

## Research Article



## Antifertility Effect of Aqueous Extract of Piper betel Leaf Stalk on Seminal Quality of Swiss Albino Male Mice

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### ABSTRACT

The present study was designed to study the impact of Piper betel leaf stalk extract on sperm count, pH, motility, and mortality on semen of Swiss albino male mice. The betel leaf stalk aqueous extracts was administered orally at the dosage of 0.15ml (50mg/kg/body weight) for 10, 20, 30, 40, and 50 days. The investigation was carried out on seminal parameters analysis. Treatment of aqueous extract of Piper Betel Leaf Stalk caused significant decrease in sperm count during 20 (P<0.01), 30 (P<0.01), 40 (P<0.001) and 50 (P<0.001) days treatment than the control. Motility of spermatozoa also decrease in treated mice significantly after 20 (P<0.01), 30 (P<0.001), 40 (P<0.001) and 50 (P<0.001) days treatment than the control. The pH of seminal plasma also decline significantly in treated male mice after exposure during 30 (P<0.01), 40 (P<0.001) and 50 days (P<0.001) than the control. However mortality of the spermatozoa increases significantly and rapidly in treated mice during 20 (P<0.01), 30 (P<0.001), 40 (P<0.001) and 50(P<0.001) days than the control. Such significant alteration suggest that aqueous extract of Piper betel leaf stalk produced adverse effects in seminal parameters and thus cause infertility among the treated groups of mice.

**Keywords:** Antifertility, Piper betel leaf stalk, Seminal parameters.

### INTRODUCTION

Population explosion is the main problem among developing countries like India. There are various contraceptive methods in practice for population control. Various methods are adopted among female to control fertility and spacing of child birth. However, among male very little attempt is made to control fertility with few exception. India has vast resources of natural products and people have been using many of the medicinal plants for inducing abortion and permanent sterility.<sup>1</sup> A large number of herbal drugs are used to control fertilization with considerable success.

However, the search for an orally active, safe and effective plant preparation or its compounds is yet to be needed for fertility regulation due to incomplete inhibition of fertility or side effects due to synthetic antifertility compounds. One such plant, Piper betel leaf stalk (Family Piperaceae) is commonly known as "Pan". It is extensively grown in Sri Lanka, India, Thailand, Taiwan & other Southeast Asian countries. The leaves are pungent, bitter and show various medicinal properties. The betel leaf (*Tambula patrabrint*) is extensively cultivated in warm moist parts of India for its leaves. The antifertility properties of the betel plant were studied in both male and female rats.<sup>2</sup> It was suggested that the contraceptive effect of the extract of leaf stalk of piper betel is mainly on the maturation process of spermatozoa in epididymis without influencing hormonal profiles. Withdrawal of the Piper betel leaf stalk extracts dose result in restoration of fertility after 60 days.<sup>3</sup> The aqueous extract of betel leaves also possess activities like anti-mutagenic<sup>4</sup>, anti-tumor<sup>5</sup>, anti-bacterial<sup>6</sup>, anti-microbial<sup>7</sup>, anti-oxidant<sup>8</sup> etc. Adhikary *et al.* (1998)<sup>9</sup> had

reported antifertility activity of Piper betel leaf stalk. Recently, it was reported that an aqueous extract of Piper betel leaves impairs masculine sexual behavior of rats when given orally. This effect was potent and reversible<sup>10</sup>. The reduction in the caudal epididymal sperm count, sperm motility as well as seminal pH and increase in sperm mortality clearly indicates that piper betel leaf stalk extract can affect one or more aspects of spermatogenesis as well as spermiogenesis.

Present study is undertaken to understand the effects of Piper betel leaf stalk on seminal profile of mice for fertility control.

### MATERIALS AND METHODS

#### Collection of Plant material

The fresh mature stalks of Piper betel were washed under tap water and dried in open air. The mature leaf stalks of Piper betel were taken approximately 1mg in weight for this experiment.

#### Preparation of plant extract

The mature leaf stalks of Piper betel were taken for making aqueous extract. Mature leaf stalks (1mg) and distilled water (100ml) were grind and mix properly, then the crude leaf stalk aqueous extract was filtered with clean cotton cloth and this aqueous extract of betel leaf stalk is ready for the use.

#### Experimental animal

Healthy adult Swiss albino male mice weighting 25 to 30g body weight were taken from University Department of Zoology, T.M.B. University, Bhagalpur. They were divided into six groups each consisting of six mice. All



experimental mice were maintained under hygienic condition in well ventilated room in polypropylene cages, with 10 hour photoperiod (7am to 5pm) along with  $25\pm 2^{\circ}\text{C}$  temperature. One group was considered as control group while other were considered as experimental. All the experimental as well as control groups were fed twice with bread, dalia, green vegetable, milk, and supplemented with germinated grown seed along with tap water *ad libitum*.

