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Antifertility Efficacy of *Piper betle* Linn. (Petiole) on Female Albino Rats

J.D. Sharma*, Lalita Sharma and Poonam Yadav
Reproductive Physiology and Environmental Toxicology Laboratory,
Department of Zoology,
University of Rajasthan, Jaipur - 302004, India.

Abstract : Normal cyclic female albino rats (*Rattus norvegicus*) of Wistar strain weighing between 150-200 gm were treated with *Piper betle* (Petiole) ethanolic (50%) extract (100 mg/day/rat) for 30 days. The results revealed that *P. betle* treatment caused reduction in reproductive organ weights, circulating level of estrogen, fertility, number of litters, serum glucose concentration, enzyme activity of acid phosphatase, SGOT and SGPT as compared to control value. Whereas, the concentration of cholesterol and ascorbic acid increased following *P. betle* treatment, revealing non-utilization of cholesterol by the system and mobilization of ascorbic acid during phytodrug treatment to over come from induced stress condition. The estrus cycle was irregular and prolonged in treated group of rats indicative of anestrus condition, which resulted in infertility. However the haematological parameters remained within normal range. Withdrawal of phytodrug for 30 days restored complete/partially decreased reproductive organ weights, circulating level of estrogen, fertility, number of litters, concentration of glucose and enzyme activity of acid phosphatase SGOT and SGPT to control values. The cholesterol and vitamin –C concentration was also restored to control level. The data suggests that the *P. betle* ethanolic extract exerted antifertility and antiestrogenic effects in female rats. The effects brought by *P. betle* extract is non-toxic and transient.

Key words : Antifertility, *Piper betle*, *Rattus norvegicus*, SGOT and SGPT

Introduction :

Population explosion is an imminent hurdle for a country's development as the natural resources are limited. The population of India is multiplying at an alarming rate and has crossed one billion. Fertility regulation has therefore become the major concern of people of all walks of life. In recent years, plants are persued over synthetic contraceptive drug because plants are easily available, economic and devoid of harmful side effects.

The occurrence of high fluoride concentration in ground water has now become one of the most important health related geoenvironmental issues in many countries of the world. Our country is also confronting the same problem where the high fluoride concentration in ground water resources and the resultant disease "Fluorosis" is evenly distributed in nearly 150 districts of 15 states. It has been observed that about 25 million people in 8700 village in india are using ground water having fluoride content more than 1.5 mg/l. In Rajasthan, ground waters of the western and some southern part of the state are enriched with high fluoride

* **Corresponding author :** J.D. Sharma, Reproductive Physiology and Environmental Toxicology Laboratory, Department of Zoology, University of Rajasthan Jaipur -302004, India;
e-mail : jaishree_ajay@yahoo.co.in

concentration. The arid areas of the state are prone to both dental and skeletal fluorosis. As per P.h.e. Deptt. out of 37,889 villages and 45,311 habitations. 9741 villages and 6819 habitations, ground waters are enriched with excess fluoride content. The extent of severity was observed in Nagaur, Pali, Barmer, Jalore and Sikar district. It has also been observed that besides water other factors viz. nutritional deficiencies, high ambient temperature, high alkalinity and low calcium content and vitamin C are also responsible for endemic fluorosis. In recent years there has been a tremendous increase in the sale of sachets of Pan masala, Gutka, mouthwash and mouth ringes in north India in general and Rajasthan in particular, these are also rich sources of fluoride. Therefore, for the welfare of mankind such items must be banned. Since it is a socio-economic disease, therefore, it is imperative to mitigate the problem by adopting suitable scientific measures to protect the consumers (Ozha et al, 2003; 2007)

Drinking water quality of 17 villages of Sanganer Tehsil, Jaipur District, Rajasthan was analyzed to identify the nature of potability of water. The data suggests that the drinking water of seventeen villages of Sanganer Tehsil contain high fluoride concentration, which leads to dreaded disease called fluorosis; hence drinking water is not potable (Sharma et al, 2005). Before that Sharma et al (2004) suggests that excess fluoride water exposure to rats caused reduction in weight of kidney, altered serum and tissue biochemistry in turn causing toxic effects on liver, heart, kidney and adrenal.

