ORIGINAL ARTICLE

Antifertility activity of ethanolic and aqueous extracts of *Piper betle* petiole on female Wistar rats

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Abstract

Aim: The aim of this study was to investigate the antifertility activity of extracts of *Piper betle* petiole on female Wistar rats. **Materials and Methods:** Ethanolic and aqueous extract of petiole of *P. betle* was study antifertility activity in proven fertile female Wistar rats at the doses 500 mg/kg b.wt./day for 30 days. Different parameters were studied in female Wistar rats, including phytochemical, effect of the reproductive outcome, anti-implantation, abortifacient, and estrogenic and antiestrogenic activity, were observed. **Results:** *P. betle* showed positive test for alkaloids, steroids, flavonoids, terpenes, carbohydrates, and tannins. The extract of *P. betle* has antifertility effect the control rats showed good number of litters. Treatment of animal with different extracts resulted a significant ($P < 0.05$, $P < 0.01$). Antifertility activity 51% and 37.2% was exhibited by alcoholic extract *P. betle* (APB) and water extract *P. betle* (WPB), respectively. After 21 days of the extracts free period, the antifertility effect of the extracts was reversed. The extract treatment with APB, an increase in the percentage of resorption index indicates the failure in development of embryo. The mean percentage of anti-implantation and abortifacient was found to be highest for APB - 38.45%, WPB - 13.62%, and APB - 28.96%, WPB - 26.22%, respectively. The decrement in implantation caused by the extracts may be due to estrogenic or antiestrogenic activity. However, along with standard APB exhibiting more potent estrogenic and less potent antiestrogenic when compared with standard. **Conclusion:** Female antifertility agents should include acceptability, safety, and efficacy during and after the treatment. The above results revealed the potential, reversible female antifertility effect of APB.

Key words: Abortifacient study and estrogenic and antiestrogenic activity, antifertility, anti-implantation, *Piper betle*, reproductive outcome

INTRODUCTION

This century search for antifertility agents is continued to tackle the problem of population explosion that may lead too economic and health impact on the family in particular and the society in general, especially in developing countries like Ethiopia where the population growth is very high (Ministry of Health, 2003). The population of India is multiplying day by day at an alarming rate and has crossed on 1.5 billion. Fertility regulation has, therefore, become the major concern of people of all walks of life. In recent years, plants are practice over synthetic contraceptive drug because plants are easily available, economic and devoid of harmful side effects.[1]

Piper betle, Family: Piperaceae (commonly known in all over India as Paan), is a perennial herb that is grown in most part of India and it has been an important herb distributed throughout of world *P. betle* are the most valued part of the plant, in the past were routinely used as a chewing agent to restrict offensive breath, and they contain tannins, chavicol, phenyl, propane, sesquiterpene, cyneole, alkaloid, sugar, and some essential oil and found various medicinal value, digestive, appetizer, aromatic, expectorant, stimulant, antibacterial, euphoria-inducing, antiprotozoal,

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carminative, antifungal and aphrodisiac, etc.[2] The leaves are also supposed to harden the gum, conserve the teeth and to prevent indigestion, bronchitis, constipation, congestion. However, scientific study of this plant in relation with the potentiality as effective antifertility agent is still fragmentary.[3] The present study was therefore carried out to evaluate the claimed antifertility effect of *P. betle* petiole using different aspects of reproductive physiology in Albino mice.[4]

MATERIALS AND METHODS

Collection of Plant Material

The plant specimens for the study were collected from the Satpura region of Madhya Pradesh, India, identified and authenticated by the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, a voucher specimen no. is NISCAIR/RHMD/consult/2015/2859/52-1. Care was taken to select healthy fully grown plant and normal parts. The samples of different parts were cut suitably and removed from the plant and thoroughly washed with water to remove the adherent impurities and dried in sunlight.[5]

Determination of Physicochemical Parameters

Physicochemical parameters of P. betle petiole were determined and reported as total ash, water-soluble ash, acid-insoluble ash. Alcohol- and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content and pH were also determined (Edwin, 2010).