### Administration of extract

Control group of mice were fed with 0.15ml of distilled water orally with gastric catheter and experimental groups were fed with 0.15ml of aqueous extract of Piper betel leaf stalk aqueous extract for 10, 20, 30, 40, and 50 days.

### Experimental design

After the completion of each respective days of treatment control and treated mice were sacrificed by cervical

dislocation and the cauda epididymis were exposed and tinged in 2ml of normal saline. Seminal content were sieved by metallic filter to avoid any tissue debris in seminal content. Sperm count was done after the method of Eliasson (1975)<sup>11</sup> while motility of spermatozoa was observed after the method of Tijee and Oentoeng (1968).<sup>12</sup> pH of seminal plasma was measured with the help of pH indicator paper, which was procured from Merck Limited, Worli Mumbai.

### RESULTS

Among the Piper Betel Leaf Stalk aqueous extract treated group of male mice shows significant decrease in sperm count ( $P<0.001$ ), sperm motility ( $P<0.001$ ) and seminal pH ( $P<0.001$ ) while mortality rate of spermatozoa ( $P<0.001$ ) significantly increases after the exposure. As indicated in Table 1 sperm counts, motility and seminal pH decrease significantly during 10 to 50 days treatment. However mortality of spermatozoa increases significantly during the treatment than the control.

**Table 1:** Effect of Aqueous Extract of Piper Betel Leaf Stalk on Seminal Quality of Mice

Groups	Sperm Counts ( $\times 10^4/\text{ml}$ )	Sperm Motility (in percent)	Sperm Mortality (in percent)	Seminal pH
Control (6)	218.50 $\pm$ 4.44	82.50 $\pm$ 1.11	17.50 $\pm$ 1.16	7.36 $\pm$ 0.08
10 days treatment (6)	194.33 $\pm$ 2.55	66.83 $\pm$ 1.49	33.33 $\pm$ 1.05	6.75 $\pm$ 0.47
20 days treatment (6)	174.83** $\pm$ 2.42	46.66** $\pm$ 1.25	44.16** $\pm$ 1.21	6.08 $\pm$ 0.45
30 days treatment (6)	125.50*** $\pm$ 2.04	37.16*** $\pm$ 1.23	62.83*** $\pm$ 1.45	5.62** $\pm$ 0.42
40 days treatment (6)	85.83*** $\pm$ 1.69	26.66*** $\pm$ 0.94	71.16*** $\pm$ 1.54	5.14*** $\pm$ 0.33
50 days treatment (6)	62.50*** $\pm$ 1.44	16.83*** $\pm$ 0.75	83.33*** $\pm$ 1.63	4.61*** $\pm$ 0.39

Data presented as Mean  $\pm$  SEM, \*\*, \*\*\*, shows significance at 0.01 and 0.001 levels with the value in control. Numbers within parenthesis denote number of sample.

### DISCUSSION

The present study shows that sperm count decreases significantly in Piper betel leaf stalk treated mice than the control. Reduction in sperm counts of treated mice may be due to altered androgen synthesis which interfere spermatogenic process.<sup>13</sup> The lower titres of androgen synthesis among treated groups of male mice possibly resulted in the reduction of fertility rate. Piper betel leaf stalk extract causes significant reduction in testosterone level.<sup>14</sup> Thus it can be concluded that this treatment may cause reduction in synthesis of androgen level which impair the spermatogenesis among the treated group of male mice. Such significant reduction in sperm count cause sterility among the treated groups of mice than the control.

As indicated in Table 1, sperm motility and seminal pH also decreases significantly in Piper betel leaf stalk treated male mice than the control, which may be due to the effects of this plant extract. Inhibition of motility of human spermatozoa *in vitro* by the use of Piper betel leaf stalk extract was reported by Ratansooriya *et al.*, (1990).<sup>15</sup> Amino butric acid which is abundant in Piper betel leaves was reported to impair human sperm motility *in vitro*.<sup>16</sup>

Decrease in sperm motility suggests the alteration of sperm maturation process in the epididymis which depends on the androgen level.

Decreased seminal pH also leads to decrease in sperm motility as sperms are fragile at low pH in the treated groups of mice. As result, lower pH of seminal plasma leads to significant increases in mortality rate of spermatozoa in treated groups of male mice than control. Piperine is the major pungent substance present in these plants. Piperine could damage the epididymal environment and sperm function, and thus increases sperm mortality.<sup>17</sup>

Thus, it can be concluded that this investigation shows decreased sperm count, lower motility, low seminal pH and higher mortality of spermatozoa which adversely affect fertility in treated groups of male mice, as these parameters are very important in evaluating the fertility status of the male mice. This study suggests that Piper betel leaf stalk alters seminal quality of male mice and thus shows antifertility effects among the treated groups than the control.



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