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Contraceptive effects of aqueous seed extract of *Carica papaya* (Linn.) on M-isozyme, H-isozyme and M/H ratio in semen of male albino mice (*Mus musculus*)

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ABSTRACT

Objective: To evaluate the effects of *Carica papaya* seed extract administration on seminal M-isozyme activity, H-isozymes activity and M/H ratio of LDH in mice. **Methods:** 60 adult male Swiss albino mice weighing about 25 to 30 gm were randomly divided into two groups. Group 1st (treated group) was fed 0.1 ml of *Carica papaya* seed extract at a dose of 50 mg/kg body wt. and group II (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 for separation of M-isozyme and H-isozymes bands. **Results:** Results showed that after 10 to 50 days of papaya seed treatment M-isozyme of LDH activity showed highly significant ($p<0.001$) increase in semen of treated group of mice than the control. However H-isozyme increased slightly and insignificantly than the control. Such increase in M-isozyme of LDH causes increase in M/H ratio of LDH in semen of treated group of mice than the control. **Conclusions:** Significant increase in M-isozyme activity of LDH may impair sperm motility which lead to infertility among papaya seed treated group of mice.

Key words: Isozymes, epididymis, semen, Infertility,

INTRODUCTION

The lactate dehydrogenase (L-lactate-NAD+ oxidoreductase, EC 1.1.1.27) catalyse interconversion of pyruvate into lactate with concomitant interconversion of NADH and NAD (+) as hydrogen acceptor (Read *et al*, 2001).

It can be separated into five distinct molecular forms. These five lactate dehydrogenase isozymes are tetramer composed of two different polypeptide units designated as LDH M and LDH H by Chan *et al* (1962) and LDH A and LDH B by Markert (1962) in order of decreasing mobility these

five forms of LDH are named as LDH_1 (HHHH), LDH_2 (HHHM), LDH_3 (HHMM), LDH_4 (HMMM), and LDH_5 (MMMM) (Aydin *et al*, 1997)..

Among these 5 isoenzymes LDH_1 and LDH_2 are categorised as H-isozyme where, LDH_4, and LDH_5 considered as M-isozymes of LDH (Cahn, 1964).

The pattern of LDH isozymes may play significant role in metabolic response to alter energy metabolism. The H- isozyme is responsible for the conversion of lactate into pyruvate while M- subunits convert pyruvate into lactate (Clausen *et al*, 1970).

Hence, the present study was carried out to evaluate the effects of *Carica papaya* seed extract on seminal M-isozyme activity, H-isozymes activity and M/H ratio of LDH isozyme in male albino mice.

MATERIALS AND METHODS

Experimental animal: 60 adult, healthy Swiss albino male mice (*Mus musculus*) weighing 25 to 30 gm of 12-14 week old 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 control and hygienic conditions 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. The initial body weight of each mice was recorded.

Extract preparation: Ripen *Carica papaya* fruit were obtained from local market. The ripen seed were removed, washed, shed dried at room temperature then coarsely powered. The weighed amount of powered material was added in measured amount of distilled water for 24 hours at room temperature. Then the content was filtered and filtrate was stored in refrigerator.

Experimental design: The experiment consisted of the two groups of 30 male albino mice per group, designated as control and treated 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. 6 mice of each group 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.

Activities of seminal LDH isozymes:
Semen sample were filtered, centrifuged and processed for electrophoretic studies. Electrophoresis was performed using polyacrylamide disc gel in accordance with the methods prescribed by Smith (1976). The gels were stained and incubated at 37° C temperature in a solution containing sodium lactate, NAD, nitro-blue tetrazolium salt and phenazine methosulphate as described by Siciliano and Shaw (1976). The activities of M-isozyme, H-isozyme of seminal LDH were calculated after the quantification of different LDH isozyme bands against the known concentration of bovine serum albumin (BPB) in the term of Unit/ml/hr.

Statistical analysis: The results presented as mean ± SEM. To understand level of significance student t-test was applied.