Successive Solvent Extraction

The method is based on the extraction of active constituents present in the drug, using various solvents ranging from nonpolar to polar. The solvents used were petroleum ether, ethanol, and water. Crude drug was subjected to Soxhlet extraction with 1.5 Litters of each solvent depending on their polarity. Each time before extraction with next solvents the marc was air-dried.[6] All the extracts were concentrated by distilling the solvent at low temperature.[7] They were then weighed and percentages of different extractive values were calculated with respect to air-dried substance.[8]

Phytochemical Screening

Identification of the chemical constituents was carried out on the powdered petiole and on the petroleum ether extract (PEE), ethanol, and aqueous extracts using chemical methods.[9]

Animals

Antifertility test was performed on adult female Wistar rats weighing between 180 and 200 g and mice. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*.[10] The institutional ethical committee for animal cares and use approved all experimental procedures. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h.[11] The experimental protocols were subjected to the securitization of the Institutional Animal Ethics Committee and were cleared by the same (1587/PO/Re/S/11/CCSEA).

Acute Oral Toxicity

The acute oral toxicity studies were carried out as per the guidelines of Organization for Economic Co-operation and Development 423, Ministry of Social Justice and Empowerment, Government of India.[12,13]

Antifertility Study

Antifertility activity of plant extracts was evaluated with the help of reproductive outcome, anti-implantation, abortifacient, estrogenic, and antiestrogenic study was also performed, which further supported by the hormonal analysis.[14]

Reproductive Outcome in Rats

Five groups of mature female rates (five rat/group) were selected for received extracts for 8 days and control group received vehicle for the same period. All the experimental rats were then allowed to mate with mature fertile male rat and the treatment continued for 21 days. The number of litters was determined after the completion of one gestation period in all experimental groups.[15] The litters were allowed to grow and the growth of litters produced from the extract-administered group was compared with those of control group. The reversibility of antifertility effect of the extracts was also studied in the treated groups. For this study, the extracts were administered continuously for 21 days, and then, the extract was withdrawn. After 21 days of extracts withdrawal, animals were allowed to mate with male rate. The number of litters was determined after the completion of one gestation period (Salhad *et al*., 1997).

Anti-implantation Study

Proven fertile female Wistar rats, weighing between 150 and 200 g, were selected and left overnight with male of proven fertile in the ratio of 3:1. The extracts were administered orally to separated group rats at the dose level of 500 mg/kg from day 1 to 7 of pregnancy. Control animal received the vehicle (carboxymethyl cellulose [CMC] 0.5%). The animals were then laparotomised on day 10 of the pregnancy under excess dose of thiopentone sodium and uteri were examined to determine the number of implantation sites (Salhad *et al*., 1997).

Abortifacient Study

Female rats at 1st day of pregnancy were divided into three groups, consisting of six animals in each group. The animals were laparotomised under light ether anesthesia and semi-sterile conditions on the 10th day of pregnancy. Both horns of the uterus were observed for the number of implants. The rats were sutured and allowed to recover. The first group served as control and received vehicle only (Tween-80, 1%) and the group second and third received suspension of extract at a dose of 500 mg/kg body weight (b.wt.) in 1% Tween-80, respectively, from day 10 to 18 of pregnancy. During the experiment, animals were observed for vaginal bleeding. On 21st day, animals were laparotomised under light ether anesthesia and observed for number of litters and percentage of resorption compared with initial number of implantation observed on 10th day of pregnancy (Khanna and Chaudhury, 1968).

Estrogenic and Antiestrogenic Study

Colony breed immature ovariectomised female rats (21-23 days) weighing between 25 and 30 g were used. They were divided into experimental and control groups, consisting of six animals each group. The extracts were suspended in 0.5% CMC and administered orally for 7 days at the dose level of 500 mg/kg b.wt. Ethinyl estradiol (Unicure Remedies Pvt. Ltd., Baroda, India) in olive oil 1 µg/rat/day was injected subcutaneously for 7 days in another group to induce estrous. CMC 0.5% was administered orally to the control animals. The extract at the dose level of 500 mg/kg was also administered orally along with ethinyl estradiol in olive oil at 1 µg/rat/day subcutaneously to different groups of rat for the same period (Sharma, 2003).

On the 8th day of the experiment, all the animals were sacrificed by decapitation under light ether anesthesia, and the uteri were dissected out, surrounding tissues removed, blotted on filter paper and weighed quickly on balance sensitive to 0.0001 g.[16,17] A portion of the uterine tissues and adrenal glands from the control and treated animals were fixed in Bouin's fluid for 24 h, dehydrated in alcohol and then embedded in paraffin. The paraffin blocks were sectioned at 6 mm intervals and stained with hematoxylin-eosin for histological examinations (Pal, 1990).