Piper betle Linn. (Beng: Jhal Pan; Piperaceae) is widely cultivated in various provinces of India, Sri Lanka and Burma. The therapeutic and medicinal uses of its leaves and roots have been mentioned in literature (Sengupta and Mukherjee, 1926, Chopra *et al*., 1982, Satyavati *et al*., 1987). Mild antifertility effect in rats was noted from the amorphous compound obtained from the benzene extract of the root. But no systematic study has been carried out in female rats sofar, hence the present investigation was undertaken to focus on antifertility efficacy of *P. betle* ethanolic extract on female albino rats.

Materials and Methods :

The petiol of *Piper betle* Linn. was dried, powdered, weighed and soxhleted for 16 to 18h using alcohal (50%) as solvent. Healthy, adult female albino rats (*Rattus norvegicus*) weighing between 150-200 gm were used for experimentation. The animals were exposed to 14h day light and maintained on standard diet (Ashirvad Ltd., Chandigarh). The rats were divided into three groups, Group I - control, Group II– treated with *Piper betle* extract (100 mg/ day/rat) for 30 consecutive days, Group III– the rats were treated with *P. betle* for 30 days and drug was withdrawn for another 30 days.

After the respective treatment half of the female rats were kept for fertility test and remaining were autopsied and used for organ weights, haematology and serum biochemistry. The blood was collected through cardiac puncture and serum was separated. The estrus cycle was checked daily in the morning during the phytodrug treatment. The reproductive

Table – 1 : Body (gm) and organ weights (mg) of control, *P. betle* and withdrawal treated rats.

Treatment	Body weight (gm)		Organ weight (mg/100gm b.wt.)						
	Initial	Final	Ovary	Uterus	Vagina	Adrenal	Liver	Kidney	Heart
Control	218.50 ± 0.71	233.00 ± 0.60	23.99 ± 0.03	79.95 ± 0.29	36.91 ± 0.10	11.31 ± 0.07	3531.55 ± 6.66	384.20 ± 1.06	364.65 ± 1.28
Piper betle (100mg/day/rat for 30 days)	183.33 ± 0.68	191.67 ± 1.25	18.01[a] ± 0.18	54.99[a] ± 1.78	26.16[a] ± 0.53	14.02 ± 0.03	3444.42 ± 24.44	423.89 ± 2.13	366.05 ± 1.82
Withdrawal for 30 days	200.83 ± 1.40	206.67 ± 1.46	20.04 ± 0.13	72.68 ± 0.52	32.41 ± 0.19	13.39 ± 0.13	3617.96 ± 23.65	381.32 ± 0.86	345.95 ± 1.35

Values are mean ± S.E.
a = $p < 0.001$

Table – 2 : Estrus cycle, fertility, litters per rat and estradiol concetration of control, *P. betle* and withdrawal treated rats.

Treatment	Estrus cycle	Fertility	Litters per rat	Estradiol (pg/ml)			
				Proestrus	Estrus	Metestrus	Diestrus
Control	Regular	100% +ve	12.60 ± 0.07	55.69	68.20	30.91	9.96
Piper betle (100mg/day/rat for 30 days)	Irregular	50% -ve	3.60[a] ± 0.36	-	-	14.06	3.21
Withdrawal for 30 days	Regular	100% +ve	8.20 ± 0.15	36.50	50.98	17.90	8.60

Values are mean ± S.E.
a = $p < 0.001$

Table – 3 : Haematology of control, *P. betle* and withdrawal treated rats.

