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PIPER BETLE LEAVES REVERSIBLY INHIBITS FERTILITY OF MALE RATS

W. D. Ratnasooriya and G. A. S. Premakumara
Department of Zoology
University of Colombo
3, Sri Lanka

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Abstract

This study was undertaken to evaluate the effects of a water extract of mature leaves of Pital of betle on libido and fertility of male rats. The dose and treatment regimen used has been previously shown to inhibit male sexual behaviour of rats (1 mL of crude extract, three times on a single day, given orally). The libido and fertility were assessed on day of treatment and days 3, 7, 14, 28, or 35 post-treatment by pairing each treated male overnight with a prooestins female rat. The results showed that the extract was devoid of overt signs of toxicity but inhibited libido on the day of treatment and impaired fertility (in terms of quantal pregnancy, number of uterine implants and implantation index) on the day of treatment and on days 3, 7 and 28 post- treatment. The antifertility effec was accompanied with moderate to sever oligozoospermia and a profound enhancement in pre-implantation losses. It is likely that oligozoospermia resulted from ejaculatory disfunction and pre-implantation loss via disruption of fertilizing potential of sperm. On the other hand , the anti-libido effect may have resulted from enhance clearance of androgens and/or altered binding to androgen binding protein.

Key words : *Piper betle* leaves, male fertility, libido, oligozoospermia, pre-implantation loss, fertilizing potential of sperm.

1. Introduction

Recently, we showed that an aqueous extract of *Piper betle* (L) leaves impairs masculine sexual behaviour of rats when given orally (Ratnasooriya and Premkumara, 1995). This effect was potent and reversible(Ratnasooriya and Premakumara. 1995). However, in that study the effects of the extract on fertility was not evaluated.

The objective of the present study was to evaluate the effects of the same water extract of *P. betle* leaves on fertility of male rats using the same treatment regimen which has been shown to inhibit masculine sexual behaviour in rats (Ratnasooriya and Premakumara, 1995).

2. Materials and methods

Fresh mature leaves (500g) of *Piper betlle* were purchased from a sales outlet in central Colombo, Sri Lanka, in February 1995, cut into small strips (about 5mm width) and extracted into 1L of distilled water using a domestic blender (John Oster Manufacturing Co., New York, USA). The resulted greenish slurry was then filtered using a muslin cloth and stored at 4°C until use.

Cross-bred nulliparous female rats (225-250g) and male rats (250-275g) of proven fertility from our own colony were used. The animals were maintained under standardised animal house conditions (temperature: 28-30°C; photoperiod: approximately 12h /12h dark: relative humidity: 50-55%) in plastic cages (4 rats/cage) with free access to pelleted food (Oils & Fats Corporation, Seeduwa, Sri Lanka) and tap water.

13 male rats were randomly divided into 2 groups and were orally administered either with 1 ml of the extract (treatment, n=7) or lmL of distilled water (control. n=6) 3 times (at 9.00. 12.00 and 15.00h) a day. The day of treatment was designated as day 1. These rats were closely monitored once daily for mortality, overt signs of toxicity and changes in behaviour and appearance, The colour of the urine and the consistency of the faeces were also noted through out the experimental period (35 days), The food and water intake were also noted.

Libido, ejaculatory competence and fertility of rats were then assessed on day of treatment and days 3 , 7, 14, 28, and 35 subsequent to treatment by pairing (at 18.00 - 19.00h) overnight with a pro-oestrous female having had a regular 4 day oestrus cycle on at least 2-3 complete vaginal cycles before the commencement of the experiment. On the following morning (between 8.00-9.00h) vaginal smears were examined microscopically (at x 100) for the presence of spermatozoa. If spermatozoa were present in the smears (considered as onset of pregnancy) their gross morphology was noted (at x 400) and the number in the smear was estimated (in duplicate) using an improved Neaubauer haemocytometer (America Optical Corporation, Buffalo, USA) after flushing the vagina with 0.05ml of isotonic saline (0.9% NaCl, w/v). If spermatozoa were absent in the vaginal smear of a paired female, daily vaginal smearing was undertaken (between 9.00 - 10.00h) from that particular female to determine the occurrence of pseudopregnancy.

14 days following mating, the females were laporotomised under ether (BDH, Poole, UK) anaesthesia, and the number of foetuses (both viable and dead) and resorption sites were counted. The ovaries were then examined grossly and the number of corpora lutea was recorded. The following reproductive indices were then computed: index of libido (number mated/numbr paired x 100); quantal pregnancy (number pregnant/number mated x 100); implantation index (number of implants/ number of corpora lutea x 100); pre-implantation loss [(number of corpora lutea - number of implants)/number of corpora lutea x 100] and post implantation loss [(number of implantations - number of viable implantations)/ number of implantations x 100).

The results are expressed as means±SEM. Where appropriate, statistical comparisons were made using G - test and Mann-Whitney U-test. The significance level was set at $p<0.05$.

3. Results

The *Piper betle* extract was well tolerated There was no overt signs of toxicity or change in appearance and the behaviour of rats, colour of urine and texture of faeces of the treated animals remained almost similar to the control animals. Furthermore. no deaths were recorded throughout the study period. There was no apparent impeirment of food and water intake of both groups of rats.

