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EVALUATION OF ANTI-OVULATORY ACTIVITY OF *PAEONIALACTIFLORA* IN FEMALE WISTAR RAT

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Abstract:

In the present study, anti-ovulatory activity was evaluated in methanolic extract of Paeonia lactiflora. The extract is found to be a rich source of paeoniflorinknown for reducing fertility. Two different concentrations of methanolic root extracts of Paeonia lactiflorawere evaluated for female antifertility activity on albino wistar rats. Methanolic root extract of Paeonia lactiflora at the dose of 250 and 500 mg/kg b.w was administered orally for 15 days. Treatments were stopped thereafter and animals were sacrificed after a recovery period. The methanolic extract of Paeonia lactiflorashowed significant anti-ovulatory activity. Pre-treatment with methanolic extract showed significant effect on estrous cycle, ovarian weight &cholesterol level at a dose of 500 mg/kg.

Key words: Paeonia lactiflora, roots, anti-ovulatory activity, ovaries, estrous cycle.

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INTRODUCTION

India within, few years of time span will be the leading country as far as the population growth is concerned. Since the population is rising tremendously, this may affect drastically the economic growth of India. Family planning has been promoted through several methods of contraception, but due to side effects produced by the use of steroidal contraceptive and use of abortifaciant drugsthere is a need of drug which is effective with lesser side effects[1]. The plant *Paeonialactiflora*is proved and described in Pharmacopoeias & well stabilised documents shown the anti-bacterial, anti-influenza[2], anti-viral[2], anti-inflammatory[3], anti-spasmodic activity, anti- fertility activity[4]. The plant *Paeonia lactiflora* also used to treat dementia, headache, vertigo, spasm of calf muscle[4-5]. The dried root of *Paeonia lactiflora* contains Paeoniflorin, a monoterpene glycoside that is the major active constituent, is present in the range of 0.05–6.01%. Traditionally *Paeonialactiflora* plant used in treatment of premenstrual syndrome (PMS), for starting menstruation or causing an abortionatopic eczema boils, sores to reduce fevers, induce sterility, and treat burns. The aim of the present study was to evaluate antiovulatory activity of *paeonia Lactiflora* in female albino rats.

MATERIALS AND METHODS

Plant material

Thefreshrootsof *Paeonia lactiflora* wascollectedandauthenticatedbyDr.GajendraRao,SurveyOfficer,RegionalResearchInstitute,Bangalore.A specimensampleofthesamewaspreservedintheherbariumsectionat RRI, Bangalore, asRRCBI,AccNo.1693forfuturereference.The root of *Paeonia lactiflora*were,choppedintosmallpiecesand driedundershadeatroomtemperatureforsevendays.Thedriedrootswas powderedandpassedthroughthesieve(coarse10/40).Thepowderwasusedforthe preparation of extract.Extraction was done by soxhletextraction process by using Petroleum ether and 95% w/v methanol[6-7]. The percentage yield was found to be 14.2% w/w. Preliminary phytochemical studies showed the presence of flavonoids, glycoside, carbohydrates, tannins, amino acid and sterols etc.

Animals

Female albino rats (Wistarsrain weighing 150-200 g) were obtained from animalhouse for the study. They were housed under standard condition of temperature (24 ±10 C), relative humidity (65±10%), light and dark cycle (14:10 h) and fed with standard pellet food. The initial body weight of each animal was recorded. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experimentation on Animals and were approved by the Institutional Animal Ethics Committee. (IAEC No.- Pharm/13/06)

Acute Toxicity Study [8-9]

Acute toxicity study of methanolic extract of *Paeonia lactiflora* were carried out in mice according to OECD guidelines. Extract at different doses up to 5000 mg/kg, p.o. was administered and animals were observed for behavioural changes, any toxicity and mortality up to 48 h. There was no toxic reaction or mortality, and found to be safe. Based on acute toxicity result we have selected 250 mg/kg and 500 mg/kg for antiovulatory evaluation.

