Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Effect of plumbagin free alcohol extract of *Plumbago zeylanica* Linn. root on reproductive system of female Wistar rats

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ARTICLE INFO

Article history:
Received 9 July 2011
Received in revised form 15 September 2011
Accepted 15 November 2011
Available online 20 December 2011

Keywords:
Anti-implantation
Abortifacient
Estrogenic/antiestrogenic activity
Progesterone
Follicle stimulating hormone
Luteinizing hormone
Prolactin
Ethinyl estradiol
Overiectomized
Haematological

ABSTRACT

Objective: To assess the effect of plumbagin-free alcohol extract (PFAE) of *Plumbago zeylanica* Linn. (Plumbaginaceae) (*P. zeylanica*) root, on female reproductive system and fertility of adult female wistar rats. **Methods:** After the oral acute toxicity study, the PFAE was administered at two dose levels to perform the estrous cycle study, anti-implantation and abortifacient activity and hormonal analysis. However, the estrogenic/antiestrogenic activity was evaluated at only one most effective dose. **Results:** LD_{50} cut-off was 5 000 mg/kg body weight. The extract exhibited significant anti-implantation and abortifacient activity at the tested dose levels (300 and 500 mg/kg, *p.o.*) ($P<0.01$). The extract dose-dependently decreased the levels of serum progesterone, follicle stimulating hormone and luteinizing hormone, while a dose-dependent increase was observed in the concentration of serum prolactin. The extract did not show any significant changes in structure and function of uterus when given alone, but when given along with ethinyl estradiol, it exhibited significant antiestrogenic activity in immature overiectomized female rats($P<0.001$). Biochemical parameters in the serum/blood and haematological parameters did not show appreciable changes throughout and after the course of investigation. However, all the altered parameters returned to normalcy within 30 days following withdrawal of treatment. **Conclusions:** All findings suggest that the antifertility activity of extract could possibly be through the changes in the implantation site, altered hormonal levels, prolonged estrous cycle and anti-estrogenic activity. Hence, the extract possesses reversible antifertility activity without adverse toxicity in female rats.

1. Introduction

Plumbago zeylanica(P. zeylanica), commonly known as white chitrak (Family: Plumbaginaceae) is a perennial herb that is grown in most part of India and is used in the traditional system of Indian medicine against a number of ailments including skin disease, abdomen enlargement, anemia, diabetes, leprosy, dyspepsia, elephantiasis, diarrhea and leprosy[1]. The pharmacological studies carried out by several workers indicate that *P. zeylanica* Linn. possesses astringent, diuretic, antibacterial, antifungal, anticarcinogenic, antitumor and radiomodifying properties. The roots of this plant has been reported to be a powerful poison when given orally or applied to ostium uteri, causes abortion[2]. Plumbagin, a crystalline naphthoquinone compound isolated from root extract has shown antifertility activity but was found to be toxic. The preliminary studies on the plumbagin free alcohol extract of the root showed antifertility activity devoid of adverse reactions[3]. However, the exact mechanism of antifertility action is still not known. The present study was, therefore, carried out to confirm the exact mechanism of antifertility action in female rats using different models, and to determine its oral LD_{50}.

2. Materials and methods

2.1. Plant material

The plant specimens for the study were collected

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in December 2008 from Government Agriculture College, Bilaspur (Chhattisgarh, India) 22°06′35.83″N and 82°08′06.23″E and were positively identified and authenticated by the Botanist Dr. Shiddamallayya N, Regional Research Institute (Ay.), Central council for research in Ayurveda and Siddha, Ashoka pillar, Jayanagar, Bangalore. A voucher specimen No. is (RRCBI/Mus.5-18), Reference No. (RRI/BNG/SMP/Drug Authentication/2008-09/957), dated 28/02/2009. Care was taken to select healthy fully grown plant with normal organs. The samples of root were cut suitably and removed from the plant and thoroughly washed with water to remove the adherent impurities. Then the roots were shade dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve.

