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Plumbagin extract of Plumbago zeylanica root on reproductive system of female Albino Rats

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ABSTRACT: Plumbago zeylanica Linn. (Plumbaginaceae), a plant utilised as a widespread folk remedy and antifertility agent, was examined for its antifertility activity in the current study. The effects of 200 and 400 mg/kg of five sequential solvent extracts, including petroleum ether, chloroform, acetone, ethanol, and water, on the estrous cycle were investigated. Only the acetone extract was shown to be the most successful at stopping the rats' typical estrous cycle (p0.05, p0.01, and p0.00). The rats' diestrous portion of the estrous cycle was lengthened, which temporarily inhibited ovulation. When the extract was removed, the anovulatory activity was reversible. The functioning of oestrogen in rats was further investigated using the efficient acetone extract. In comparison to the control, the extract demonstrated strong estrogenic and antiestrogenic action (p0.05), (p0.01), and (p0.001). Studies on the uteri's histology were done to verify the estrogenic activity. The findings showed that Plumbago zeylanica stem extract had an antifertility effect on albino rats.
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1. INTRODUCTION

Plumbago zeylanica (P. zeylanica), more commonly known as white chitrak (Family: Plumbaginaceae), is a perennial herb that is grown throughout most of India. It is used in the traditional system of Indian medicine to treat a variety of conditions, including skin disease, abdomen enlargement, anemia, diabetes, leprosy, dyspepsia, elephantiasis, diarrhea, and leprosy [1]. Studies on P. zeylanica Linn. carried out by a number of researchers have revealed that the plant has pharmacological activities including those of an astringent, diuretic, antibacterial, antifungal, anticarcinogenic, anticancer, and radiomodifying agent.

It has been said that the roots of this plant contain a potent poison that, if taken orally or administered to the ostium uteri, might cause a miscarriage or an abortion[2]. Plumbagin is a crystalline naphthoquinone molecule that was obtained from root extract. Although it had effect against fertility, researchers discovered that it was hazardous. The preliminary research on the plumbagin free alcohol extract of the root demonstrated antifertility effectiveness without the presence of any side responses [3]. However, the precise mechanism by which an antifertility agent works is not yet fully understood.

As a result, the current investigation was carried out to verify the precise mechanism of antifertility action in female rats utilising a variety of models, as well as to calculate its oral LD50.

2. Materials and methods

Plant material

Roots of P. zeylanica were collected from Udhampur district of Jammu and kashmir and positively identified by Prof. Faisal Mustaq, Botanist, Govt. Degree College, Kishtwar, J&K.

Preparation of Extracts

The plant's stems were dried in the shade before being ground into powder. In a Soxhlet apparatus, the powdered material was first extracted for 72 hours using petroleum ether at temperatures between 60 and 80 degrees Celsius, and then it was extracted using chloroform, acetone, ethanol, and water for 72 hours each. After evaporating the extracts at a lower temperature and pressure, solid masses were

obtained, and the percentage yield of each extract was determined to be 1.23, 1.26, 4.67, 3.65, and 19.23% accordingly.

Phytochemical Screening

A preliminary phytochemical investigation (color reactions) using plant extracts was undertaken in order to evaluate the presence of alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, lipids, and sugars. This was accomplished by the utilisation of plant extracts.

Plumbagin in Various Extracts

The HPTLC method was used to further confirm the napthaquinone found in the petroleum ether, chloroform, and acetone extracts. 10 mg of plumbagin (National Chemicals, Baroda, India) was dissolved in 10 ml of petroleum ether to create a standard solution with a concentration of 1 mg/ml, and 100 mg of extracts were dissolved in 10 ml of the appropriate solvent to create sample solutions with a concentration of 10 mg/ml. For the investigation, a Camag HPTLC system (Switzerland) was employed together with a precoated aluminium silica gel F25 plate (Merck), a twin trough liner development chamber, a Linomat IV sample applicator, and integration software CATS4.06 (Switzerland). Using a Camag Linomat IV applicator, 5 l of reference plumbagin (1 mg/ml) and 5 l of sample solutions (extracts) (10 mg/ml) were applied as a 6 mm band width from roughly 1 cm of the border of the HPTLC plate. The solvent system used was hexane, ethyl acetate, chloroform, and acetic acid. Using a TLC scanner, the chromatogram was produced and scanned at 366 nm.

