



## Research Article

### SELECTIVE AND DIRECTIONAL INFLUENCE OF *BOUGAINVILLEA SPECTABILIS* ON ANODIC ELECTROPHORETIC PROTEINS AND M-ISOZYMES OF LDH IN SEMEN OF MICE IN RELATION TO FERTILITY CONTROL

Anita Raj Hembrom, S. Pragma, V.N. Singh\*

University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, Bihar, India

\*Corresponding Author Email: leoannie83@gmail.com

Article Received on: 27/05/14 Revised on: 21/06/14 Approved for publication: 16/07/14

DOI: 10.7897/2230-8407.0507116

**ABSTRACT**

The present study investigate the effects induced by the administration of the aqueous leaf extract of *Bougainvillea spectabilis* at the doses of 0.1 ml (800mg/kg/BW/day) on male mice for 10 to 50 days. This treatment causes significant increase in the anodic or negatively charged electrophoretic protein concentration in seminal plasma of mice, collected from cauda epididymis during 10, 20, (P<0.1), 30, 40 (P<0.01) & 50 days (P<0.001) of treatment than the control. This significant rise of anodic protein adds more negative charges on sperm surface membrane that inhibits capacitation and fertilizing ability of the sperm. M –isozymes of LDH (LDH<sub>4</sub> and LDH<sub>5</sub>) also shows significant increase during 10 to 30 days (p<0.01) and highly significant during 40 and 50 days (p<0.001) of *B. spectabilis* treated mice than the control. Increased M –Isozymes causes significant increase in the total activity of LDH which suggests a shift in the tissue respiration from aerobic to anaerobic condition resulting more conversion of pyruvate into lactate. As a result there is more accumulation of lactate in the seminal plasma. Accumulated lactate in the seminal plasma of treated mice may cause decreased cellular respiration than the control, which adversely affect the sperm metabolism in the epididymis. It is concluded that the aqueous leaf extract of *Bougainvillea spectabilis* show antifertility effects in male mice by impairing capacitation power of spermatozoa due to rise in anodic protein and by altering sperm metabolism due to change in M- Isozyme pattern and thus helps in fertility regulation.

**Keywords:** *B. spectabilis*, Anodic electrophoretic protein, M-Isozymes of LDH, fertility regulation

**INTRODUCTION**

Population explosion is leading cause of poverty and pollution in developing countries like India. There are several medicinal plants associated with antifertility properties. Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous system of medicine. A large numbers of plant species with antifertility effects have been screened both for men and women<sup>1,2</sup>. *Bougainvillea spectabilis* is the most common evergreen ornamental woody plant belonging to the family Nyctaginaceae. The leaves of this plant are attributed with medicinal properties which include antidiabetic<sup>3,4</sup>, antiviral<sup>5</sup>, antibacterial<sup>6</sup>, insecticidal<sup>7</sup>, hepatoprotective<sup>8</sup> etc. Recently it has been reported that *B. spectabilis* possesses antifertility activity<sup>9,10</sup>. Any alteration in the biochemical parameters like increase in anodic electrophoretic protein concentrations and M-Isozymes of LDH can affect the sperm metabolisms which will interfere with normal sperm production and their function. Increase in anodic protein concentration in uterine luminal fluid of mice during pre and post implantational stages had been caused by administration of neem oil<sup>11,12</sup>. Kumar *et al.*, (2009)<sup>13</sup> had also reported that neem oil shows antifertility effects among male mice by increasing anodic electrophoretic protein concentration and M-isozymes of LDH.

The present investigation has been undertaken to understand the effect of *B. spectabilis* on seminal anodic proteins and M-Isozymes patterns in relation to fertility control.

**MATERIALS AND METHODS**

Adult Swiss albino male mice of 25-30 g bodyweight were divided into six groups each consisting of six mice. One group was considered as control group while rest were considered as experimental. All the experimental as well as

control group of mice were maintained at uniform animal husbandry condition (12h photoperiod, 25±2 °C temperature). Fresh and mature leaves of *Bougainvillea spectabilis* were taken and washed under tap water. 12g of leaves were grinded in 12ml of distilled water. The mixture was filtered with the clean cotton cloth and centrifuged at 500rpm for 10 minutes. After centrifugation, supernatant was diluted up to 50 ml with distilled water and considered as aqueous extract. The experimental groups were fed with 0.1 ml (800mg/kg/BW/Day) aqueous leaf extract of *B. spectabilis* while control group was fed with equal amount of distilled water with the help of gastric catheter.