2.2. Preparation of PFAE

Plumbagin was freed from the root by Soxhlet extraction with petroleum ether (60-80℃), then re-extracted with ethyl alcohol. The extract obtained contained only traces of plumbagin which were removed by further extraction with petroleum ether (60-80℃). The extract obtained was decanted, and concentrated at reduced pressure below 40℃ through rotary evaporator to obtain dry extract (19.56%). A suspension of this was prepared in distilled water using Tween-80 (1%).

2.3. Phytochemical screening

Phytochemical tests of PFAE of *P. zeylanica* Linn. root were carried out following the methods of Kokate[4]. Thin-layer chromatography studies were carried out following Wagner *et al*[5] to confirm the absence of plumbagin. The standard plumbagin was obtained from Hi Media, Mumbai, India. Silica gel 60 F_{254} pre coated sheets (E. Merck, Mumbai, India) was used as adsorbent. The plates were developed using chloroform: methanol (93:7) as mobile phase, in a Camag twin trough chamber to a distance of 8 cm each and iodine vapor was taken as a viewing medium.

2.4. Animals

Wistar albino rats of either sex were used for this study. The animals were procured and housed in the animal house of School of Pharmacy, Chouksey Engineering College, Bilaspur (Chhattisgarh). Six rats were taken for each group. The rats were acclimized to the laboratory environment for 7 days prior to the study. Animal house was well maintained under standard hygienic condition at (26±2)℃, relative humidity of 44%-55%, 12 h day and night cycle, and with food and water *ad libitum*. The animal experiment was performed as per Committee for the Purpose of Control and Supervision of Experiments on Animals norms after obtaining Institutional Animal Ethics Committee clearance.

2.5. Acute oral toxicity studies

Acute toxicity studies were carried out following Ghosh [6] to study the acute toxic effects and to determine the minimum lethal dose of the drug extracts. Swiss albino rats of either sex were used for the study. The alcohol and aqueous extracts were administered orally to overnight fasted animals at doses of 30, 100, 300, 1 000, 2 000 and 5 000 mg/kg of body weight (bw). After administration of the extracts, the animals were observed individually for mortality and clinical signs of toxicity.

2.6. Study of estrous cycle

Matured female Wistar albino rats (200-210 g) having regular estrous cycle were divided into three groups consisting of six animals in each group. One group served as control and received vehicle orally for 21 days. The other two groups received PFAE at a dose of 300 and 500 mg/kg bw/day respectively for 21 days. The estrous cycle was determined by stained preparation of vaginal smear of the animals[7]. After 21 days of treatment, extract was withdrawn from the rats and estrous cycle was studied for another 21 days, ie., post-extract period.

2.7. Study of reproductive outcome in rat

Three groups of mature female rats (six rats/group) were selected as mentioned above. Two groups received PFAE 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 rats and the treatment continued for 21 days. The number of litters was determined and compared after the completion of one gestation period. The reversibility of antifertility effect of the extract was also studied. For this study, the extract was administered continuously for 21 days and then the extract was withdrawn. After 21 days of extract withdrawal, animals were allowed to mate with male rats. The number of litters was determined after the completion of one gestation period[7].

2.8. Postcoital antifertility activity

Wistar strain female albino rats (150-200 g) of proven fertility and regular estrous cycle were caged with males of proven fertility in a ratio of 2:1. The females were examined the following morning for evidence of copulation. The animals, which showed thick clumps of spermatozoa in the vaginal smears, were separated and that day was designated as day 1 of pregnancy. The suspension of PFAE were administered orally through intragastric catheter from day 1 to 7 of pregnancy at 300 and 500 mg/kg bw, respectively to different groups of pregnant rats. Control animals received the vehicle (Tween-80, 1%, *p.o.*) only. The animals were laparotomised under light ether anesthesia and semi-sterile conditions on day 10 of pregnancy. Both horns of the uterus were observed for the number and size of implants. The rats

were allowed to recover and deliver after full term. Each fetus was weighed and examined for gross defects. The litters were allowed to grow to check their postnatal growth and monitor any congenital abnormalities[8].

2.9. Abortifacient activity

Rats at day 1 of pregnancy were divided into three groups, consisting of six animals in each group. The first group served as control and received vehicle only (Tween-80, 1%,) and group 2-3 received suspension of PFAE of *P. zeylanica* root (300 and 500 mg/kg) 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 on 10th day of pregnancy[9].