Animal

Albino rats (females) of wistar strain (150 ± 10 g b.w.) and swiss albino female mice (20 ±10 g b.w.) were used in this study. The rats were placed in plastic cages of 36x35x19 cm in dimensions. Animals were housed under standard husbandry condition of temperature (24 ± 2C), light (photocycle of 14 h light and 10 h dark) and relative humidity (60 % to 70 %). The animals were fed on standard pellet diet (Pranav agro industries, New Delhi) and water ad libitum. Animals were treated and cared for in accordance with the guidelines recommended by “the Committee for the Purpose of Control and Supervision of Experiments on Animals”, Govt. of India.

Anovulatory Activity

Research using female Wistar rats weighing between 150 and 200 g was done [16]. Each rat's vaginal smear was inspected everyday between 9 and 10 in the morning for 15 days to identify the rats with regular cycles. The chosen rats were split into 11 groups of six each. For five days, the extracts were taken orally to cover one typical estrous cycle. Group I functioned as the control group and got the vehicle (1% Tween 80, p.o. daily). Petroleum ether, chloroform, acetone, ethanol, and aqueous extracts of P. zeylanica stems were given to Groups II to XI at doses of 200 mg/kg and 400 mg/kg body weight. Each animal's vaginal smear was examined daily between the hours of 9 and 10 in the morning for the first five days of therapy and for the next 15 days.

Estrogenic and Antiestrogenic Activity

The extract, which demonstrated anovulatory activity, was subsequently examined for the estrogenic and antiestrogenic activity. Six groups (n = 6) of immature female Wistar strain rats were created. The rats were 21–23 days old and weighed between 35–45 g. The first group functioned as control and received only vehicle (1% Tween 80). The second group was given 0.02 mg/kg body weight of ethinyl estradiol (standard) (Rajesh m Chemicals, Indore, India) dissolved in distilled water with Tween-80 (1%). The third and fourth groups received acetone extract of P. zeylanica at two dose levels, 200 and 400 mg/kg body weight correspondingly. The test dose of the plant's acetone extract was given to groups five and six at the same dose together with ethinyl estradiol. Three days were spent administering each of the aforementioned treatments. The rats were killed by decapitation on the fourth day, and the uteri and surrounding tissues were taken. The uteri were promptly weighed on a sensitive balance after being wiped on filter sheets, and they were then fixed in Bouin's solution for 24 hours.

Statistical Analysis

The data underwent statistical analysis, and the results were expressed using mean and standard error of the mean. The student's t-test was utilised in the process of doing statistical analysis on the differences in value between the control group and the experimental group.

Table 1. Preliminary Phytochemical Studies on Various Extract of P. zeylanica

Plant extracts	Constituents
Pz-P	Fats, steroids and napthaquinone
Pz-C	Steroids and napthaquinone
Pz-A	Tannins, flavonoids, triterpenoids and napthaquinone
Pz-E	Carbohydrates, glycosides, tannins, flavonoids and saponins
Pz-W	Carbohydrates, glycosides, tannins, flavonoids and saponins

Pz- Plumbago zeylanica,P-petroleum ether extract, C-chloroform extract, A-acetone extract, E-ethanolic extract, W-aqueous extract.

3. RESULTS

Examination of Phytochemicals

The phytochemical analysis of a number of different extracts revealed the presence of a number of distinct ingredients, which are detailed in table 1. The Rf value of the standard plumbagin was determined to be 0.94, and so was the plumbagin peak in the extracts (Fig 1). The amount of plumbagin that was present in the extracts was determined by comparing the peak area of the standard to that of the extracts. It was discovered that the amounts of plumbagin present in stems extracted using petroleum ether, chloroform, and acetone were respectively 1.94%, 0.54%, and 2.65%. These percentages may be seen in Figures.

Acute Toxicity Studies

Treatment groups up to 2000 mg/kg body weight had no mortality or behavioural, neurological, or autonomic abnormalities. The results selected 200 and 400 mg/kg dosages for future testing.

