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Antifertility Effects of Aqueous Leaf Stalk Extract of *Piper betel* on Seminal LDH Isozymes in Mice

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ABSTRACT

The aqueous stalk leaf extract of *Piper betel* administered orally at the dose of 0.15 ml (50 mg/kg/BW/day) for 10, 20, 30, 40, and 50 days resulted in significant increase in total activity of seminal LDH isozymes in Male Swiss Albino mice due to increased level of M-isozymes of LDH in treated groups than the control. The increased levels of M-isozymes of LDH in seminal plasma of *Piper betel* leaf stalk treated mice caused more accumulation of lactate and decreased cellular respiration. Possibly this impairs sperm motility and causes higher mortality of spermatozoa in treated groups of mice. Thus, aqueous leaf stalk extract of *Piper betel* adversely affects fertility in mice and showed antifertility effects.

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INTRODUCTION

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide $(NAD)^+$ as hydrogen acceptor. The reaction is reversible and the reaction equilibrium strongly favors the reverse reaction, namely the reduction of pyruvate (P) to lactate (L). (Lott JA et al., 1987; Moss DW et al., 1986; and Beckman Instruments Inc. 1993).

Lactate dehydrogenase occurs in five different forms (Market, 1968) as isozymes and catalyzes conversion of pyruvate into lactate and vice versa. These five LDH isozymes are made up of two subunits, the heart type (H-LDH) and the muscle type (M-LDH) (Cahn *et al*, 1964). LDH_1 and LDH_2 are called H-Isozymes while LDH_4 and LDH_5 are called M-Isozymes. M-Isozyme of LDH is responsible for conversion of pyruvate into lactate (Battellino *et al*. 1971). Kumar & Singh (2011) and Hembrom et al. (2013) had also reported that significant increase in seminal LDH isozymes of treated mice with Neem oil and *Bougainvillea spectabilis* respectively. The present study was investigated to investigate the antifertility effect of aqueous leaf stalk extract of *Piper betel* on seminal LDH isozymes in mice.

MATERIALS AND METHODS

Adult swiss albino mice of 25 and 30 gm body weight were divided into six groups each consisting of six mice. One group was considered as control group while rest were considered as experimental group. All the mice were maintained at uniform animal husbandry condition and provided with normal food and water *ad libitum* for 50 days. The treated groups of mice were fed with aqueous leaf stalk extract of *Piper betel* (0.15 ml) for 10 to 15 days orally with the help of gastric catheter, while control group of mice was fed with same amount of distilled water.

Semen samples from the cauda epididymis were collected from each mice. These samples were filtered, centrifuged and processed for electrophoretic studies. Separation of LDH isozymes was made as per the method of Smith (1976) and staining solution was prepared by the method of Siciliano and Shaw (1976). Quantification of LDH isozymes was made by gel scanner against the known concentration Bovine Serum Albumin. Student's t-test wad applied for statistical evaluation.

RESULT AND DISCUSSION

The Total LDH activity shows significant increase when duration of *Piper betel* leaf stalk extracts exposure increases. After 10 and 20 days, the total LDH activity shows significant increase ($p>0.01$) while after 30, 40 and 50 days record highly significant increase ($p>0.001$) in the total LDH activity among the treated group of mice than the control group (Table 1).

Table 1: Effect of aqueous leaf stalk of *Piper betel* on LDH Isozymes in semen of mice

Groups	LDH_1 (Units/ml/ hr)	LDH_2 (Units/ml/ hr)	LDH_3 (Units/ml/ hr)	LDH_4 (Units/ml/h r)	LDH_5 (Units/ml/h r)	Total LDH activity (Units/ml/h r)
Control (6)	0.84 ±0.02	1.14 ±0.08	1.39 ±0.02	1.61 ±0.04	1.72 ±0.03	6.39 ±0.04
10 day treatment (6)	0.87 ±0.04	1.17 ±0.02	1.48 ±0.02	1.74 ±0.12	1.80 ±0.02	7.05 ±0.22
20 day treatment (6)	0.99 ±0.03	1.26 ±0.04	1.59 ±0.20	1.93 ±0.25	2.24* ±0.12	7.94* ±0.29
30 days treatment (6)	1.24* ±0.20	1.35* ±0.03	1.66* ±0.05	2.56** ±0.34	2.86** ±0.24	9.61** ±0.22
40 days treatment (6)	1.28* ±0.06	1.39* ±0.07	1.56* ±0.11	2.66** ±0.28	2.94** ±0.28	9.74** ±0.04
50 days treatment (6)	1.30** ±0.07	1.42* ±0.02	1.60* ±0.08	2.70** ±0.29	2.98** ±0.28	9.94*** ±0.19

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

Such increase of total LDH activity in Semen of treated mice is due to significant increase in M-isozymes of LDH during 10 to 30 days ($p>0.01$) and highly significant during 40 and 50 Days ($p>0.001$) of *Piper betel* leaf stalk treated mice than the control. Similarly, M/H ratio also exhibit significant increases in *Piper betel* leaf stalk extract treated mice as duration of exposure increases (Table 2).

Table 2: Effects of aqueous leaf stalk extract of *Piper betel* on M-isozymes of LDH and M/H Ratio in semen of mice

Groups	**M-Isozyme (Units/ml/hr)**	**H-Isozyme (Units/ml/hr)**	**M/H ratio**
Control (6)	3.33 ±0.04	1.96 ±0.20	1.70 ±0.08
10 day treatment (6)	3.56 ±0.16	2.07 ±0.08	1.74 ±0.17
20 day treatment (6)	4.16 ±0.09	2.24 ±0.17	1.85 ±0.13
30 days treatment (6)	5.43* ±0.11	2.59* ±0.09	2.14* ±0.16
40 day treatment (6)	5.63* ±0.09	2.62* ±0.12	2.16* ±0.13
50 days treatment (6)	5.70** ±0.06	2.67** ±0.18	2.19** ±0.10

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

As indicated in Table 1 and 2, M-isozymes of LDH increased significantly the seminal plasma of *Piper betel* leaf stalk extract treated mice leading to significant increase in total LDH activity during 10 to 50 days of exposure.

Increased M-isozymes and total LDH activity in the seminal plasma of treated mice cause antifertility effects. Hembrom and Singh (2013) shows antifertility effects of aqueous leaf extract of *Bougainvillea spectabilis* on seminal LDH isozymes in mice. Kumar & Singh (2011) has also reported antifertility effect in neem treated mice with increased M-Isozymes of LDH and total LDH activity in the seminal plasma. Rani *et al*. (2009) also reported selective and directional influence of neem oil on M-isozymes of LDH in the uterine fluid of mice. In women increased M- LDH isozymes in the uterine fluid is one of the cause of infertility (Singh, 1994).Increased M-Isozymes and activity of LDH suggests a shift in the tissue respiration from aerobic to anaerobic condition resulting in more conversion and accumulation of lactate in seminal plasma (Cahn *et al*., 1962). More conversion and accumulation of lactate in the seminal plasma of treated mice may cause decreased cellular respiration (Free *et al*., 1969) than the control, which impaired sperm motility and increased mortality of spermatozoa. This showed that increased M-isozymes of LDH in the seminal plasma of *Piper betel* leaf stalk treated mice possibly impair fertility among them.

Thus, it was clearly shown that aqueous leaf stalk extract of *Piper betel* cause antifertility effects in mice by selective modulation on M-isozymes of LDH which may act as fertility control.

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