2.10. Estrogenic and antiestrogenic activity

PFAE at 500 mg/kg bw was found to be the most active in the post-coital antifertility testing. Hence, it was subjected to a detailed investigation for potential estrogenic and antiestrogenic activity. The uterine weight and vaginal cornification method was employed for this assay. Colony-bred immature ovariectomised female albino rats (Wistar strain), 21-23 days old and weighing between 30 and 40 g were used. They were divided into four groups consisting of eight animals in each group. The first group served as control and received the vehicle only (Tween-80, 1%). The second group received a suspension of ethinyl estradiol (Unicure Remedies Pvt. Ltd., Baroda, India) in distilled water using Tween-80, (1%) at a dose of 0.02 mg/kg bw. The third group received the PFAE at 500 mg/ kg bw and the fourth group received, in addition to 0.02 mg/kg bw of ethinyl estradiol, a test dose of PFAE at 500 mg/kg bw. All the above treatments were given orally for 7 days. Body weights and vaginal smears were recorded daily. Positive smears were those containing nucleated or cornified cells and not more than a few leucocytes.

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 0.000 1 g. 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 haematoxylin-eosin for histological examinations. The other portion of the uterus was homogenized with ice-cold distilled water in a pre-cooled mortar and pestle to contain 10 mg of tissue/mL. The homogenate was centrifuged in cold at 3 000 rpm for 15 min and the supernatant was used for the estimation of glucose, cholesterol and alkaline phosphatase using the standard methods[10].

2.11. Quantitative determination of hormones

The serum progesterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin were quantitatively determined following the procedure outlined in the manufacturer's protocol version which adopted the principle of Tietz[11] using ELISA kit (Kruise Pathline, Ahmedabad, India). Protein concentration of the homogenate was determined using the biuret method[12].

2.12. Toxicological investigations

Blood samples of each animal were collected by cardiac puncture. Haematological parameters were recorded[13]. Biochemical parameters were also estimated using reagent kits (Span Diagnostics, Surat, India).

2.13. Statistical analysis

All values are expressed as mean±SEM. Means were statistically analyzed by one-way analysis of variance (ANOVA), and values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Phytochemical screening

Phytochemical studies revealed the presence of glycosides, terpene and tannin. TLC of extract revealed the presence of four spots at R_f 0.06, 0.13, 0.22, 0.31 with no spot corresponding to that of plumbagin reference standard (R_f 0.66). The most prominent spot is at R_f 0.06.

3.2. Acute oral toxicity studies

Acute toxicity studies were carried out to evaluate toxicity and to determine the minimum lethal dose of the drug extracts, using Swiss albino rats. No mortality as well as any clinical sign of toxicity has been observed at a dose level of 5 000 mg/kg indicating that all the extracts comes under category 5 or unclassified according to Globally Harmonized Classification System, and hence, LD_{50} cut-off was found to be 5 000 mg/kg bw. Hence, one-tenth of this dose, *i.e.* up to 500 mg/kg bw, was used for antifertility investigation.

3.3. Effect of the extract on the estrous cycle and reproductive outcome

Treatment of rats with extract of 300 and 500 mg/kg bw for 21 days caused a dose dependent prolonged estrus cycle with significant increase in the duration of diestrus phase($P<0.01$) (Table 1). A dose dependent decrease was observed in case of number of litters produced, however

both the post treatment groups indicated an increase in the number of litters.

3.4. Postcoital antifertility activity

Anti-implantation activity is calculated as the percentage of animals indicating the absence of implantations in the uteri when laparatomy was performed on day 10 of pregnancy (Table 2). A significant anti-implantation activity was observed in a dose dependent manner($P<0.01$). All treatments significantly reduced the number of litters born confirming the antifertility activity of PFAE($P<0.01$). None of the treatments altered the number of corpora lutea, which was similar to that of the controls. The number of rats without implantation on day 10 was 0 in control group (0.00%), 2 in 300 mg/kg bw PFAE group (33.33%), and 3 in 500 mg/kg bw PFAE group (50.00%).

