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SELECTIVE AND DIRECTIONAL INFLUENCE OF PIPER BETEL LEAF STALK ON ANODIC ELECTROPHORETIC PROTEINS AND M-ISOZYMES OF LDH IN SEMEN OF MICE IN RELATION TO FERTILITY CONTROL

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ABSTRACT

The present study investigate the effects induced by the administration of the aqueous leaf stalk extract of Piper betel at the doses of 0.15 ml (50mg/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 Piper betel leaf stalk 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 stalk extract of Piper betel show antifertility effects in male mice by impairing capacitating 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: *Piper Betel Leaf Stalk, 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 man and women[1, 2]. Piper betel leaf stalk (Faimly Piperaceae) is commonly known as "Pan". The aqueous extract of Piper betel leaf stalk are attributed with medicinal properties which include anti mutagenic[3], anti- tumor[4], anti bacterial[5], anti-oxidant[6], etc. Recently it has been reported that Piper betel leaf stalk also possesses antifertility activity[7]. 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 [9]. Kumar et al., (2009)[10] 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 *Piper betel* leaf stalk on seminal anodic proteins and M-Isozymes patterns in relation to fertility control.

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Materials and Methods

Adult (age 12-14 weeks) Swiss albino mice weighting 25 to 30g were selected for the investigation. Mice will be maintained under hygienic condition in well ventilated room with 10 hour photoperiod (7am to 5pm) along with 25±2˚C temperature. All animals will be fed twice with bread, dalia, green vegetable, milk, and supplemented with germinated grown seed along with Tap water ad libitum. Male mice were caged in separate polypropylene cages. Fresh mature leaf stalk of Piper betel will be taken. This leaf stalk were washed under tap water and dried. For making aqueous extract of leaf stalk of betel, 100mg of stalk were dissolved in 100ml of distilled water. The dose of male mice will be provided at the rate of 50mg of leaf stalk of betel aqueous extract per kg body weight. The experimental groups of mice were with 0.15ml (50mg/kg/BW/day) aqueous extract of betel leaf stalk orally by gastric catheter. After feeding, mice were sacrificed by cervical dislocation and both the cauda epididymis were taken into watch glass and tinged with 2 ml 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)[11] and the staining solution for LDH was prepared after the method of Siciliano and Shaw (1976)[12].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 stalk extract of Piper betel during 10 to 50 days of exposure as shown in **Table 1**.

Table 1: Effects of Aqueous Leaf Stalk Extract of Piper Betel 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)	2.35±0.07	3.33±0.04
10days treatment (6)	2.69±0.11	3.56±0.16
20days treatment (6)	2.83±0.09*	4.16±0.09
30days treatment (6)	3.18±0.08**	5.43±0.11*
40days treatment (6)	3.39±0.10**	5.63±0.09*
50days treatment (6)	3.75±0.08***	5.70±0.06**

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($P<0.1$), 40($P<0.1$) and 50 ($P<0.01$) days of Piper betel leaf stalk treatment than the control (Table 1). The significant increase in anodic protein concentration after the treatment of Piper betel leaf stalk may affect the capacitation power of spermatozoa as these anodic proteins adds more negative charges on sperm surface membrane[13] and has detrimental effects on sperm motility[14] that inhibits the process of fertilization and may be one of the factor causing infertility among the male mice. Rani et. Al. (2009a)[15] 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)[16] 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. More conversion and accumulation of lactate in the seminal plasma of Piper betel leaf stalk treated mice may cause decreased cellular respiration than the control, which adversely affect the sperm metabolism in the epididymis. Thus, it can be concluded that aqueous leaf extract of Piper betel leaf stalk 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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