Hormonal Analysis

Blood (2 ml) was drawn by retro-orbital puncture and was immediately transferred into EDTA coated vacationer. The samples were mixed gently and were left for more than ½ h at room temperature, and finally centrifuged at 3000 rpm for 15 min. Serum was separated and assayed for follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol, and prolactin and 17-OH progesterone using enzyme-linked immunoassay technique (ELISA reader) (BIORAD 680 Microplate Reader).[18,19]

Statistical Data

All values are expressed as mean ± standard error of mean. Means were statistically analyzed by one-way analysis of variance, and values of $P < 0.05$ were considered statistically significant.[20]

RESULTS AND DISCUSSION

Physicochemical Parameters

Physicochemical parameters of *P. betle* petiole were determined. In physicochemical parameter, total ash is approximately 2 and 4 times more than acid insoluble ash and water soluble ash, respectively.[21] Ethanol soluble extractive is approximately 2 times higher than water soluble extractive. Moisture content was <2.2% and pH was 6.4 [Table 1].

Preliminary Phytochemical Investigation

A number of phytoconstituents from natural sources have been proved efficacy to prevent the pregnancy. Many scientific reports were published for antifertility activity of flavonoids (Hiremath *et al.*, 2000), glycosides, and alkaloids (Sadik *et al.*, 2001). Phytochemical investigation of *P. betle* showed Table 2, the preliminary phytochemical study of *P. betle* petiole showed that Alkaloid, Steroid, Terpene, Flavonoid Glycoside, Tannin, were present in Alcoholic Extract. Whereas Glycoside, Tannin, Sugar were present in Petroleum Ether Extract and Alkaloid, Terpene, Glycoside and Tannins were present in Aqueous Extract. The successive solvent extraction with petroleum ether, alcohol, and water gave 17.5%, 21.45%, and 26.56%, respectively, practical yield.

Acute Oral Toxicity

Acute toxicity studies were carried out to evaluate toxicity and to determine the minimum lethal dose (LD) of the drug extracts, using Swiss albino rats. No clinical signs were evident in any animal during treatment period (Clinical observations

Table 1: Various physicochemical parameters

Physicochemical parameter	Value % w/w* mean±SD
Total ash	20.75±0.5
Acid insoluble ash	10.8±0.25
Water soluble ash	7.2±0.15
Water soluble extract	12.5±0.5
Ethyl alcohol soluble extract	18.8±0.3
Moisture content	2.2
pH	6.4

Value (%) mean±SD. w/w*: Weight/weight, SD: Standard deviation

include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern, tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma). No mortality as well as any clinical sign of toxicity has been observed at a dose level of 2000 mg/kg indicating that all the extracts comes under Category 5, and hence, LD_{50} cut-off was found to be 2000 mg/kg b.wt. Hence, one-five of this dose, i.e., up to 500 mg/kg b.wt, was used for antifertility investigation.

Hematological and biochemical parameters were also performed before and after treatment and no significant changes were observed.[22,23]

Reproductive Outcome Study

Table 3 shows the effect of different extracts on the fertility of female rats. The control rats showed good number of litters. Treatment of animal with different extracts resulted a significant ($P < 0.05$, $P < 0.01$). A significant antifertility activity (51% and 37.2%) was exhibited by APB and WPB respectively.

It was also found that the litters of the extract treated rats did not show any physical deformity.[24] All litters grew up to normal adult stage, which indicates that the extracts do not have teratogenic effect and the absence of teratogenic effect of extracts at a given dose justifies the safety of the plant. The present observations agree with Salhad *et al.* (1997), who reported the reversible antifertility effect of *Ricinus communis* (castor beans) on female rabbits and also supported by Endalk *et al.* (2005), who reported the same effect of the methanolic root extract of *Rumex steudelii* on female rats.[25]

After 21 days of the extracts free period, the antifertility effect of the extracts was reversed for all animals. An increase in the number of litters observed in all the posttreatment groups may indicate the reversible antifertility effect of all extracts. These observations correlate the findings of Ganguly *et al.* (2007) and Gebrie *et al.* (2005), who reported the reversible antifertility effect with similar observations on the treatment with methanolic extract of *Cissampelos pareira* leaves in mice and methanolic root extract of *R. steudelii* in rats, respectively. The animal groups gave 7.89 ± 0.05 Litters at an average. This showed that there was no statistically significant change from the control group (10.00 ± 0.03).