3.5. Abortifacient activity

A dose dependent abortifacient response was confirmed by the number of implants versus number of litters with

Table 1
Effect of PFAE on the estrous cycle of rat for 21 days and number of litters produced in different groups of rat (mean±SEM, n=6).

Groups		Observation parameters					
		Duration of cycle (days)	Proestrus phase (days)	Estrus phase (days)	Metestrus phase (days)	Diestrus phase (days)	No. of litters
Control		4.66±0.16	0.93±0.04	0.95±0.09	0.87±0.05	1.91±0.09	7.66±0.33
PFAE treatments	300 mg/kg bw/d	5.95±0.24*	0.51± 0.15*	1.21±0.08	0.56±0.03	3.67±0.12**	4.16±0.31**
	Post-treatment of 300 mg/kg bw/d	4.65±0.38	0.87±0.17	1.11±0.22	0.81±0.15	1.86±0.22	7.16±0.47
	500 mg/kg bw/d	5.91±0.56*	0.42±0.06**	1.37±0.11	0.45±0.08*	3.76±0.08**	2.66±0.33**
	Post-treatment of 500 mg/kg bw/d	4.83±0.32	0.41± 0.01**	1.31±0.08	0.73±0.09	2.38±0.14	5.83±0.61*

Data are analysed one-way ANOVA followed by Dunnet multiple comparison test. * $P<0.05$, ** $P<0.01$, significantly different from control.

Table 2
Postcoital antifertility activity of PFAE (mean±SEM, n=6).

Treatment	Dose(mg/kg bw)	No. of implantation sites	No. of corpora lutea	Total litter size
Control	–	9.00±0.26	9.16±0.16	9.16±0.31
PFAE	300	6.50±0.23**	8.83±0.31	4.16±0.47**
PFAE	500	5.16±0.41**	8.33±0.33	3.33±0.42**

Data are analysed one-way ANOVA followed by Dunnet multiple comparison test. ** $P<0.01$, significantly different from control.

Table 3
Abortifacient activity of PFAE (mean±SEM, n=6).

Treatment	Dose(mg/kg bw)	No. of implantation sites	Total litter size
Control	–	6.66±0.62	6.50±0.43
PFAE	300	5.66±0.98	4.33±0.49*
PFAE	500	5.83±0.41	3.50±0.81**

Data are analysed one-way ANOVA followed by Dunnet multiple comparison test. * $P<0.05$, ** $P<0.01$, significantly different from control.

Table 4
Histological changes in the uterus and endometrium after treatment with PFAE (mean±SEM, n=6).

Treatment	Dose(mg/kg bw)	Diameter of uterus(μ m)	Thickness of endometrium (μ m)	Height of endometrium epithelium (μ m)
Control	–	331.42±8.75	202.15±4.59	12.49±2.91
Ethinyl estradiol	0.02	819.3±13.14△△	382.34±7.35△△	44.44± 3.72△△
PFAE	500	308.12±9.26	190.35±3.95	11.08±1.51
Ethinyl estradiol + PFAE	0.02+500	501.95±4.97**△△	286.46±5.48**△△	29.61±3.07*△△

Data are analysed one-way ANOVA followed by Tukey multiple comparison test. △△ $P<0.001$, significantly different from control. * $P<0.01$, ** $P<0.001$, significantly different from ethinyl estradiol.

Table 5
Biochemical changes in the uterus after treatment with PFAE (mean±SEM, n=6).

Treatment	Dose(mg/kg bw)	Glucose (mg/100 g)	Cholesterol (mg/100 g)	Alkaline phosphatase (IU/100 g)
Control	–	0.98±0.03	5.68±0.08	0.63±0.07
Ethinyl estradiol	0.02	1.52±0.06△△	7.81±0.23△△	0.98±0.04△△
PFAE	500	0.93±0.05	5.67±0.05	0.57±0.01
Ethinyl estradiol + PFAE	0.02+500	1.12±0.06**△△	5.96±0.12**△△	0.68±0.02**△△

Data are analysed one-way ANOVA followed by Tukey multiple comparison test. △△ $P<0.001$, significantly different from control, ** $P<0.001$, significantly different from ethinyl estradiol.

