The results of seminal electrophoretic protein concentration of control as well as treated group of mice were summarised in **table- 1** and depicted as Mean ±SE.

The present study showed that anodic protein concentration increased significantly in semen of papaya seed treated group of mice after 10 to 50 days of treatment than the control. However cathodic protein concentration of treated group of mice semen increased insignificantly than the control. Due to increase in anodic protein concentration, total electrophoretic protein concentration of papaya seed treated group of mice also increased significantly (P<0.001) after 10(P<0.1), 20(P<0.01), 30(P<0.001) and 40 (P<0.001) days of treatment than the control. Such gradual and significant increase in total electrophoretic protein concentration in papaya treated mice semen attain maximum level after 50 days of treatment and is highly significant (P<0.001) than the control group.

TABLE 1: EFFECT OF CARICA PAPAYA SEED TREATMENT ON SEMINAL PROTEIN CONCENTRATION OF MICE IN COMPARISON WITH CONTROL GROUP.

Groups	Anodic protein conc. (mg/ml)	Cathodic protein conc. (mg/ml)	Total protein conc. (mg/ml)
Control (6)	2.42 ± 0.09	2.56 ± 0.06	4.98 ± 0.10
10 Days of treatment (6)	2.78 ± 0.08*	2.64 ± 0.17	5.42 ± 0.08*
20 Days of treatment (6)	3.19 ± 0.11**	2.72 ± 0.13	5.91 ± 0.11**
30 Days of treatment (6)	3.48 ± 0.10**	2.77 ± 0.12	6.25 ± 0.08***
40 Days of treatment (6)	3.93 ± 0.12***	2.81 ± 0.09	6.74 ± 0.07***
50 Days of treatment (6)	4.20 ± 0.09***	2.87 ± 0.10	7.07 ± 0.13***

Data presented as Mean± SEM; *, **, *** shows significance at 0.1, 0.01 and 0.001 levels respectively with value in control. Number within parenthesis denote number of samples.



DISCUSSION

Results of present study showed **(table-1)** that papaya seed extract mainly induced the anodic protein concentration in semen of treated group of mice than the control. However cathodic protein concentration increase slightly and insignificantly than the control. Due to increase in anodic protein concentration, total electrophoretic protein concentration of treated group of mice also increased significantly ($P<0.001$) after 10 to 50 days of treatment than the control.

Negatively charged proteins migrate toward the anode and known as anodic protein. In this study increase in anodic protein concentration in semen of treated group of mice may add more negative charge on the sperm surface membrane which may inhibit capacitation as well as fertilizing ability of spermatozoa among treated group of mice than control [13, 14].

Stewart-savage [15] concluded that albumin is the most negatively charge protein migrate towards the anode. When concentration of albumin is increased in the semen of Golden Hamster, sperm fertilizing ability decreased significantly. This indicates that the fertilizing ability of the sperm inhibited due to increase in anodic protein concentration in the semen of mice.

It has been reported that protein level is directly correlated with the secretory activities of the testis which is androgen dependent. [16] In the present study alteration of total electrophoretic protein concentration in semen may be due to anti-androgenic nature of papaya seed extract. [16]

Hasim *et al* [17] also reported similar findings by administration of papaya seed extract (50 mg/kg body weight for 20 days) to Wister male rats which causes accumulation of proteins in epididymis. Due to this treatment spermatozoa did not undergo proper maturation, which leads to infertility among treated mice. In this present study, similar findings were observed in semen of treated group of mice. Administration of papaya seed extract caused increase in total seminal electrophoretic protein concentration in treated group of mice than the control. Due to the papaya seed extract administration there is accumulation of proteins in epididymis which impair the maturation of spermatozoa that lead to infertility among treated group of mice. [17]

In a similar study, Goverdhan *et al* [18] reported increase in testicular proteins of rather when treated with *Piper betel* leaf extract administration. It may be due to alternation in the synthetic activity of testis. In this study, the accumulation at protein occurred in testis due to anti-androgenic action of the *Piper betel* extract. In the present study probably through a similar reason, total protein concentration in seminal fluid increased significantly [16].

CONCLUSION

The oral exposure of *Carica papaya* seed extract inhibit, a low fertilizing ability of sperms among treated group of mice by increasing anodic protein concentration in semen. Increase in anodic protein concentration inhibits capacitation as well as fertilizing capability of spermatozoa. Thus it may be concluded that *Carica papaya* shows antifertility effects in mice.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest.

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