RESULTS AND DISCUSSION

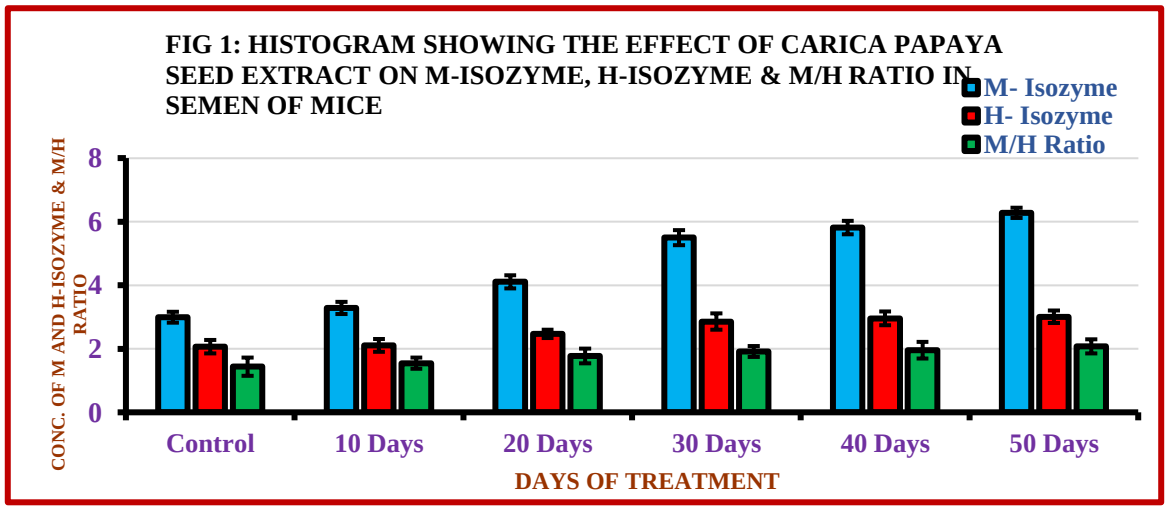
The data represented in table 1 shows that during 10 to 50 days of papaya seed treatment M-isozyme of LDH activity showed highly significant increase in semen of treated group of mice than the control. However, H-isozyme activity also increased in the semen of papaya seed treated group of mice but such increase is insignificant than the control. This increase in M-isozyme of LDH caused increase in total LDH activity of semen of treated group of mice than the control. While M/H ratio also showed significant increase in treated group of mice semen than the control. Thus, it is clear that papaya seed treatment mainly inducing M-isozyme activity significantly in the semen of treated group of mice than the control.

In this study, seminal M-isozyme & H-isozyme activity of both control as well as papaya seed treated group were statistically examined. Results revealed the variation in M-isozyme & H-isozyme activity in semen of both control and treated group of the mice. On the basis of results of present study, it is clear that papaya seed treatment mainly induces M-isozyme activity significantly in the semen of treated group of mice than the control and this extract has selective and directional influence on M-isozyme of LDH in semen of treated group of mice than control.

Table 1: Data showing effect of aqueous seed extract of carica papaya on m-isozyme, h-isozyme & m/h ratio in semen of mice

Groups	M-Isozyme (Units/ml/hr)	H-Isozyme (Units/ml/hr)	M/H Ratio
Control (30)	3.00 ± 0.17	2.07 ± 0.21	1.44 ± 0.29
10 Days treatment (6)	3.29 ± 0.19*	2.11 ± 0.20	1.55 ± 0.18*
20 Days treatment (6)	4.11 ± 0.21**	2.47 ± 0.13	1.78 ± 0.23*
30 Days treatment (6)	5.50±0.24***	2.86 ± 0.25*	1.92 ± 0.17**
40 Days treatment (6)	5.82±0.22***	2.96 ± 0.22*	1.96 ± 0.26**
50 Days treatment (6)	6.28±0.31***	3.01 ± 0.20**	2.08 ± 0.22***

Data presented as mean ± SEM **, *** shows significant at 0.01 and 0.001 levels with the value in control. Numbers within parenthesis denote number of samples.



Clausen (1970) stated that M-isozyme of LDH is responsible for the conversion of pyruvate into lactate and H-isozyme converts lactate into pyruvate. Increased M-isozyme activity in present study causes more conversion of pyruvate into lactate which leads to shifting of aerobic to anaerobic condition (Battellino *et al*, 1971) causing loss of oxygen availability. More accumulation of lactate decrease cellular respiration (Free *et al*, 1969) lead to decline in sperm motility and fertility rate of treated mice.

The present study revealed in conformity with the findings of earlier workers carried out in our laboratory using neem oil (Rani *et al*, 2009; Kumar and Singh, 2011) aqueous extract of *Bougainvillea spectibilis* leaves (Hembrom *et al*, 2013) and *Piper betel* stalk extract (Verma *et al*, 2017). All these extract treatments caused increase in total LDH isozyme activity of treated group of mice semen significantly due to significant increase in M-isozyme activity.

Rani *et al* (2009) reported that increase in M-isozyme activity in the luminal fluid of neem treated female mice is due to estrogenic nature of neem oil. Similarly we found the increase in M-isozyme activity of seminal fluid of papaya seed treated mice may be due to the estrogenic nature of *Carica papaya* seed extract (Lohiya and Goyal, 1992; Pathak *et al*, 2000).

Similar findings were reported by Yakubu *et al* (2007) in which oral administration of *Chromolaena adoratum* leaf extract (200 and 500 mg/kg body weight) for 14 days to male albino rats revealed a significant increase ($p<0.05$) in testicular and serum lactate dehydrogenase activities.

Overall, we found that papaya seed extract has selective and directional influence on M-isozyme of LDH in semen of treated group of mice than control.

CONCLUSION

Thus, it can be concluded that papaya seed treatment alter the quality of seminal plasma significantly by inducing M-isozyme activity of LDH in semen of treated group of mice than the control. The increase in M-isozyme activity of LDH in semen of treated group of mice may leads to anaerobic conditions in the seminal plasma which affect motility and viability of spermatozoa. Thus these alterations in M-isozyme activity of LDH in semen of treated group of mice lead to infertility among treated group of mice than the control.

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Conflict of Interest
The author declares that there is no conflict of interest.

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