2% resorption in control group, 40% at 500 mg/kg bw dose, and 23% at 300 mg/kg bw dose. All treatments significantly reduced the number of litters born indicating the antifertility activity of PFAE (Table 3) (P<0.01).

3.6. Estrogenic and antiestrogenic activity

Oral administration of the extract alone at 500 mg/kg bw did not show any significant changes in uterine weight [(70.48±2.13) mg/100 g bw *vs.* (67.31±1.51) mg/100 g bw] and uterine content of glucose, cholesterol and alkaline phosphatase compared with control group. The vagina remained closed and cornification was not induced (versus control). The uterotrophic changes such as diameter of the uterus, thickness of endometrium and height of endometrial epithelium were also insignificantly changed (versus control)(Table 4). A highly significant increase in the uterine weight[(312.36±5.24) mg/100 g bw] and uterine contents was observed in estrogen treated group (P<0.001) (Table 5). However, co-administration of ethinyl estradiol and extract caused a highly significant (P<0.001) decrease in uterine weight[(218.18±1.97) mg/100 g bw] when compared to estrogen treated group. The uterotropic changes were also significantly decreased (versus standard, P<0.001) (Table 4). A significant decrease in the uterine content of glucose, cholesterol and alkaline phosphatase was also observed (versus standard, P<0.001) (Table 5). Vaginal opening and cornification were also reduced as compare to estrogen treated group.

3.7. Hormonal analysis

The extract dose dependently decreased the concentration of serum progesterone, FSH and LH, while a dose dependent increase was observed in the concentration of serum prolactin (Figure 1).

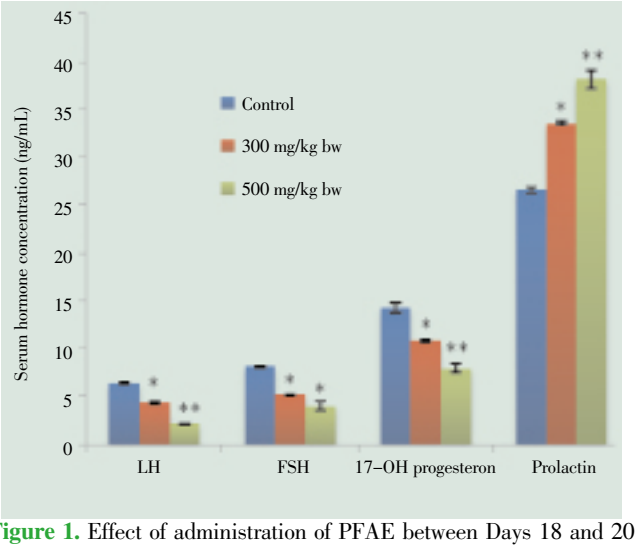

Figure 1. Effect of administration of PFAE between Days 18 and 20 of pregnancy on some reproductive hormones of female rats.
* P <0.05, ** P <0.01, significantly different from control.

3.8. Txicological investigation

Biochemical parameters in the serum/blood and haematological parameters did not show appreciable changes throughout and after the course of investigation

Table 6
Blood/serum biochemical profile of control and experimental female rats treated with PFAE (mean±SEM, n=6).

Group	Dose (mg/kg bw)	Protein (g/dL)	Albumin (g/dL)	Glucose (mg/dL)	SGOT (IU/L)	SGPT (IU/L)	Cholesterol (mg/dL)	ALP (U/L)	Creatinine (mg/dL)
Control	–	7.13±0.27	3.10±0.24	110.22±5.27	104.0± 6.76	85.99±4.51	130.01±3.27	328.69±5.68	0.71±0.10
Extract	300	6.41±0.11	2.91±0.10	102.08±5.25	111.24±3.21	87.28±6.21	97.09±1.15	311.10±4.12	0.52±0.12
Extract	500	6.98±0.31	3.23±0.12	104.13±5.91	96.11±4.32	82.26±4.27	108.02±1.19	302.15±5.12	0.65±0.04
Post-extract (30 days)	300	6.40±0.12	2.80±0.12	112.08±5.15	109.14±2.22	89.20±7.21	107.14±1.15	320.12±2.12	0.50±0.11
Post-extract(30 days)	500	6.80±0.22	3.18±0.10	108.12±9.21	94.12±3.22	78.16±5.28	118.12±2.10	312.11±4.12	0.63±0.02

Table 7
Haematological profile of control and experimental female rats treated with PFAE (mean±SEM, n=6).

