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Fertility regulation of male mice by selective and directional influence of aqueous leaf extract of *Ocimum sanctum* L. on anodic electrophoretic proteins and m-isozymes of LDH in the semen of mice

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ABSTRACT

The present study investigates the effects induced by the administration of the aqueous leaf extract of Ocimum sanctum at the doses of 0.1 ml (250mg/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 (LDH4 and LDH5) also shows significant increase during 10 to 30 days (p<0.01) and highly significant during 40 and 50 days (p<0.001) of Ocimum sanctum 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. Therefore, it is concluded that the aqueous leaf extract of Ocimum sanctum 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: Ocimum sanctum, Anodic electrophoretic protein, M-Isozymes of LDH, fertility regulation

INTRODUCTION

Population control is a major problem for the developing countries like India. There are various contraceptive measures available for fertility control in both male as well as female. However, due to their side effects and growing contradictions they couse reproductive morbidity and thus affect reproductive health as well as reproductive performance of the individuals. 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 medicine1,2. *Ocimum sanctum* is the most common, effective, indigenous, familiar medicinal plant belonging to the family Labiatae, attributed with medicinal properties which include antibacterial (Sharma, 2010; Kumar et al. 2011b), antifungal (AMamdi *et al.,* 2010), antiviral (Deepthi *et al.,* 2007), immuno modulator (Rathore *et al.,* 2012a), antioxidant (Madhuri and Pandey, 2010) etc. Recently it has been reported that *Ocimum* sanctum also possesses antifertility activity (Pragya *et al.,* 2012; 2013). 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. There is an 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 (Singh and Rani, 2003, Rani *et al.,* 2009a). Kumar *et al.,* (2009) 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 aqueous leaf extract of *Ocimum sanctum* on seminal anodic proteins and M-Isozymes patterns in relation to fertility control.

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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 (12 h photoperiod, 25±2 °C temperature). Fresh and mature leaves of *Ocimum sanctum* were taken and washed under tap water. 10g of leaves were grinded in 10ml of distilled water. The mixture was filtered with the clean cotton cloth and centrifuged at 5000 rpm for 10 minutes. After centrifugation, supernatant was diluted up to 30 ml with distilled water and considered as aqueous extract.

The experimental groups were fed with 0.1 ml (250 mg/kg/BW/Day) aqueous leaf extract of *Ocimum sanctum* while the 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 was 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) and the staining solution for LDH was prepared after the method of Siciliano and Shaw (1976). Concentration of protein bands were done by scanning of gels against the known concentration of Bovine Serum Albumin (BSA). Relative mobility (Rm) 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 *Ocimum sanctum* during 10 to 50 days of exposure as shown in Table 1 and the fig. given below showing the graphical presentation of effect of aqueous leaf extract of *Ocimum* sanctum L. on M isozyme of LDH in mice.

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

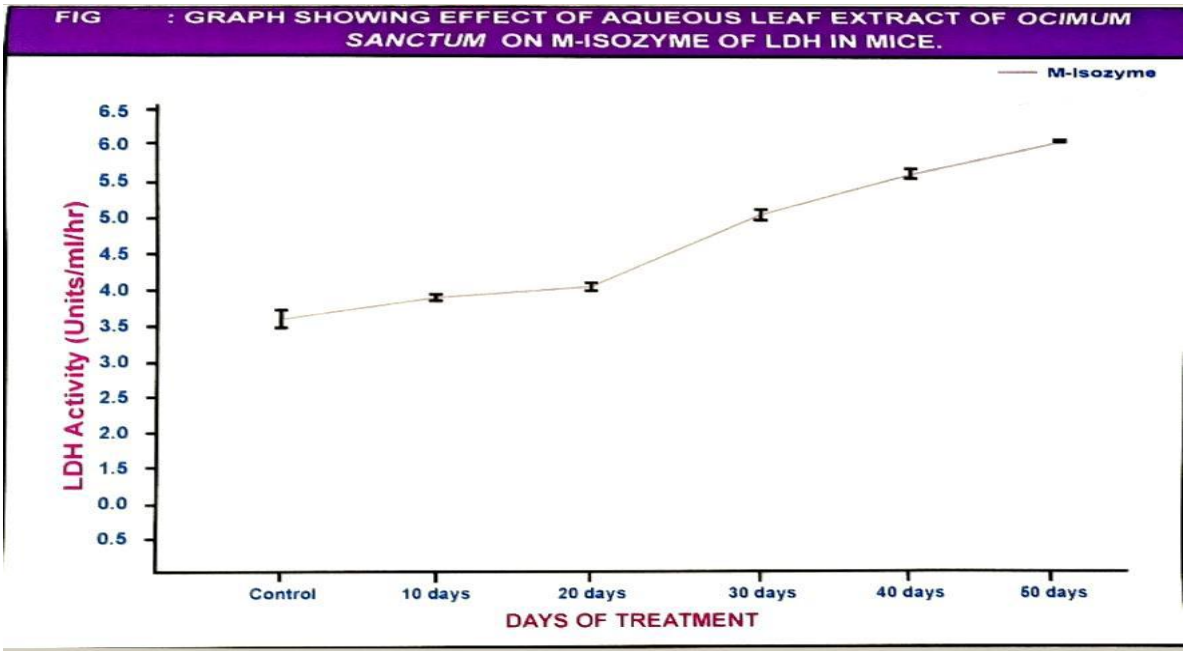
	Anodic Protein Conc.(mg/ml)	M- Isozymes of LDH(Units/ml/hr)
Control (6)	2.53±0.04	3.67±0.22
10days treatment (6)	2.79±0.02*	3.81±0.08*
20days treatment (6)	3.13±0.09**	4.02±0.10**
30days treatment (6)	3.35±0.06***	5.07±0.13***
40days treatment (6)	3.65±0.08***	5.65±0.12***
50days treatment (6)	4.01±0.10***	6.04±0.03

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($P<0.1$), 20($P<0.01$), 30, 40 and 50 days ($P<0.001$) of experiment than the control. M-Isozymes of LDH also shows significant increases after 10($P<0.1$), 20($P<0.01$), 30, 40 and 50 days ($P<0.001$) days treatment of *Ocimum sanctum* than the control (Table 1). The significant increase in anodic protein concentration after the treatment of *Ocimum sanctum* may affect the capacitation power of spermatozoa as these anodic proteins adds more negative charges on sperm surface membrane (Singh and Singh, 1988) and have detrimental effects on sperm motility (AL-Somani et al. 1994) that inhibits the process of fertilization and may be one of the factors causing infertility among the male mice (Singh *et al.* 1993). Rani et al (2009a) 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) 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 (Chan *et al*. 1962). More conversion and accumulation of lactate in the seminal plasma of *Ocimum sanctum* treated mice may cause decreased cellular respiration (Free *et al*. 1969) than the control, which adversely affect the sperm metabolism in the epididymis (Anitha *et al*. 2006).



Thus, it can be concluded that aqueous leaf extract of *Ocimum sanctum* 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.

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