The results of the fertility experiment are summarized in Table 1. As shown, the extract significantly (G-test, $p<0.05$) impaired the index of libido only on day 1. The treatment caused a significant ($p<0.05$) inhibition in vaginal sperm count (moderate to severe oligozoospermia): on day 1 (by 99%). day 3 (by 57%), day 7 (by 87%) and day 28 (by 98%). However, azoospermia was ,vident only on day 28 : in 3 out of 7 rats (43%). in contrast, a mild hyperzoospermia (by 35%) was evident on day 1.4. However, this effect was not significant ($p>0.05$). The external gross morphology of the ejaculated sperms of the treated rats appeared almost identical to that. of control without any marked decapitation.

Table I: Effect of *Piper betle* leaf water extract on reproductive parameters of male rats

			Day of pairing with a prooestrsus female					
Parameter	Treatment	n	1	3	7	14	28	35
Index of libido(%)	Control	6	100	100	100	100	100	100
	Extract	7	33.33**	100	100	100	100	100
Vaginal Sperm count (x 10^6/ml)	Control	6	11.6±1.05	8.93±1.05	8.41±1.77	8.56±1.45	10.85±2.78	13.25±3.79
	Extract	7	0.01±0.01**	3.83±2.23*	1.10±0.36**	11.57±2.43	0.17±0.16	12.73±1.82
Quantal pregnancy (%)	Control	6	100	100	100	100	100	100
	Extract	7	0.00**	28.57**	71.42	100	33.33**	100
Number of Implants	Control	6	7.5±0.42	8.0±0.73	7.83±0.47	7.83±0.47	7.66±0.8	9.0±0.36
	Extract	7	0.0**	1.0±0.84**	3.74±1.28*	8.14±0.76	1.33±0.98**	7.0±0.51
Implantation index(%)	Control	6	72.58	80.0	79.66	83.92	83.63	90.0
	Extract	7	0.00**	13.5**	48.14	76.0	14.54**	72.41
Pre implantation Loss (%)	Control	6	26.85±4.16	20.58±3.45	20.04±3.38	24.58±5.41	14.73±3.91	10.06±0.36
	Extract	7	100*	90.12±8.45*	55.5±14.9*	22.78±7.04	85.18±10.9**	27.43±4.22
Post implantation Loss	Control	6	0.0	0.0	0.0	3.95±3.67	2.38±2.38	0.0
	Extract	7	-	0.0	14.28±14.28	2.04±2.04	0.00	0.0

* = $p<0.05$, ** = $p<0.01$;- = not applicable

The extract caused a significant inhibition in quantal pregnancy: on day 1 (by 100%), day 3 (by 72%) and day 28 (by 67%). In parallel with this effect there was also a significant ($p<0.05$) reduction in implantation index : day 1 (by 100%), day 3 (by 100%), day 28 (by 83%). The number of uterine implants was also significantly impaired on these days: day 1 (by 100%), day 3 (by 88%) and day 28 (by 83%). In addition, the number of implants was also significantly ($p<0.05$) reduced on day 7 (by 53%). At these matings the corpora lutea displayed lustre and size characteristics of mid-gestation corpora lutea. It is note worthy that the extract induced complete sterility in all females mated with treated rats on day I (100%), 4 out of 7 (57%) on day 3, 1 out of 7 (14%) on day 7 and 4 out of 7 (57%) on day 28. The extract caused a large and a significant enhancement ($p<0.05$) in pre-implantation loss on day 1(by 273%), day 3 (by 337%), day 7 (by 173%) and day 28 (by 478%). However, the extract was devoid of a significant ($p>0.05$) effect on post-implantation loss during the period of study.

4 Discussion

The objective of this study was to examine the effects of *P. betle* leaves on libido and fertility of males. This was done using rats, an aqueous extract of *P. betle* leaves, and a dose and treatment regimen which has been previously shown to impair masculine behaviour of rats (Ratnasooriya and Premakumara, 1995). The results show that the extract, under the experimental conditions used, inhibited fertility (in terms of quantal pregnancy, number of uterine implants and implantation index) in a reversible manner. The appearance of this antifertility effect was rapid (within 12h) and so was its decay (by day 14 post treatment). This is a novel finding which in our opinion is also important. Firstly it shows the potential of developing a male contraceptive agent based on *P. betle* leaves: currently abut 30 alkaloids and 19 non-alkaloid compounds isolated from higher plants are incorporated as single purified ingredients into medicinal preparations in the USA (Phillipson, 1994). Secondly, it hints the possible risks to fertility of men of reproductive age who are habitual betel leaf chewers.

In agreement with the results of our previous study (Ratnasooriya and Premakumara, 1995) the extract was well tolerated: there were no overt signs of toxicity, behavioural abnormalities or apparent suppression in food and water intake. Thus, the antifertility action is unlikely to be due to stress and/or toxicity: both stress (Kimmel, 1993) and reprotoxicants (Working, 1988) impair male fertility.