After feeding, mice were sacrificed by cervical dislocation and both the cauda epididymis were taken into watch glass and tinged with 2ml of normal saline. Then both the cauda epididymis of each male mice were teased and seminal content were sieved by metallic filter to avoid any tissue debris in seminal content. The seminal content was centrifuged and processed for electrophoretic studies. Electrophoretic proteins and LDH isozymes were separated after the methods of Smith (1976)<sup>14</sup> and the staining solution for LDH was prepared after the method of Siciliano and Shaw (1976)<sup>15</sup>. Concentration of protein bands were done by scanning of gels against the known concentration of Bovine Serum Albumin (BSA). Relative mobility (R<sub>m</sub>) of different protein bands were calculated against the movement of marker Bromophenol Blue (BPB). Quantitation of total electrophoretic proteins and LDH –Isozymes were done by gel scanner. Student's t- test was applied for test of significance.

**RESULTS**

The anodic electrophoretic protein concentrations and M-Isozymes increases significantly in the semen of mice treated

with aqueous leaf extract of *Bougainvillea spectabilis* during 10 to 50 days of exposure as shown in Table 1.

**Table 1: Effects of aqueous leaf extract of *Bougainvillea spectabilis* on anodic electrophoretic proteins and M-Isozymes of LDH in seminal plasma**

Groups	Anodic Protein Conc.(mg/ml)	M- Isozymes of LDH (Units/ml/hr)
Control(6)	2.37±0.11	3.31±0.03
10days treatment (6)	2.62±0.16*	3.53±0.19**
20days treatment (6)	2.95±0.18*	4.14±0.15**
30days treatment (6)	3.15±0.06**	5.41±0.11**
40days treatment (6)	3.32±0.10**	5.59±0.03***
50days treatment (6)	3.74±0.08***	5.68±0.04***

Data represented as mean ± SE. Values in parenthesis indicate number of samples.

\*, \*\*, \*\*\* indicate significance with control at 0.1, 0.01 and 0.001 level respectively.

## DISCUSSION

The anodic electrophoretic protein increases significantly in seminal plasma of treated mice after 10, 20 (P<0.1), 30, 40 (P<0.01) and 50 days (P<0.001) of experiment than the control. M-Isozymes of LDH also shows significant increases after 10, 20, 30 (P<0.01), 40 and 50 (P<0.001) days of *B. spectabilis* treatment than the control (Table 1). The significant increase in anodic protein concentration after the treatment of *B. spectabilis* may affect the capacitation power of spermatozoa as these anodic proteins adds more negative charges on sperm surface membrane<sup>16</sup> and have detrimental effects on sperm motility<sup>17</sup> that inhibits the process of fertilization and may be one of the factor causing infertility among the male mice<sup>18</sup>. Rani *et al* (2009a)<sup>12</sup> also reported selective and directional influence of neem oil on anodic electrophoretic proteins and M-isozymes of LDH in the uterine fluid of mice. Earlier Singh (1994)<sup>19</sup> had reported that increased M- LDH Isozymes in the uterine fluid is one of the causes of infertility in women. Increased M –Isozymes caused significant increase in total activity of LDH which suggests a shift in the tissue respiration from aerobic to anaerobic condition resulting more conversion of pyruvate into lactate which accumulates in the seminal plasma<sup>20</sup>. More conversion and accumulation of lactate in the seminal plasma of *B. spectabilis* treated mice may cause decreased cellular respiration<sup>21</sup> than the control, which adversely affect the sperm metabolism in the epididymis<sup>22</sup>. Thus, it can be concluded that aqueous leaf extract of *B. spectabilis* show antifertility effects among Swiss Albino male mice by affecting motility and capacitating power of spermatozoa caused by increased seminal anodic electrophoretic protein concentration and M-Isozymes of LDH in seminal plasma.

## ACKNOWLEDGEMENT

The authors are grateful to UGC, New Delhi for financial assistance during the tenure of this research work.