Group	Dose (mg/kg bw)	RBC (10^6/μ L)	WBC (10^3/μ Ll)	Hb (g/dL)	PCV (%)	MCHC (%)	Clotting time(Sec.)	Platelet (10^3/μ L)	Differential leukocyte count				
									N(%)	B(%)	L(%)	M(%)	E(%)
Control	–	7.70± 0.39	14.55±5.78	15.03± 0.96	50.00± 3.68	32.18± 3.44	150.19± 12.83	450.86± 60.29	20.50±0.22	0.25±0.19	75.08±6.38	1.75±0.27	1.25±2.84
Extract	300	6.22± 0.23	14.22±6.09	12.35± 1.90	42.67± 4.23	32.42± 1.26	137.23± 19.92	553.43± 77.37	18.90±0.18	0.22±0.21	83.00±8.12	1.45±0.32	0.94±1.22
Extract	500	5.98± 0.21	12.20±3.23	12.14± 0.52	42.98± 3.12	27.89± 9.08	178.23± 6.73	552.11± 78.23	21.22±0.21	0.23±0.11	84.12±3.8	1.79±0.45	1.24±1.98
Post-extract (30 days)	300	6.10± 0.20	13.34±4.23	14.50± 1.32	46.23± 2.12	32.15± 1.34	147.20± 16.72	512.40± 66.23	19.02±1.23	0.18±0.20	82.00±7.22	2.35±0.12	0.95±1.02
Post-extract (30 days)	500	6.24± 0.12	14.14±2.34	14.34± 0.43	46.98± 2.13	29.09± 6.78	160.16± 6.18	502.96± 68.23	20.19±0.99	0.19±0.12	89.01±2.34	1.99±0.12	1.34±1.21

* P <0.05, ** P <0.01, significantly different from control.

(Table 6 & 7). However, all the induced effects were reversible following withdrawal of treatment.

4. Discussion

Study on the effect of extract on the estrus cycle of rat indicating the significant increase in the length of diestrus phase. These findings are comparable with the studies of Gebrie *et al*[14], Pattanayak and Mazumder[10] who had reported antifertility effect with similar observations in guinea pigs, and in rats on the treatment with root extract of *Rumex steudelii* and extract of aerial parts of *Dendrophthoe falcate*. However, a significant decrease in the duration of proestrus and metestrus phase in the treated groups was recorded than those of control animals. These changes were found to revert back after withdrawal of the treatment except the proestrus phase in groups treated with higher group. These observations are correlated with the findings of Ganguly *et al*[15] who had reported antifertility effect with similar observations in mice on the treatment with leaf extract of *Cissampelos pareira*.

The prolongation of diestrus phase and significant decrease in proestrus and metestrus phase may lower the chances of pregnancy by interfering with the secretion of estrogen and progesterone because a proper estrogen/ progesterone balance is required for uterine receptivity to the embryo. A decrease in the mean number of litters in the treated groups is also suggesting the antifertility activity of the extract. The number of litters was found to decrease more with higher dose of treatment, which may suggest dose dependent antifertility effect. All the litters of treated rats grew up normally without having any physical abnormality indicating that the extract is not teratogenic. An increase in the number of litters observed in both the post treatment groups may indicate the reversible antifertility effect.

Postcoital antifertility study showed the anti-implantation activity in the treated animals. The number of litters born due to this treatment was significantly less than controls. This indicates the abortifacient nature of extract which was evidenced by the study on abortifacient activity. Both response were dose dependent and increased at higher doses. An increase in the resorption index (%) by the extract is an indication of failure in the development of the embryo, a rate which was dose-dependent in this study. Such occurrence of fetal resorption suggests that interruption of pregnancy also occurred after implantation. 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. Hence, the present investigation clearly reveals that the extract is effective before and after the implantation occurred.