Table 2: Preliminary phytochemical study of *P. betle* petiole

Test for constituent	*P. betle* petiole		
	PEE	AE	WE
Alkaloid	–ve	+ve	+ve
Steroid	–ve	+ve	–ve
Terpene	–ve	+ve	+ve
Flavonoid	–ve	+ve	–ve
Glycoside	+ve	+ve	–ve
Sugars	+ve	+ve	+ve
Saponins	–ve	+ve	–ve
Tannin	+ve	+ve	+ve
Carbohydrate	–ve	+ve	+ve
Color and consistency	Dark green	Dark green	Dark brown
Yield	17.8	21.45	26.56
Code	PPB	APB	WPB

P. betle: Piper betle, PEE: Petroleum ether extract, EE: Ethanol extract, WE: Water extract, PPB: Petroleum *Piper betle*, APB: Alcoholic *Piper betle*, WPB: Water *Piper betle*

Table 3: Effect of extracts on reproductive outcome

Group	Eestrous cycle	Fertility (%)	Litters present
Control	Regular	100 + ve	10.00±0.03
APB	Irregular	51 – ve	4.90±0.10[a]
WPB	Irregular	37.2 – ve	6.28±0.25[b]
W.D-APB	Regular	81.6 + ve	8.16±0.08
W.D-WPB	Regular	82 + ve	8.20±0.02[a]

[a]$P<0.05$, [b]$P<0.01$, when compared with normal control. Values are expressed as mean±SD. SD: Standard deviation APB: Alcoholic *Piper betle*, WPB: Water *Piper betle*

Anti-implantation and Abortifacient Activities

Postcoital antifertility study showed the anti-implantation activity in the treated animals. Treated animals delivered litters, which was significantly less than control [Table 4]. The extract treatments with APB, significantly ($P < 0.001$) reduced the number of litters born [Table 5]. This indicates the abortifacient nature of extracts. An increase in the resorption index (%) by the extract is an indication of failure in the development of the embryo (Dhanwad *et al.*, 2005). Such occurrence of fetal resorption suggests that interruption of pregnancy also occurred after implantation (Elbetieha, 2000). These observations indicate the pregnancy terminating potential of the extract. Embryonal resorption could be due to modifications of uterine lining function or maternal toxicity which consequently may increase early resorption and late fetal death (Chaves, 1985; Khera, 1987). Hence, the present investigation clearly reveals that the extracts are effective before and after the implantation occurs (Vasudeva and Sharma, 2006).

Both these activities were calculated on the basis of number of implants and number of litters. The mean percentage of anti-implantation and percent resorption (abortifacient) were found to be highest for APB - 38.45%, whereas in the case of percent resorption; APB - 28.12%, these results indicated that all the extracts inhibited the conversion or development of implants into litters.[26] The decrement in implantation caused

Table 4: Effect of extracts on anti-implantation activity

Treatment (dose)	Anti-implantation activity		
	Number of implants	Number of litters	Mean percentage anti-implantation
Control	7.23±0.52[a]	7.20±0.65[a]	Nil
APB	4.45±0.22[a]	4.40±0.55[a]	38.45[a]
WPB	6.10±0.10[b]	6.05±0.12[b]	13.62[c]

[a]P<0.05, [b]P<0.01, [c]P<0.001 when compared with normal control. Values are expressed as mean±SD. SD: Standard deviation, APB: Alcoholic *Piper betle*, WPB: Water *Piper betle*

Table 5: Effect of extracts on abortifacient activity

Treatment (dose)	Abortifacient activity		
	Number of implants	Number of litters	Percentage resorption
Control	7.42±0.62[a]	7.52±0.30[a]	5.45[a]
APB	5.62±0.53[b]	4.43±0.41[b]	28.12[b]
WPB	5.42±0.47[b]	4.33±0.30[b]	26.22[c]

[a]P<0.05, [b]P<0.01, [c]P<0.001 when compared with normal control. Values are expressed as mean±SD. SD: Standard deviation, APB: Alcoholic *Piper betle*, WPB: Water *Piper betle*

by the extracts may be due to estrogenic or antiestrogenic activity as described by Hafez (1970).