Effect of Extract on the Estrous Cycle of Rats

The results of the current investigation suggested that the antifertility effect was caused by the acetone extract of P. zeylanica stems. We employed rats that displayed a typical estrous cycle. As shown in Table 2, administration of acetone extract to rats for 5 days significantly (p0.05), (p0.01), and (p0.001) lengthened the estrous cycle in comparison to the control group. Rats given acetone extract for their estrous cycles saw shorter estrous and metestrous phases, while on the other side, their diestrous phases lasted longer. Only in the diestrous phase did the two dosages of acetone extract differ significantly from one another. When the treatment was stopped, it was discovered that the estrous cycle may be reversed (Table 3). All remaining extracts were discovered to be inert, with the exception of the acetone extract. Table illustrates how P. zeylanica stems' acetone extract affected developing rat uteri. When compared to controls, oral administration of the extract at 200 and 400 mg/kg body weight significantly increased the weight of the uterus in immature rats (p0.05, p0.01, and p0.001). There was no discernible difference between the two dosages of the acetone extracts in terms of estrogenic and antiestrogenic action. When compared to the control rats, the endometrial epithelium's height and thickness both dramatically increased. Endometrial glands were dilated, and the epithelium of the endometrium was made up of spindle-shaped cells with basal nuclei. The stroma was made up of fibroblast-like cells that were loose and oedematous. The treated rats displayed an open vagina while the control rats had closed vagina.

Table 4. Estrogenic and Antiestrogenic Activity of Acetone Extract of P. zeylanica Stem

Groups	Dose (mg/kg body weight)	Uterine weight (mg/100 g body weight)
Control	(Tween-80, 1%)	45.50 ± 2.27
Ethinyl estradiol	0.02	142.00 ± 4.83***
Pz. Acetone	200	60.17 ± 2.98**
Pz. Acetone	400	66.19 ± 3.48***
Ethinyl estradiol + Pz. Acetone	0.02 + 200	128.13 ± 3.70***†
Ethinyl estradiol + Pz. Acetone	0.02 + 400	120.87 ± 3.97***‡

Pz- P. zeylanica

Values are expressed in mean ± SEM, n=6, **P<0.01, ***P<0.001 Vs Control, P<0.05 Vs Ethinyl estradiol (Students ‘t’ test)

DISCUSSION

Compared to control, P. zeylanica stem acetone extract had considerable antifertility effect ($p<0.05$, $p<0.01$, $p<0.001$). Rats have 4-5-day estrous cycles. A normal rat estrous cycle's vaginal smear exhibits three cell types under a microscope. The stages of the rat's estrous cycle can be detected by their presence and proportion. Only acetone extract altered the estrous cycle temporarily. Diestrous phase extension explains rats' low fertility. The extract's antifertility action is reversible because the diestrous and estrous cycles did not change after withdrawal from the control. The extract inhibited ovulation and cyclicity. Ovarian oestrogen production, controlled by pituitary gonadotropins and hypothalamic-releasing factor, controls the estrous cycle. The acetone extract was tested for estrogenic and antiestrogenic activity after showing anovulatory activity. Compared to the control, the extract increased uterine diameter, weight, and endometrial epithelial thickness, indicating estrogenic activity. The acetone extract also inhibited ethinyl estradiol. With ethinyl estradiol, the extract was slightly antiestrogenic, but alone it was estrogenic. Extract competed with the strong ethinyl estradiol. In preliminary phytochemical analyses, the acetone extract contained tannins, flavonoids, triterpenoids, and napthaquinone. Flavonoids and plumbagin (napthaquinone) are reported to have antifertility properties. Flavonoids and napthaquinones may explain our study's activity. The petroleum ether and chloroform extracts had napthaquinones, but no action. The rationale may be that petroleum ether, chloroform, ethanol, and aqueous extract do not contain enough of the active ingredient (flavonoid or napthaquinone) responsible for antifertility action, or that acetone extract has antifertility activity due to napthaquinone-flavonoid synergism.

CONCLUSION

The results of the present study conclude that the acetone extract of P. zeylanica stems have significant antifertility activity and it could be used as an alternative medicine instead of roots. The extract of this plant can further be developed into a contraceptive and uprooting of this plant could be avoided.

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