A large drop in sperm output (in terms of vaginal sperm count) was evident in extract-treated rats throughout the period of impaired fertility. Profound decrease in sperm numbers in rats is also reported with chronic administration of an alcoholic extract of *P. betle* stalks (Adhikary, *et. al.*. 1989). Since positive correlations exist between sperm numbers and fertility (Aitken. *et. al.*, 1995) the extract induced severe oligozoospermia by itself should be sufficient to account for the antifetility action evident in this study. However, now it is claimed that if the female partner

is normal, over time' pregnancy can be achieved with seemingly very low sperm counts (Seibel and Zilberstein 1995). The onset of the oligozoospermic ejaculates was rapid (within 12h). Therefore' it is likely that the reduction in sperm count results from an impairment of orgasmic contraction of epididymis and vas deferens at ejaculation. *P. betle* leaves are rich in phenolic compounds (The Wealth of India, 1969) and phenolic compounds inhibit smooth muscle contractions by interfering either specifically or non-specifically with contractile machinery (Ogata, *et.al* 1992). The oligozoospermia observed cannot result from an obstruction in the sperm passage in view of the reversibility of the effect: Usually, obstructions in sperm, passage lead to permanent oligo and/or azoospermia (Martin-da-Pan and Campana. 1993). Retrograde ejaculation is another possibility but we have no evidence in favour or against such a mechanism. In contrast, the oligozoospermia produced in the latter stages of the study could be due to Spermatogenic arrest. Indeed, alcoholic extract of stalks of *P. betle* leaves have shown to disorganize spermatogenesis (Adhikary, *et. al,* 1989).

Along with the *P. betle* leaf extract-induced antifertility action was a significant and large elevation in pre-implantation loss. Such action can result from several mechanisms. Inhibition of sperm motility is one possible mechanism. Indeed *P. betle* leaf extract has been shown to inhibit motility of human sperm in *vitro* (Ratna sooriya, *et. al.* 1990). Furthermore, amino butric acid which is abundant in *P. betle* leaves (The Wealth of India, 1969) also impairs motility of human sperm *in vitro* (Ratnasooriya and Kaluarachchi 1991). In this respect, it is worth noting that in Sri Lanka, according to anecdotes *P. betle* leaves have been used by prostitutes as a vaginal contraceptive.

The presence of morphologically defective sperms in the ejaculate can also cause an elevation in pre-implantation loss as evident in this study. However, this mechanism seems unlikely as the gross external characteristics of the ejaculated sperm in the extract-treated rats were essentially similar to those of vehicle-treated rats. Alternatively, a disruption in fertilizing potential of sperm by the extract, can induce an elevation in pre-implantation loss. Indeed, lycine, an amino acid which is present in *P. betle* leaves has claimed to inhibit fertilizing potential of rat sperm when administered locally to the epididymis (Ratnsooriya and Ananda, 1990). Failures to acrosome reaction following binding to zona pellucida and sperm oocyte fusion have been implicated with disruption of fertilizing potential of sperm in humans when all the parameters of semen quality appear normal (Liu and Baker 1994). *P. betle* leaf extract inhibit the frequency of intromissions during coital behaviour of rats (Ratnasooriya and Premakumara, 1995) and reduction in the number of intromissions can increase pre-inplantation loss in rats (see Coopersmith and Erstitne, 1994 and reference therein). Thus, it is possible that this mechanism too may have played a supportive role for the antifertility action of *P. belle* leaf extract. On the other hand, the extract induced no significant effect on post-implantation losses.

The extract inhibited libido. This effect had a rapid onset and a transient action only on day[1] (Ratnsooriya and Premakumara 1995). An imparment of libido can result from hyperprolactinemia (Chambers and Phoenix, 1992), sedative (Horowitz and Goble, 1979), or by an antiandrogenic action (Neumann, 1994) of the extract. However, the first and the second mechanisms are unlikely to precipitate the antilibido action evident in this study: the aqueos extract of *P. betle* have been shown to possess no such activity in rats (Ratnsooriya and Premakumara, 1995). An antiandrogenic action of the extract also seems unlikely because the extract has been shown to have no depressive effect on the wet weights of the sexual accessory glands of rats: the structural and functional integrity of sexual accessory organs are androgen dependent (Neumann, 1994) and their weights have been used as an index of androgen status in animals (Neumann, 1994). However, it is possible that such a transient inhibition of libido may result from an abrupt enhancement of clearance of androgens (Von Schoultz and Carbstrom, 1989) induced by extract and/or altered binding with androgen binding protein (Von Schoultz and Carbstrom, 1989).

In conclusion, this study showed that an aqueous extract of *P. betle* leaves can temporarily inhibit fertility of male rats. On the day of treatment this effect was accompanied with an impairment of libido. The antifertility action was primarily via enhancement of preimplantaion loss.

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Short running head: *Piper betle* leaves and male fertility

Reprint requests to : Prof, W.D.Ratnasooriya, Department of Zoology, University of Colombo, Colombo 3, Sri Lanka.

***Piper betle.* leaves reversibly inhibits fertility of male rats**

W.D.Ratnasooriya and G.A.S.Premakumara