Estrogenic and Antiestrogenic Study

Antifertility activity of all the extracts was finally evaluated with the help of estrogenic and antiestrogenic activity associated with the hormonal level and histological parameter like uterine weight, diameter of uterus, thickness of endometrium, and height of endometrium epithelium.[27] The stages of estrous cycle and its duration were determined as described by Makonnen *et al*. (1997). The detailed data have given in Tables 6 and 7. A highly significant increase in the uterine weight ([410.67 ± 09.09[a]] mg/100 g b.wt.) and uterine contents was observed in estrogen-treated group ($P < 0.001$) [Table 6]. However, coadministration of ethinyl estradiol and extract caused a highly significant ($P < 0.001$) decrease in uterine weight ([102.65 ± 7.43[b]] mg/100 g b.wt..) when compared to estrogen-treated group.[27]

However, along with standard APB exhibiting strong estrogenic property, increase in uterine weight, diameter of uterus, thickness of endometrium and height of endometrial epithelium and WPB exhibiting strong anti-estrogenic property, decrease in uterine weight, diameter of uterus, thickness of endometrium and height of endometrial epithelium when compared with standard. These observations are similar to the finding of Ravichandran *et al*. (2007) and Vishnukant and Rana (2010) on the effect of hydroalcoholic extract of *Ailanthus excels* (Roxb.) stem bark and *Plumbago zeylanica* leaves on uterus of female Wistar rats. These observations revealed that these extracts acted as competitive antagonist to ethinyl estradiol. Hence, the anti-implantation activity of these extract may be due to their anti-estrogenic nature, which antagonise the action of estrogen and cause structural and functional changes in uterus and finally decreases the implantation.

Hormonal Analysis

Sex hormones were assayed based on their roles in maintaining pregnancy since a failing pregnancy could be correlated to the levels of these hormones in the body fluids (Yakubu and Bukoye, 2009). The reduction in the concentration of FSH is an indication of disturbance of estrus cycle and ovulation (Ganguly *et al*., 2007). LH is required for continued development and normal function of corpora lutea. The significant reduction in the level of serum LH could be associated with the physiological process of luteolysis preceding parturition (Yakubu and Bukoye, 2009). It could possibly be attributed to pregnancy failure resulting from a luteal phase that is not being maintained.[28] The reduced level of hormone may also be due to inactivation of luteinization of ovarian follicles, which could be responsible for the reduction in the concentration of serum progesterone in this study. Elevated level of progesterone during pregnancy plays a key role in maintaining the conditions and is an important factor in the implantation process. Therefore, luteolysis and reduction in the blood levels of progesterone may contribute to abortion and anti-implantation activity of all extracts. The findings of the present study were agreed with previous studies which reported the effect of *Inula viscose* and *Bambusa vulgaris* leaf extract on implantation and abortion in rats and rabbits (Yakubu and Bukoye, 2009). In this study, an increase in prolactin level was observed [Table 8]. These findings were also supported by Ganguly *et al*. (2007), who reported that a combination of enhanced prolactin and suppressed LH secretion is due to prolongation of estrus cycle (Ganguly *et al*., 2007). An imbalance in endogenous estrogen and progesterone levels could be responsible for anti-implantation activity (Dhanwad *et al*., 2005).

CONCLUSION

The present findings inferred that the gathering treated with the most noteworthy convergence of plant concentrate indicated great come about as that of the standard medication

Table 6: Effect of extracts on estrogenic and antiestrogenic study

Group	Treatment (dose)	Uterine weight (mg/100 g BW; mean±SD)	Vaginal cornification
1	Control	70.24±5.35[a]	Nil
2	Ethinyl estradiol (1 µg/rat per day)	335.40±7.56[a]	+++
3	APB (500 mg/kg)	242.65±7.43[c]	+ to ++
4	WPB (500 mg/kg)	180.05±5.20[c]	++
5	Ethinyl estradiol (1 µg/rat/day) + APB (500 mg/kg)	420.67±9.09[a]	+++
6	Ethinyl estradiol (1 µg/rat/day) + WPB (500 mg/kg)	315.58±8.27[b]	+++

