



Effects of *Piper Betel* Aqueous Extract on Electrophoretic Protein in Semen of Male Mice

KEYWORDS

Piper betel leaf stalk, Anodic, Electrophoretic proteins, Fertility regulation.

AARTI VERMA*

Department of zoology, T.M. Bhagalpur University, Bhagalpur (Bihar)

V.N.SINGH

Department of zoology, T.M. Bhagalpur University, Bhagalpur (Bihar)

ABSTRACT

The present study shows that the effects of *Piper betel* leaf stalk aqueous extract was administered orally at the dosage of 0.15ml(50mg/kg/BW/day) on male mice for 50 days. This treatment causes significant increase in total electrophoretic protein concentration in seminal plasma of mice collected from cauda epididymis during 10, 20 ($P<0.01$), 30, 40 and 50 ($P<0.001$) of treatment than control. Significant increase in electrophoretic protein concentration causes increase in viscosity of seminal plasma of *Piper betel* leaf stalk treated mice that result into low motility and high mortality rate of spermatozoa. *Piper betel* leaf stalk treated mice shows rise in anodic protein concentration which adds more negative charges on sperm surface membrane that inhibits capacitation and fertilizing capability of the sperm. This suggests that *Piper betel* leaf stalk shows antifertility effects in male mice by increasing viscosity of semen plasma causing lower motility and also impair capacitation of spermatozoa, thus helps in fertility control.

INTRODUCTION

The population is rising tremendously this may affect drastically the economic growth of India and other developing country. Fertility regulation with plant and plant preparations has been reported in the ancient literature of indigenous system of medicine. In our country as well as in the world there are several medicinal plants associated with antifertility properties.(Madhumitha G,2009; Mwangwa et al.,2012; Hiremath SP,1999; and Ahirwar, 2011). *Piper betel* leaf stalk (Family Piperaceae) is commonly known as "Pan". The aqueous extract of betel leaves are attributed with medicinal properties which include anti-mutagenic (Amonkar et al.,1989) , anti-tumor (Bhide et al.,1991) anti-bacterial (Ramji et al.,2002) anti-oxidant (Choydhury et al.,2002) etc. Recently it has been reported that *Piper betel* leaf stalk also possesses antifertility activity (Adhikary et al.,1998 and Verma et al.,2015). 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 et al.,2003). Kumar et al. (2009) had also reported that neem oil shows antifertility effects among male mice by increasing protein concentration. and Hembronm et al.(2013) had also reported that *B.spectabilis* shows antifertility effects among male mice by increasing protein concentration. The present study to investigate to understand the effect of *Piper betel* leaf stalk on seminal protein pattern in reation to fertility control.

MATERIAL 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 $25\pm 2^{\circ}\text{C}$ 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. These leaf stalk were washed under tap water and dried. For making aqueous extract of leaf stalk of be-

tel, 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 were separated after the methods of Smith (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 band was concluded against the movement of marker Bromophenol Blue (BPB). Quantitation of anodic, cathodic and total electrophoretic proteins was done by gel scanner.Student's t-test was applied for test of significance.

RESULTS

As shown in Table 1 the total electrophoretic concentration and anodic protein concentration increases significantly in the semen of mice treated with aqueous leaf extract of *Piper betel* leaf stalks during 10 to 50 days. However, cathodic protein concentration shows no significant change in the semen of *Piper betel* leaf stalk treated group of mice than the control. This clearly indicates that significant increase in total electrophoretic protein concentration in semen of treated mice than control was due to significant increase in anodic protein concentration. The electrophoretic protein increases significantly in seminal plasma of treated mice after 10,20($p<0.01$),30,40days($p<0.001$)of experiment than control (Table.1).This rise in total electrophoretic seminal protein is due to significant increase in anodic protein concentration during 10, 20, $(p<0.1)$,30 ($p<0.01$),40 and 50 days ($p<0.001$) of exposure than control group.

Table. 1 Effects of aqueous leaf stalk extract of Piper betel on electrophoretic proteins in seminal proteins.

Groups	Anodic Protein Conc. (mg/ml)	Cathodic Protein Conc. (mg/ml)	Total Electrophoretic Protein (mg/ml)
Control (6)	2.35±0.07	2.43±0.10	4.78±0.06
10 days treatment (6)			5.22±0.07
20 days treatment (6)	2.69±0.11	2.53±0.09	5.44±0.04**
30 days treatment (6)	2.83±0.09*	2.61±0.07	5.82±0.11***
40 days treatment (6)	3.18±0.08**	2.64±0.06	6.13±0.09***
50 days treatment (6)	3.39±0.10**	2.74±0.05	6.64±0.08***
50 days treatment (6)	3.75±0.08***	2.89±0.06	

Values are mean ± S.E. Values in parenthesis indicate number of samples. *, **, *** indicate significance with control at 0.1, 0.01 and 0.001 level respectively.