Anti-ovulatory Activity

Experiments were carried out in female wistarrats. Thetreatmentwasgivenfor15daystocover threeregularestrouscycles. The selected rats were divided into three groups ofsix animals each.The treatment wasstarted when the animalswere in the estrous phase[10].Vaginalsmearwasobservedeverymorning at9-10A.M.Onthe16th day,24hrafterthelasttreatmentthe animalsfromeachgroupweresacrificed,ovariesanduteriweredissectedoutfreedfromextradispositionandweighedonasensitivebalance.Oneovaryfromeachanimalwasprocessedforbiochemicalanalysisofcholesterol.Theotherovarywasfixed in formalin bufferfor histological studies.

Statistical Analysis

Theresultsareexpressedasmean±SEM.Comparisonbetweenthetreatment groupsandcontrolgroupswereperformedby Student's*t*–test.

RESULTS

Phytochemical Screening

The phytochemical screening of different various extract of *Paeonia lactiflora* roots revealed the presence of various constituents as shown in table no.1

Table 1: Phytochemical Screening of Different Various Extract of *Paeonia Lactiflora* Roots

Phytochemical constituents	Petroleum Ether extract	Methanolic extract
Alkaloids	-	-
Flavanoids	+	+
Carbohydrate	+	-
Saponins	+	+
Triterpens	+	+
Tannins	+	+
Glycosides	-	+

+ VE = Present -VE = Absent

Table2: Effect of Methanolic Extract of *Paeonia Lactiflora* on Different Phases of Estrouscycles

S.No	Treatment	Dose	Duration of cycles(Days)	Duration of different phases of estrous cycle (days)			
				Proestrous	Estrous	Metestrous	Diestrous
1	Control	--	4.82±0.29	1.02±0.39	0.96±0.31	0.98±0.24	1.86±0.40
2	Methanolic extract-I	250	5.15±0.32	1.32±0.00 ^{a}	0.86±0.21	0.93±0.21 ^{c}	2.04±0.30
3	Methanolic extract-II	500	4.57±0.49	1.12±0.33 ^{b}	0.65±0.21 ^{a}	0.71±0.22 ^{c}	2.09±0.21

P< 0.05,when compared with control. b-P<0.01, when compared with control.c-P<0.001, when compared with control Values are represented as mean ± S.E.M (n=6)

Effectofextracton Estrouscycles of Female Wistar Rats

The present study revealed that the methanolic extractso f *Paeonia lactiflora root*showeda n a n t i o v u l a t o r y e f f e c t . T r e a t m e n t s o f r a t ' s w i t h methanolicextract prolonged the estrous cycle significantly as indicated in table no.2. The estrous cycle in rats treated with methanolic extracts showed significantly decrease in the duration of estrous and metestrous phase by prolongation in the diestrous phase and proestrous phase [11].Withdrawal of the treatment did not indicate any significant change either in the four phases of the estrous cycle or in the duration of the cycle.