In the present study, specific hormones were assayed based on their roles in maintaining pregnancy, since a failing pregnancy could be correlated to the levels of these hormones[16] in the body fluids. The reduction in the concentration of FSH is an indication of disturbance of estrus cycle and ovulation[15]. 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[16]. It could possibly be attributed to pregnancy failure resulting from a luteal phase that is not being maintained. The reduced level of hormone may also be due to inactivation of lutenization 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 the *P. zeylanica* root extract in this work. These findings in the present study agree with that of Yakubu and Bukoye[16] on the effect of *Inula viscose* and *Bambusa vulgaris* leaf extract on implantation and abortion in rats and rabbits. In this study, an increase in prolactin level was observed. This finding is comparable with studies made by Ganguly[15] who reported that a combination of enhanced prolacin and suppressed LH secretion is due to prolongation of estrus cycle. An imbalance in endogenous estrogen and progesterone levels could be responsible for Anti-implantation activity[17].

In another set of experiment on immature rats, we observed that the PFAE exhibited anti-estrogenic activity along with ethinyl estradiol as shown by the significant decrease in uterine weight, diameter of uterus, thickness of endometrium, height of endometrial epithelium and vaginal cornification compared with standard. These findings are similar to that of Ravichandran *et al*[18] and Vishnukanta and Rana[2] on the effect of hydroalcoholic extract of *Ailanthus excelsa* (Roxb) stem bark and *P. zeylanica* L. leaves on uterus of female wistar rats. The treatment also caused a significant decrease in uterine content glucose, cholesterol and alkaline phosphatase compared with standard. This study revealed that the extract acted as a competitive antagonist to much more potent ethinyl estradiol. It is well known that for implantation the exact equilibrium of estrogen and progesterone is essential, and any disturbance in the level of these hormones may cause infertility[19]. The compounds having hormonal values usually disturb the hormonal milieu in the uterus and provoke an infertility effect[8]. In species such as rat, mouse and gerbil that exhibit facultative delay of implantation, nidatory estrogen secreted within about 24 h of initiation of implantation is necessary for induction of endometrial receptivity to blastocyst signal (s). Estrogen within a very narrow range determines the duration of window of uterine receptivity, and while the window of uterine receptivity remains open for an extended period at lower estrogen levels, it rapidly closes at higher levels. The uterine refractoriness that follows the receptive state at high estrogen levels is accompanied by aberrant endometrial expression of receptivity/implantation-related

genes. Based on this, careful regulation of estrogen levels for improvement of female fertility in *in-vitro* fertilization/ embryo transfer programs has been suggested[20]. Hence, the anti-implantation activity of the extract may also be due to antiestrogenic activity of extract which antagonizes the action of estrogen, causes structural and functional changes in uterus and finally decreases the implantation.

Nontoxicity of PFAE was evident by the unaltered haematological and serum clinical parameters which indicate normal functioning of vital organs.

The Phytochemical test and TLC study on PFAE revealed the absence of steroid and plumbagin which was removed by petroleum ether extraction. The root has been reported to be devoid of any alkaloid as confirmed by us[21]. However, the antifertility activity may be due to the presence of other phytoconstituents like glycoside, terpene and tannin. In conclusion, the results of this study confirm the reversible antifertility potentiality of PFAE of *P. zeylanica* Linn root which may be attributed, at least in part, to the phytoconstituents like glycoside, terpene and tannin. The extract has significant anti-implantation, abortifacient and antiestrogenic activity and is safe at the effective antifertility doses used in this study. It also possesses a significant effect on estrous cycle and hormonal level of female rats. Further work on isolation of active component(s) responsible for the antifertility activity is underway.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

I express my sincere thanks to Dr. Dheeraj Ahirwar (Principal) and Management of School of Pharmacy, Chouksey Engineering College, Bilaspur (C.G.), who took interest in looking into our research needs and thus providing us with the best available resources.

References

[1] Kirtikar KR, Basu BD. *Indian medicinal plants*. Dehradun: Shiva publishers; 1993, p. 1466-1468.

