



## Antifertility effect of aqueous leaf extract of *Aegle marmelos* (Linn.) on M-isozyme, H-isozyme and M/H ratio in semen of Swiss albino mice

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### ABSTRACT

**Objective:** To evaluate the effects of *Aegle marmelos* leaf extract treatment on seminal LDH isozymes activity in mice. **Methods:** 60 adult male Swiss albino mice weighing about 25 to 30 gram were selected and divided into two groups, control group and treated group. Mice of control groups were fed 0.1 ml of distilled water and treated groups fed 0.1 ml *Aegle marmelos* leaf extract. After 10 to 50 days of treatment mice were sacrificed. Semen samples were collected from cauda epididymis. These samples were filtered, centrifuged and processed for electrophoresis for separation of different LDH isozymes. **Results:** In the *Aegle marmelos* leaf extract treated group of mice M-isozyme of LDH showed significant increase during 10 ( $P<0.01$ ), 20 ( $P<0.01$ ), 30 ( $P<0.001$ ), 40 ( $P<0.001$ ) and 50 ( $P<0.001$ ) days of exposure than the control. H-isozyme of LDH also increased but it is insignificant than the control. Due to increase in M-isozyme, the M/H ratio also increased highly significantly as the duration of exposure ages than the control. The increased level of M-isozyme to LDH in seminal plasma of *Aegle marmelos* leaf extract treated group of mice caused more accumulation of lactate and cellular respiration decreased. This may impair motility of spermatozoa and increased mortality of spermatozoa. Thus, *Aegle marmelos* leaf extract adversely affect fertility of male mice. **Conclusions:** From the study, it can be concluded that the aqueous leaf extract of *Aegle marmelos* has potential effect on seminal LDH isozyme activity which is an important parameter for analyzing the fertilizing ability of male mice.

**Key Words:** Infertility, Seminal fluid, Isozyme, LDH.

### INTRDUCTION

Growing population is a serious problem in India. It is our great concern to stop such growing population. Therefore it is necessary to search a safe and effective contraceptive agent for fertility control. Some plants possess antifertility property such as *Azadirachta indica*, *Bougainvillia spectabilis*, *Ocimum sanctum*, *Carica papaya*, *Alium sativum*, *Momordica charantia*, etc. *Aegle marmelos* belonging to family rutaceae possess various medicinal properties (Sharma, 1997). Sathyaraj *et al* (2010) showed the antifertility property of *Aegle marmelos*.

Lactate dehydrogenase (Lactate: NAD oxidoreductase EC. 1.1.1.27) found in five different forms (Markert, 1968). It catalyzes the conversion of pyruvate into lactate and lactate into pyruvate (Fox *et al*,

1969). The five isozymes of LDH are made up of two sub units H-isozyme and M-isozyme. The M-isozyme is indicates for LDH<sub>4</sub> and LDH<sub>5</sub>. It is responsible for conversion of pyruvate into lactate (Battellino *et al*, 1971). In a study Kumar *et al* (2011) reported that neem oil influences the M-isozyme in the semen of mice. The M-isozyme increased highly significantly after treatment of neem oil from 10 to 50 days of exposure

Therefore it was decided to evaluate antifertility effect of aqueous leaf extract of *Aegle marmelos* on M-isozyme, H-isozyme and M/H ratio in semen of Swiss albino mice.

## MATERIALS AND METHODS

60 adult male Swiss albino mice (*Mus musculus*) 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.

Fresh and mature leaves of *Aegle marmelos* were taken from Bhagalpur district. The leaves were washed and dried at room temperature. Dried leaves were powdered with electric blender. 1000 ml distilled water was added in 100 gm of *Aegle marmelos* leaf powder and left it to whole night. The mixture was filtered and centrifuged at 1000 rpm for 15 minutes. After centrifugation, the supernatant was taken for administration.

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.

After completion of days of exposure, all mice were sacrificed. The testis of the mice were operated out from the testis and tinged with 2 ml of normal saline (1 epididymis/ml of normal saline). After separating cauda epididymis each of the cauda were crushed and seminal content were sieved by metallic filter.