[a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ when compared with normal control. Values are expressed as mean±SD. SD: Standard deviation. +: Nucleated epithelial cells, ++: Nucleated epithelial cells and cornified cells, +++: Cornified cells, APB: Alcoholic *Piper betle*, WPB: Water *Piper betle*

Table 7: Histological changes in the uterus and endometrium after treatment with extracts

Treatment (dose)	Diameter of uterus (µm±SD)	Thickness of endometrium (µm±SD)	Height of endometrial epithelium (µm±SD)
Control	330.54±5.25[a]	54.14±2.12[a]	17.4±0.25[a]
Ethinyl estradiol (1 µg/rat/day)	821.45±6.25[a]	245.45±15.15[a]	45.10±4.18[a]
APB (500 mg/kg)	625.15±8.66[c]	256.20±4.59[c]	65.00±1.43[b]
WPB (500 mg/kg)	225.40±2.45[a]	158.04±5.45[b]	28.14±1.70[a]
Ethinyl estradiol (1 µg/rat/day) + APB (500 mg/kg)	905.15±8.66[c]	256.20±4.59[c]	45.50±1.43[a]
Ethinyl estradiol (1 µg/rat/day) + WPB (500 mg/kg)	545.05±4.04[c]	179.12±5.10[c]	24.12±2.52[a]

[a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ when compared with normal control. Values are expressed as mean±SD. SD: Standard deviation, APB: Alcoholic *Piper betle*, WPB: Water *Piper betle*

Table 8: Hormonal levels in various groups of animals

Treatment 500 mg/kg	LH	FSH	Prolactin	17β estradiol	17 OH progesterone
Control	6.25±2.42	8.64±5.20	40.25±6.10	745.12±45.40	14.54±1.10
APB	6.15±2.44[a]	7.54±4.10[a]	41.70±3.25[c]	714±25.14[a]	34.42±1.10[a]
WPB	5.14±01.25[b]	5.24±4.10[a]	31.10±2.10[b]	524±14.02[a]	20.14±1.10[c]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ when compared with normal control. $N = 5$, Data representation as mean ± SD. SD: Standard deviation, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, APB: Alcoholic *Piper betle*, WPB: Water *Piper betle*

and was underpinned by histopathological investigations of the antifertility activity on albino rats. The result of our study clearly demonstrates that extract of *P. betle*. The control rats showed good number of litters. Treatment of animal with different extracts resulted a significant ($P < 0.05$, $P < 0.01$). A significant antifertility activity (62.2%) was exhibited by APB. It was also found that the litters of the extract treated rats did not show any physical deformity. All litters grew up to normal adult stage, which indicates that the extracts do not have teratogenic effect and the absence of teratogenic effect of extracts at a given dose justifies the safety of the plant. Post-treatment groups indicate the reversible antifertility effect of all extracts.

Antiestrogenic in nature at the dose of 500 mg/kg b.w. as evident form the significance increases in the diameter of uterus, height of endometrial epithelium, and thickness of endometrium in extracted animal compared with control, whereas the animal treated with aqueous extract (500 mg/kg p.o.) showed increase height of luminal epithelium with stimulated uterine glans. The extract did not exhibit any estrogenic activity. Proper equilibrium between estrogen and progesterone is essential for implantation, and any disturbance in the level of these hormones may affect the fertility.

It is a suitable plant for developing antifertility drug *P. betle* is recommended for working out and should be experimented for the antifertility program. *P. betle*, antifertility reproductive outcome, anti-implantation, abortifacient, estrogenic, and antiestrogenic activity. Further studies on mechanism of antifertility action and isolation of the active components responsible for antifertility effect are in progress.

The results of the present study provide that evidence for the antifertility activity of *P. betle* as claimed in the traditional

use. The flavonoids, phytosterol, and terpenoid present in the extracts may be responsible for their activity. Further studies on the mechanism of antifertility action and isolation of the active components responsible for antifertility effect are in progress. With these preliminary results, we can conclude that the ethanolic and aqueous extracts showed significant evidence that antifertility activity using potent reproductive outcome, anti-implantation, abortifacient, estrogenic, and antiestrogenic activity.

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