[2] Vishnukanta, Rana AC. Evaluation of antifertility activity of the hydroalcoholic extract of the leaves of *Plumbago zeylanica* L. (Plumbaginaceae) in female wistar rats. *Ind J Pharm Educ Res* 2010; **44** (1): 49-55.

[3] Azad Chowdhury AK, Sushanta KC, Azadkhan AK. Antifertility activity of *Plumbago zeylanica* Linn root. *Indian J Med Res* 1982; **76**: 99-101.

[4] Kokate CK. *Practical pharmacognosy*. New Delhi: Vallabh Prakashan; 1999, p.107-108.

[5] Wagner H, Baldt S, Zgainski EM. *Plant drug analysis*. Berlin, Heidelberg, New York, Tokyo: Springer-Verlag; 1984, p. 276.

[6] Ghose MN. *Fundamentals of experimental pharmacology*. Kolkata: Hilton; 2005, p. 182-183.

[7] Ganguly M, Borthakur MK, Devi N, Mahanta R. Effect of *Mimosa pudica* root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. *Contaception* 2007; **76**: 482-485.

[8] Vasudeva N, Sharma SK. Post-coital antifertility activity of *Achyranthes aspera* Linn. root. *J Ethnopharmacol* 2006; **107**: 179-181.

[9] Savadi RV, Alagawadi KR. Antifertility activity of ethanolic extracts of *Plumbago indica* and *Aerva lanata* on albino rats. *Int J Green Pharmacy* 2009; **3** (3): 230-233.

[10] Pattanayak SP, Mazumder PM. Effect of *Dendrophthoe falcata* (L.f.) Ettingsh on female reproductive system in Wistar rats: a focus on antifertility efficacy. *Contraception* 2009; **80**: 314-320.

[11] Tietz NW. *Clinical guide to laboratory tests*. 3rd ed. Philadelphia: W.B. Saunders, Co.; 1995.

[12] Plummer DT. *An introduction to practical biochemistry*. 2nd ed. London: Mc Graw-Hill; 1978.

[13] Sharma N, Jacob D. Assessment of reversible contraceptive efficacy of methanol extract of *Mentha arvensis* L leaves in male albino mice. *J Ethnopharmacology* 2002; **80**: 9-13.

[14] Gebrie E, Makonnen E, Debella A, Zerihun L. Phytochemical screening and pharmacological evaluations for the antifertility effect of the methanolic root extract of *Rumex steudelii*. *J Ethnopharmacology* 2005; **96**: 139-143.

[15] Ganguly M, Borthakur MK, Devi N, Mahanta R. Antifertility activity of the methanolic leaf extract of *Cissampelos pareira* in female albino mice. *J Ethnopharmacology* 2007; **111**: 688-691.

[16] Yakubu MT, Bukoye BB. Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Contraception* 2009; **80**: 308-313.

[17] Dhanwad R, Purohit MG, Patil SB, Satyanarayan ND. Antiimplantation activity of ethanolic extract of *Cardiospermum halicacabum* L. (Sapindaceae) in albino rats. *Indian Drugs* 2005; **42**: 726-730.

[18] Ravichandran V, Suresh B, Sathishkumar MN, Elango K, Srinivasan R. Antifertility activity of hydroalcoholic extract of *Ailanthus excelsa* (Roxb): An ethnomedicines used by tribals of Nilgiris region in Tamilnadu. *J Ethnopharmacol* 2007; **112**: 189-191.

[19] Madhavan V, Kumar BHP, Murali A, Yoganarasimhan SN. Antifertility activity of *Drosera burmannii*. *Pharmaceutical Biol* 2009; **47**(2): 128-131.

[20] Keshri G, Kumar S, Kulshreshtha DK, Rajendran SM, Singh MM. Postcoital interceptive activity of *Wrightia tinctoria* in Sprague-Dawley rats: a preliminary study. *Contraception* 2008; **78**: 266-270.

[21] Gupta S, Ahirwar D, Jhade D, Sharma NK, Ahirwar B, Muthal N. Pharmacognostic standardization, physico and phytochemical evaluation of *Plumbago zeylanica* Linn. root. *Drug Invention Today* 2010; **2**(9): 408-410.