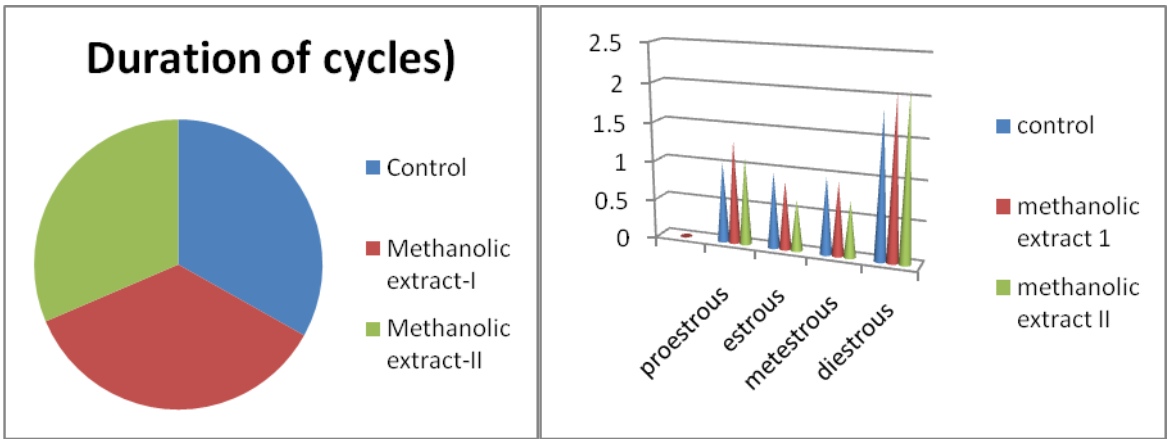


Fig 1 and Fig 2: Mean duration of estrous cycle

Table3: Effect of methanolic extract of *Paeonia lactiflora* on ovarian weight

Group	Treatment	Dose	Ovarian weight in mg
1	Control	----	48.30 ±0.22
2	Methanolic extract- I	250	41.22 ±0.21*
3	Methanolic extract-II	500	36.01 ±0.24

** P<0.01 when compared to the control.Values are represented as mean ± S.E.M (n=6)*

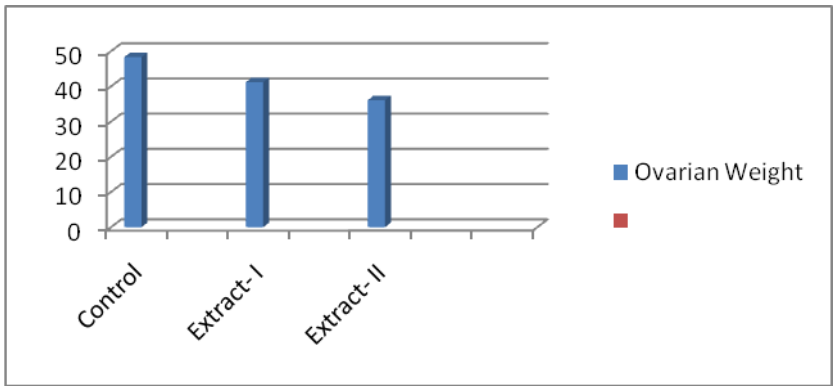


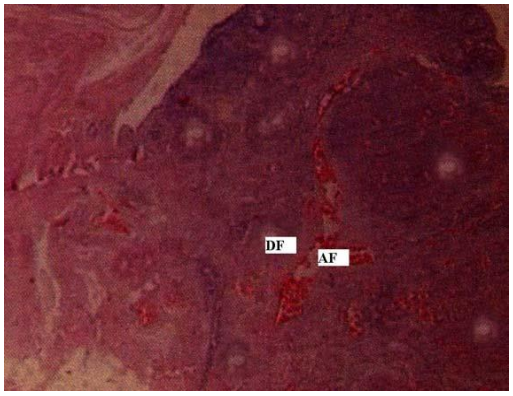
Fig 3: Mean ovarian weight

Effect of extract on Ovarian Weight of Female Wistar Rats

Th e effect of methanolic extract of roots at doses of 250 and 500 mg/kg body weight caused a significant decrease in the ovarian weight when compared with the control group.

Histopathology Effectofextractin Ovary of Female Wistar Rats

Section of the ovary treated with the control showed matured graffain follicle and developing follicles butt hetreated groups at 250 and 500mg/kg body weight did not show matured graffianfollicles and showed increased number of developing follicles, atreticfollicles and disorganized stroma cells.