## REFERENCES

- Ahirwar D. Antifertility activity of *Acacia leucophloea*. Scholars Research Library, Der Pharmacia Letter 2011;3(3): 411-413
- Mwangwa EK. Antifertility effects of ethanolic extract of *Xylopi aethiopia* on male reproductive organ of Wister rats. American Journal of Medicine and Medical Sciences 2012; 2(1): 12-15
- Bates SH, Jones RB, Bailey CJ. Insulin – like effect of pinitol. British Journal of Pharmacology 2000; 130: 1944-1948
- Narayanan CR, Joshi DD, Mujumdar AM, Dhekne V. Pinitol a new anti- diabetic compound from the leaves of *Bougainvillea spectabilis*. Current Science 1987; 56: 139-141

- Narwal S, Balasubrahmanyam A, Lodha ML, Kapoor HC. Purification and properties of antiviral proteins from the leaves *B. xbutiana*. Indian Journal of Biochemistry and Biophysics 2001; 38: 342-347
- Patel RB, Gandhi TP, Chakarvarthy BK, Patel RJ, Pundarikakshudu K, Dhyani HK. Glycosides from *Bougainvillea glabra*. Indian Drugs 1986; 23: 595
- Thangem TS, Kathiresan K. Synergistic effects of insecticides with plant extracts on mosquito larvae, Tropical Biomedicine 1990; 7: 135-1
- Chang W, Lee Y, Lu F, Chiang H. Glycosides from *Bougainvillea glabra*. Anticancer Research 1994; 14: 501-506
- Mishra N, Joshi S, Tandon VL, Munjal A. Evaluation of antifertility potential of aqueous extract of *Bougainvillea spectabilis* leaves in Swiss albino mice. International Journal of Pharmaceutical Sciences and Drug Research 2009; 1(1): 19-23
- Hembrom AR, Pragma S, Kumar J, Singh VN. Effects of aqueous leaf extract of *Bougainvillea spectabilis* on seminal quality of mice. Journal of Advanced Zoology 2011; 32(2): 119-122
- Singh VN, Rani B. Effects of neem oil (*Azadirachta indica*) on luminal protein pattern in mice during pre and post implantational stages. International Journal of Comparative Animal Physiology 2003; 21: 86-89
- Rani B, Singh VN, Kumar J. Selective and directional influence of neem oil on anodic protein and isozyme pattern in uterine fluid of mice in relation to fertility control. Biospectra 2009a; 4: 303-306
- Kumar J, Singh PP, Singh VN. Effects of neem oil (*Azadirachta indica* A. Juss.) on electrophoretic protein pattern in semen of mice. The Bioscan 2009; 4(4): 679-681
- Smith I. Chromatographic and electrophoretic techniques, Vol-II Zone electrophoresis, Williams Heinmann Med. Book Ltd., London, 1976, pp. 185-209
- Siciliano MJ, Shaw CR. Separation and visualization of enzymes in gels. In: Chromatographic and electrophoretic technique Vol. II. (ed. Smith I) William Heinemann Medical Book Limited London, 1976, 185-209
- Singh VN, Singh JN. Total and electrophoretic proteins in luminal fluid of rats. Journal of Biological Research 1988; 8(2): 63-67
- al – Somai N, Vishwanath R, Molan PC, Shannon P. Anionic and Cationic components from protein aggregates in bovine seminal plasma and their effects on sperm motility. Molecular Reproduction and Development 1994; 39(3): 328-336
- Singh VN, Quadros M, Singh JN. Human uterine protein in relation to infertility. Hormones and Metabolic Research 1993; 25: 231-233
- Singh VN. Lactate dehydrogenase isozymes in uterine fluid of infertile women. Hormones and Metabolic Research 1994; 26: 250-252
- Chan RD, Kaplan NO, Levine L, Zwilling E. Nature and development of subunits. Science 1962; 136: 962
- Free MJ, Massie ED, Vandemark ML. Glucose metabolism by the cryptorchid rat testes. Biology of Reproduction 1969; 1: 354-366
- Anitha P, Indira M. Impact of feeding ethanolic extract of root bark of *Cananga odorata* (Lam.) on reproductive functions in male rats. International Journal of Experimental Biology 2006; 44: 976-980

## Cite this article as:

Anita Raj Hembrom, S. Pragma, V.N. Singh. Selective and directional influence of *Bougainvillea spectabilis* on anodic electrophoretic proteins and M-isozymes of LDH in semen of mice in relation to fertility control. Int. Res. J. Pharm. 2014; 5(7):576-577 <http://dx.doi.org/10.7897/2230-8407.0507116>