Antifertility effects of aqueous leaf extract of *Aegle marmelos* on seminal LDH isozymes in male mice (*Mus musculus*).

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ABSTRACT

After 10 to 50 days of *Aegle marmelos* leaf extract 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 showed slight and insignificant increase. Increase in M-isozyme of LDH caused highly significant (P<0.001) increase in total LDH isozyme activity and M/H ratio in semen of treated group of mice than the control. Thus, it can be concluded that the aqueous leaf extract of *Aegle marmelos* has significant effect on seminal LDH isozyme activity which is an important parameter for analyzing the fertilizing ability of male mice.

Key Words: Antifertility, Isozyme, LDH, Relative mobililty.

INTRODUCTION

Population explosion is great problem in India. It is our great concern to stop such a growing population. Therefore, it is required to search a safe and effective contraceptive agent of plant origin. Various contraceptive agents are available with less assurance to male. *Aegle marmelos* belongs to family rutaecae is a common plant in India. It is commonly known as bael. *Aegle marmelos* is a sacred tree possesses various medicinal properties (Sharma, 1997). Thus it was decided to evaluate effect of aqueous leaf extract of *Aegle marmelos* on seminal LDH isozyme in association with antifertility.

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a tetrameric enzyme. There are five isoenzymes of LDH that are made up from M and H subunits. According to electrophoretic mobility these five possible tetramers (isoenzymes) of LDH are known as LDH_1 (HHHH), LDH_2 (HHHM), LDH_3 (HHMM), LDH_4 (HMMM), and LDH_5 (MMMM). Among these five isoforms of LDH_1 and LDH_2 are categorized as H-isozymes where as LDH_4 and LDH_5 known as M-isozymes of LDH (Chan, 1964).

Mature human testis and spermatozoa possess a unique lactate dehydrogenase named as LDH-C4 (Fadiloglu *et al*, 1998). It is found in the serum of various mammalian and avian species designated as LDH-X by Balanco and Zinkham (1963). The LDH-C4 isozyme is one of the many regulatory mechanism involved in the metabolic process needed for fertility. Synthesis of LDH-C4 is required for active spermatogenesis (Goldberg, 1977). Markert (1971) showed that LDH-C4 deficiency is associated with decreased forward motility and immotile or dead spermatozoa. Duan and Goldberg (2003) reported that Lactate dehydrogenase C4 (LDHC4) is a tissue-specific enzyme that plays an important role in sperm capacitation, acrosome reaction and fertilization. Kumar and Singh (2011) reported that 10 to 30 days of neem oil treatment caused increase in total LDH activity among treated group of mice than the control. Singh *et al* (1995) also reported a new LDH isozyme pattern in mice uterus which is estrogen dependent during early pregnancy and named as LDH-Y. This LDH-Y appears in the mice uterus during pre-implantation period and disappears during implantation and post-implantation period.

MATERIALS AND METHODS

EXPERIMENTAL ANIMAL

60 Swiss albino male mice (*Mus musculus*) of 25 to 30 gm body wt. were included in the investigation. These mice were procured from the animal stalk of University department of Zoology, T.M. Bhagalpur University, Bhagalpur and maintained at uniform animal husbandry conditions of food, light, temperature (12hrs photoperiod 25±2˚C temp.).

PLANT LEAF EXTRACT PREPARATION

Fresh and mature leaves of *Aegle marmelos* were taken from the gardens of Bhagalpur district. The leaves were washed under tap water and dried at room temperature. Dried leaves were grinded with electric blender. 100 gm of *Aegle marmelos* leaf powder were kept in a jar and added 1000 ml distilled water and left it to overnight. The mixture was filtered with filter paper and centrifuged at 1000 rpm for 15 minutes. After centrifugation, the supernatant was taken out from centrifuge tube for administration.

DOSE AND DURATION OF TREATMENT

The total mice were divided into two groups the treated and control. Each group containing 30 mice. The treated groups of mice were fed 0.1 ml (350 mg/kg body wt./day) (Sathyaraj *et al*, 2010) aqueous extract of *Aegle marmelos* leaves for 10, 20, 30, 40 and 50 days of exposure. The control group of mice were fed 0.1 ml of distilled water.

KILLING OF THE ANIMALS

After completion of treatment all mice were sacrificed. The testis of the mice were operated out and kept them in a cleaned watch glass and tinged with 2 ml of normal saline (1 epididymis/ml of normal saline). Each of the cauda were crushed and seminal content were sieved by metallic filter.

RESULTS

In this study, as shown in the result **(Table-1, fig; 1)** the LDH_1 and LDH_2 isozyme activity in *Aegle marmelos* leaf extract treated group of mice increased insignificantly. While the activity of LDH_4 and LDH_5 increased highly significantly (P<0.001) from 30 to 50 days of treatment than the control. Due to significant increase in LDH_4 and LDH_5 activity, total LDH isozyme activity increased highly significantly (P<0.001) in the *Aegle marmelos* leaf extract treated group from 30 to 50 days of treatment than the control.