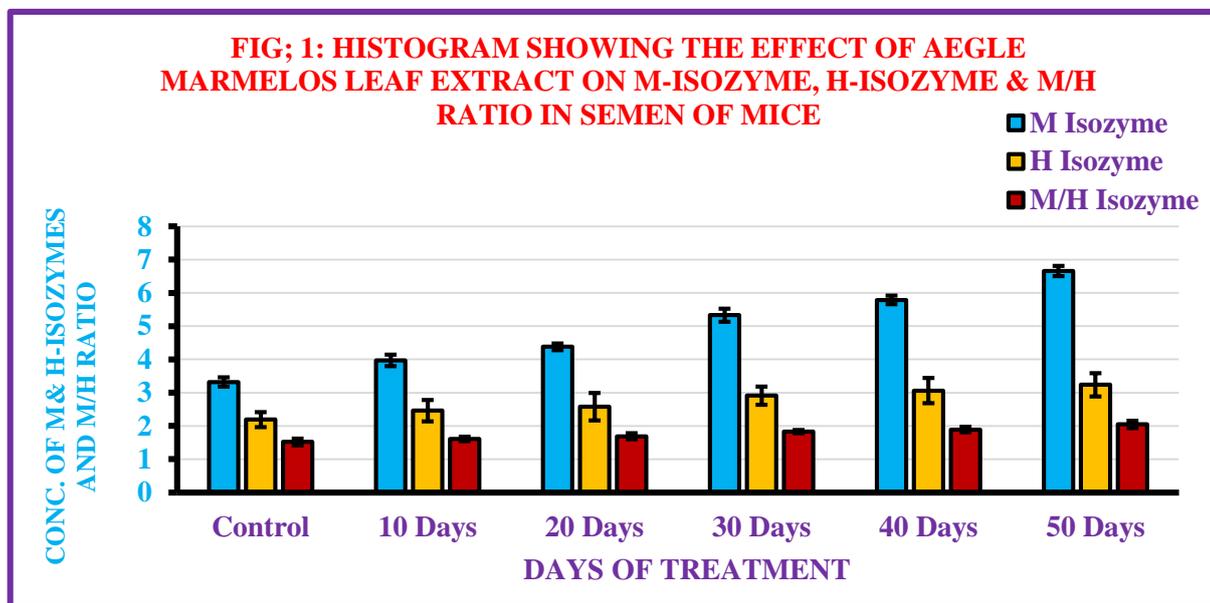
## RESULT

In this study, result shown in the **table-1 and fig; 1**, M-isozyme activity in the semen of *Aegle marmelos* leaf extract treated group of mice increased highly significantly ( $P<0.001$ ) after 30 to 50 days of treatment, while H-isozyme activity increased slightly which is insignificant than the control. Due to significant increase in M-isozyme activity in the semen of *Aegle marmelos* leaf extract treated group of mice M/H ratio also increased highly significantly ( $P<0.001$ ) after 30 to 50 days of treatment.

**TABLE- 1: Data showing effect of aqueous leaf extract of *Aegle marmelos* on M and H-Isozyme in semen of mice**

Groups	M-Isozyme (U/ml/hr)	H-Isozyme (U/ml/hr)	M/H Ratio
Control (30)	3.32± 0.14	2.19± 0.23	1.52± 0.10
10 Days of Treatment (6)	3.97± 0.17**	2.46± 0.32	1.61± 0.07*
20 Days of Treatment (6)	4.38± 0.10**	2.58± 0.41	1.69± 0.09**
30 Days of Treatment (6)	5.33± 0.20***	2.91± 0.27	1.83± 0.05***
40 Days of Treatment (6)	5.79± 0.13***	3.06± 0.38	1.89± 0.08***
50 Days of Treatment (6)	6.66± 0.15***	3.24± 0.35	2.05± 0.11***

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.



## DISCUSSION

LDH isozymes are very important for spermatozoa, as it provides energy for viability, motility, fertilization and other activities (Nale *et al*, 2012). LDH isozymes are specific for spermatocytes, spermatids and spermatozoa. In this study M-isozyme activity in the semen of treated group of mice increased significantly ( $P < 0.001$ ) while, H-isozyme activity increased slightly which is insignificant than the control. The increase in M-isozyme activity leads to increase in M/H ratio in treated group of mice than the control (**Table- 1, Fig; 1**).

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

Higher activity of M-isozymes of LDH in the uterine fluid of female albino mice cause infertility. Increase in M-isozyme resulted into higher conversion of pyruvate into lactate which leads to anaerobic condition in the uterus of mice (Singh, 1994). Kumar and Singh (2011) reported that increase in M-isozyme activity in the seminal fluid of male albino mice cause antifertility due to lack of oxygen in seminal plasma. Due to insufficient energy, viability of spermatozoa is affected which results into higher rate of mortality has been seen.

Increase in M-isozyme of LDH cause anaerobic condition in the uterine fluid for the sperms (Battellino *et al*, 1971). It is also reported that due to anaerobic condition cellular respiration decreases which cause insufficient energy supply for the spermatozoa that ultimately leads to higher mortality rate in spermatozoa. In a study Verma *et al* (2017) reported that due to increase in M-isozyme activity of LDH cause higher activity of total LDH isozymes. Pragyā *et al* (2015) showed that increase in M-isozyme activity seminal plasma may cause infertility among male albino rats Talluri *et al* (2017) found that due to increase in LDH isozyme activity cause damage in the acrosomal integrity of spermatozoa that may leads to infertility among male mice.

## CONCLUSION

From the above study it can be concluded that the aqueous leaf extract of *Aegle marmelos* affects the M-isozyme, H-isozyme and M/H ratio. The increase in M-isozyme activity in seminal fluid of male mice is responsible of more accumulation of lactate that may leads to anaerobic condition in the seminal plasma. The anaerobic condition affect motility, viability and capacitation of spermatozoa which may cause

infertility in male mice. Thus, the study shows that *Aegle marmelos* could be a good, safe and effective herbal contraceptive agent for male.

## **CONFLICT OF INTEREST**

The author declares that there is no conflict of interest.

## **ACKNOWLEDGEMENT**

I am sincerely thankful to Zoology department T.M. Bhagalpur University, Bhagalpur for providing laboratory and library facility.

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