DISCUSSION

Significant increase in total electrophoretic protein concentration in seminal plasma of Piper betel leaf stalk treated mice increased due to significant increase in anodic protein concentration (kumar et al., 2009). The Piper betel leaf stalk treated mice shows elevated protein content suggests that accumulation of proteins, which were not utilized for spermatogenesis in the form of proteins or in the form of enzymes (Changamma., 2013). As the result the plasma protein that selectively alters the protein secretions (Brooks.,1983; El-Kashoury et al.,2010) the viscosity of seminal plasma may increase that makes the seminal plasma less conductive for motility and survival of spermatozoa thus causing infertility among them. After the treatment of Piper betel leaf stalk the significant increase in anodic protein concentration may affect the capacitation of spermatozoa as these anodic proteins adds more negative charges on sperm surface membrane (Singh et al.,1988) and have detrimental effects on sperm motility that inhibits fertilization process. Significant increase in total electrophoretic proteins in the seminal plasma of Piper betel leaf stalk treated mice is mainly due to significant increase in anodic protein concentration which adds negative charge on sperm surface membrane thus influencing fertilizing capacity of spermatozoa that may be one of the factors causing infertility (Singh et al.,1993) among them.

Thus, it can be concluded that aqueous leaf stalk extract of Piper betel showed antifertility effects among Swiss Albino male mice by increasing seminal protein concentration and viscosity of semen which affecting motility and capacitation power of spermatozoa and helps in fertility control.

REFERENCES

- Ahiwar, D. (2011). Antifertility activity of *Acacia leucophloea*, Scholars Research Library, Der Pharm. Letter,3 (3):411-413.
- Amonkar AJ, padma PR, Bhide S, Protective effect of hydroxychavicol, a phenolic component of Piper betel leaf against the tobacco specific carcinogen Mutation research Fundamental and molecular mechanism of mutagenesis,210, 1989, 249-253.
- Adhikari P, Chowdhury D, Beneerji J, Chatterjee A, Antifertility effects of crude alcoholic extract of Piper Betel Stalk, Indian journal of Physiology & Allied Sciences, 52, 1998, 22-27.

- Bhide SV, Zariwala MB, Amonkar AJ, Ajuine MA, Chemopreventive efficiency of Piper betel leaf extract against benzo(a) pyrene induce for stomach tumour in mice, Journal of Ethenopharmacology, 34, 1991, 207-13.
- Brooks DE. Effect of Androgen on protein synthesis and secretion in various regions of the rat epididymis, as analysed by two dimensional gel electrophoresis. Mol.Cell Endocrinol. 1983; 29: 255-270.
- Choudhury D, Kale RK, Antioxidant and nontoxic properties of Piper betel leaf extracts: In vitro and In vivo studies, Phytother. Res., 16, 2002, 461-466.
- Chanagamma, C.(2013).Effect of betel leaf stalk extraction on testicular metabolism in albino rats.I.J.P.R.D.,5(1):501-506.
- El-Kashoury AA, Tag El-Din HA.Chlorpyrifos (From different sources): Effect on testicular biochemistry of male albino rats. J Am Sci.2010; 252-261.
- Hembrom, A.R.; Shail, P.; and Singh, V.N. (2013).Effects of Aqueous Leaf Extract of *Bougainvillea Spectabilis* on Electrophoretic Protein Pattern in Semen of Mice. National Journal of Life Science, Vol. 10(1) 83-84
- Hiremath SP, Rudresh K, Badani S, Patil SB, Patil SR: Post-coital antifertility of *Aealypha indiac* L. J Ethnopharmacol 1999; 67: 226-8.
- Kumar, J.; Singh, P.P and Singh, V.N. (2009). Effects of neem oil (*Azadirachta indica* A.Juss) on electrophoretic protein pattern in semen of mice. The Bioscan, 4 (4):679-681.
- Madhumitha G, Saral AM: Free radical scavenging assay of the vetia *neriifolia* leaf extracts. Asian Journal of chemistry. 2009; 21: 2468-2472;
- Mwangwa, E.K. (2012).Antifertility effects of ethanolic extract of *Xylopi-aaethiopia* on male reproductive organ of Wister rats. American Journal of Medicine and Medical Sci., 21(1): 12-15
- Ramji N, Iyer R, Chandrasekaran S, Phenolic antibacterial from Piper betel in the prevention of halitosis, J. Ethonpharmacology, 83, 2002, 149-152.
- Singh V. N. and Rani B. (2003). Effects of neem oil (*Azadirachta indica*) on luminal protein pattern in mice during pre and post implantation stages. Ind.J.Comp. Anim.Physio., 21: 86-89
- Singh V.N. and Singh, J.N. (1988). Total and electrophoretic proteins in luminal fluid of rats, J.Biol.Res., 8(2): 63-67.
- Singh V.N.; Quadros M. and Singh, J.N. (1993). Human uterine protein in relation to infertility. Horm. Metab. Res., 25: 231-233.
- Smith, I. (1976). Chromatographic and electrophoretic techniques, Vol-II Zone electrophoresis. Williams Heinmann Med. Book Ltd., London,pp. 185-209.
- Verma, A.; Hembrom,A.R.; Shail,P.; and Singh,V.N. (2011 to 2015). Antifertility Effect of Aqueous Extract of Piper Betel Leaf Stalk on Seminal Quality of Swiss Albino Male Mice. Int.J.Pharm.Sci.Rev.Res.,31 (2) Article No.19,116-118.