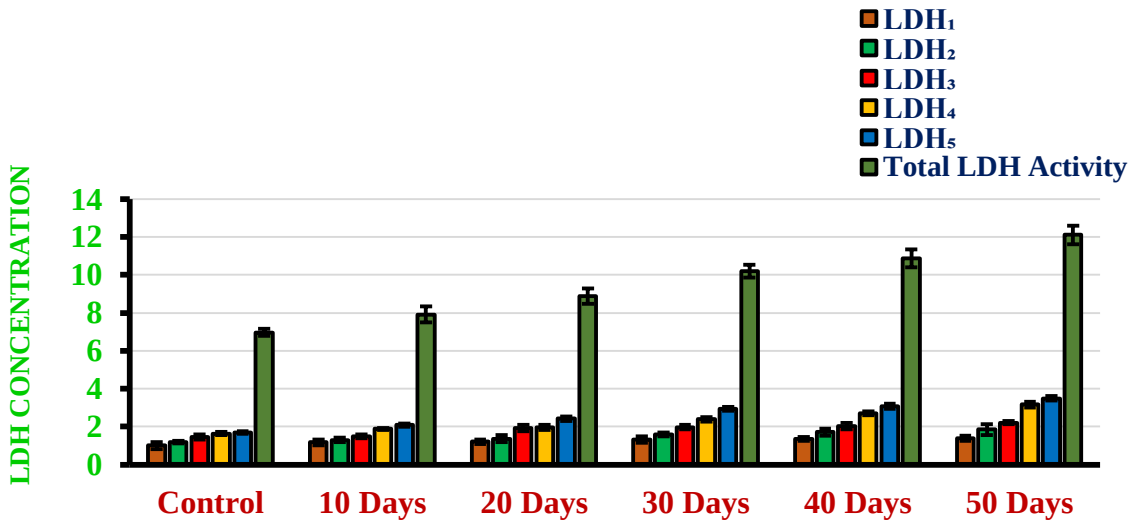
TABLE- 1: Data showing the effect of aqueous leaf extract of *Aegle marmelos* on LDH isozymes activity in semen of mice.

Groups	LDH_1 (U/ml/hr)	LDH_2 (U/ml/hr)	LDH_3 (U/ml/hr)	LDH_4 (U/ml/hr)	LDH_5 (U/ml/hr)	Total LDH Activity (U/ml/hr)
Control (30)	1.01± 0.19	1.18± 0.13	1.46± 0.13	1.63± 0.08	1.69± 0.07	6.97± 0.19
10 Days of Treatment (6)	1.17± 0.15	1.29± 0.12	1.49± 0.10	1.88± 0.06*	2.09± 0.09*	7.92± 0.43*
20 Days of Treatment (6)	1.21± 0.11	1.37± 0.17	1.92± 0.16	1.96± 0.14**	2.42± 0.12**	8.88± 0.40**
30 Days of Treatment (6)	1.32± 0.16	1.59± 0.10	1.97± 0.12	2.39± 0.11***	2.94± 0.10***	10.21± 0.33***
40 Days of Treatment (6)	1.35± 0.09	1.71± 0.18	2.02± 0.17*	2.71± 0.09***	3.08± 0.13***	10.87± 0.47***

50 Days of Treatment (6)	1.39± 0.13	1.85±0.28*	2.21± 0.09*	3.17± 0.15***	3.49± 0.11***	12.11± 0.49***

Data presented as Mean ± SEM *, **, *** shows significant at 0.1, 0.01 and 0.001 levels with the value in control. Numbers within parenthesis denote number of samples.

FIG; 1: HISTOGRAM SHOWING THE EFFECT OF AEGLE MARMELOS LEAF EXTRACT ON CONCENTRATION OF DIFFERENT LDH ISOZYMES IN SEMEN OF MICE



DISCUSSION

LDH isozymes are very important for spermatozoa, as it provides energy for viability, motility, fertilization and other activities (Nale *et al*, 2012). In this study, the LDH_1 and LDH_2 isozyme activity in *Aegle marmelos* leaf extract treated group of mice increased insignificantly. While the activity of LDH_4 and LDH_5 increased highly significantly ($P<0.001$) than the control. Total LDH isozyme activity increased significantly ($P<0.001$) in the *Aegle marmelos* leaf extract treated group.

Kumar *et al* (2011) reported that the administration of neem oil to the male albino mice up to 50 days the LDH_4 and LDH_5 activity increased significantly which leads to increase in total LDH isozyme activity. Rani *et al* (2009) also reported that the neem oil treatment to the rats increases the LDH_4 and LDH_5 activity in luminal fluid of female mice uterus due to estrogenic nature of neem oil.

The increase in total LDH isozyme activity in the semen of neem leaf extract treated group of mice may be due to decreased level of androgen in the seminal plasma which cause anaerobic condition by converting pyruvate to lactate (Aladakatti *et al*, 2011). Kumar and Singh (2011) reported that the increase in total LDH isozyme activity in semen of male albino mice cause antifertility due to lack of oxygen in the seminal plasma which is very important for the aerobic respiration to the spermatozoa. Due to insufficient energy, viability of spermatozoa is affected which results into higher rate of mortality has been seen.

In a study Verma *et al* (2017) reported that the increase in LDH_4 and LDH_5 activity is the main reason of higher activity of total LDH isozyme activity. The increase in total LDH isozyme activity may cause antifertility in male albino mice. Pragya *et al* (2015) also reported the increase in LDH_4 and LDH_5 activity may cause infertility among male albino rats. Duan and Goldgerg (2003) stated in their study that LDH isozyme is an important factor in sperm capacitation and fertilization, because it provides energy to the sperms for various activities. Talluri *et al* (2017) found that the increase in LDH isozyme activity represent the damage in the acrosomal integrity of spermatozoa which may cause infertility among male.

CONCLUSION

From the above study it may be concluded that aqueous extract of *Aegle marmelos* leaf has selective and discretional influence on LDH and LDH in semen of treated group of mice than the control. The significant increase in LDH and LDH in semen of treated group of mice may lead to anaerobic condition due to more conversion of pyruvate into lactate which affect motility, viability and capacitation of spermatozoa that may lead to infertility among the treated group of mice than the control. Thus this study shows that *Aegle marmelos* may be a good herbal contraceptive agent for male which is cheaper safe and effective.

INTEREST OF CONFLICT

The author declares that there is no conflict of interest.

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