Treatment	WBC (per cmm)	RBC million/cmm	Hb (gm%)	HCT (%)
Control	7150.00 ± 342.28	4.86 ± 0.09	11.73 ± 0.03	35.77 ± 0.11
Piper betle (100mg/day/rat for 30 days)	10500.00 ±772.89	3.58 ± 0.28	11.43 ± 0.24	35.08 ± 0.67
Withdrawal for 30 days	9825.00 ± 908.40	4.06 ± 0.17	12.40 ± 0.30	38.77 ± 0.08

Values are mean ± S.E.

Table – 4 : Serum biochemistry of control, *P. betle* and withdrawal treated rats.

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	Total protein (g/l)	AcP KA	AlP KA	SGOT (U/l)	SGPT (U/l)	Vitamin C mg/dl
Control	195.57 ± 9.31	66.33 ± 2.43	6.11 ± 0.08	4.93 ± 0.17	20.60 ± 1.78	336.00 ± 9.45	68.20 ± 3.39	1.76 ± 0.05
Piper betle (100mg/day/rat for 30 days)	180.04 ± 32.82	136.38[a] ± 6.24	7.98 ± 0.18	2.03[a] ± 0.12	38.71[a] ± 2.22	20.68[a] ± 2.39	31.88[a] ± 2.83	2.97[a] ± 0.05
Withdrawal for 30 days	170.17 ± 4.34	93.33 ± 1.29	5.00 ± 0.07	4.01 ± 0.02	25.00 ± 2.20	256.67 ± 3.47	81.64 ± 1.73	1.42 ± 0.00

Values are mean ± S.E.
a = $p < 0.001$

organs (ovary, uterus, vagina) and vital organs (adrenal gland, liver, kidney and heart) were excised blotted free of blood and weighed. The relative weight of the organ per 100 gm of body weight was calculated.

Results and Discussions :

The results revealed that *P. bettle* ethanolic extract (50%) (100mg/day/Rat) for 30 days did not change the body and vital organ weights, but the reproductive organ weights diminished significantly ($p<0.001$) as compared to control rats (Table 1). As the structural and functional integrity of reproductive organ depend on circulating level of estrogen, any small change in estrogen level may lead to altered structural and functional activity of reproductive organs. Jain (2005) also reported decreased reproductive organ weights following *P. betle* treatment to male rats. The estrus cycle was of 4 to 5 days in control rats and the same was prolonged and irregular following *P. betle* treatment for 30 days, leading to anestrus condition. The fertility was inhibited up to 40% and average number of litters declined significantly ($p<0.001$) following *P. betle* therapy (Table 2). These altered parameters may attribute to diminished circulating level of estrogen. The plant extract may brought about its effect through pituitary-gonadal axis, which resulted in diminished gonadolrophine release, in turn reduced reproductive organ weights and estrogen level affecting ovarian cyclicty. Antiferlility effect of *P. betle* in female rats has been reported by Adhikary *et al*. (1989).

The haematoglogical parameters WBCs, RBCs, haemoglobin content and haemotocrit values were all found within normal range (Table 3). This indicate that the ethanolic plant extract of *P. betle* has no toxic effect on physiology of rat.

Serum biochemistry revealed that glucose level was declined but cholesterol and Vitamin C concentrations were elevated beyond control value; indicate non-utilizaton of cholesterol by the system, hence decrease in estrogen level, whereas increase in Vitamin-C concentration reveal mobilization of antioxidant to over come from induced stress condition due to plant extract. However, non-significant change in protein concentration was observed as compared to control value. The enzyme activity of serum acid phosphatase, SGOT, SGPT diminished significantly ($p<0.001$) (Table 4), revealing enzyme inhibiting effects of plant extract. The alkaline phosphatase activity enhanced beyond control value. Non-toxic effects of acid and alkaline phosphatase has been reported by Adhikary *et al* (1989).

The effects brought by *P. betle* are antiestrogenic, leading to antifertility in female rats. Withdrawal of *P. betle* treatment restored above altered parameter partially/completely to control level, indicate that plant extract effects were reversible and transient. The data suggests that *P. betle* extract brought about antifertility and antiestrogonic effects in female rats, and these effects were reversible on cessation of treatment.

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