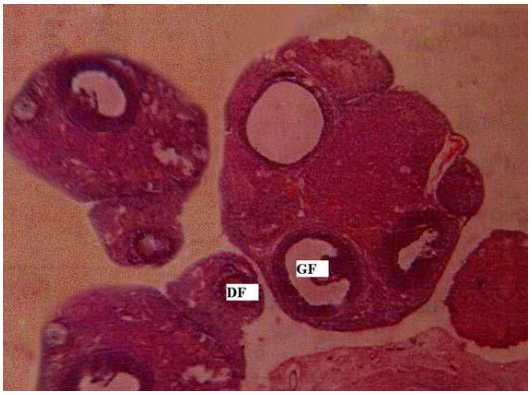


Fig 4: Photomicrograph of a transverse section of the ovary of Methanolic extract,500mg/kg p.o treated rats

Fig 5: Photomicrograph of a transverse section of the ovary of control rats

Table4: Effect of Methanolic Extract of *Paeonia Lactiflora* on cholesterol Level in Ovary

Group	Treatment	Dose (mg/kg body weight)	Cholesterol level in ovary *(mcg/mg of ovary)*
I	Control	----	2.39 ±0.005
II	Methanolic extract-I	250	3.87 ±0.07 *
III	Methanolic extract-II	500	4.68 ±0.02 *

** P<0.01 when compared to the control ,Values are represented as mean ± S.E.M (n=6)*

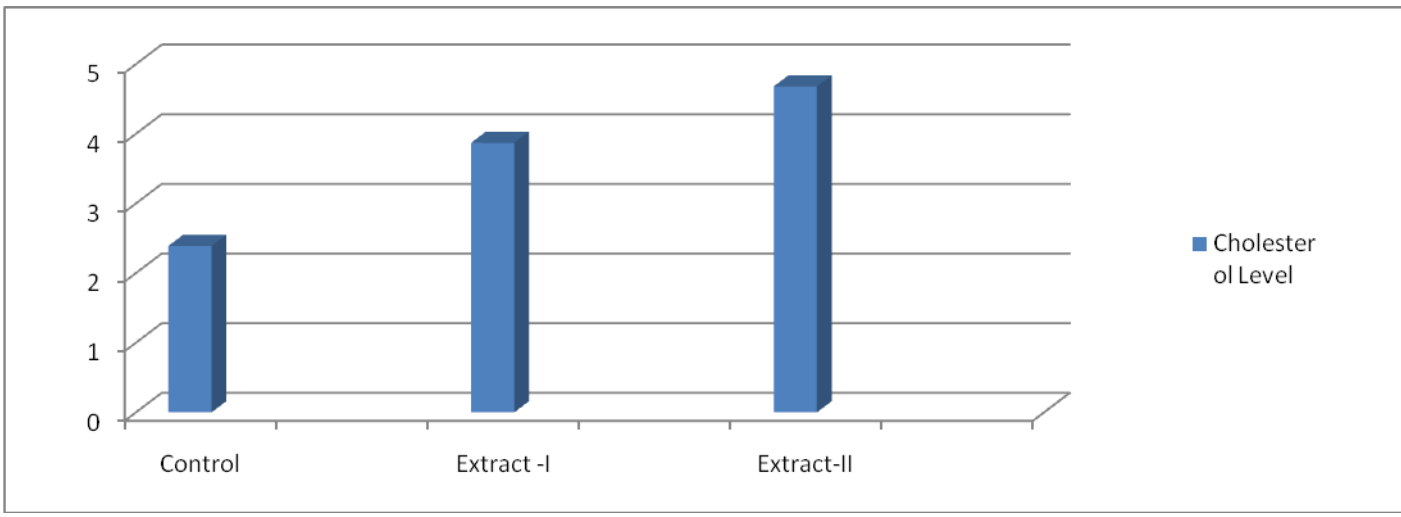


Fig 7: Mean cholesterol level

Effect of extract on Cholesterol level in ovary of Female Wistar Rats

It has been found that administration of Methanolic extract showed dose dependant effect in the cholesterol level. Increase in Cholesterol level was significant at 250 & 500mg/kg body weight when compared to control.

DISCUSSION [12-15]:

Preliminary phytochemical studies indicated the presence of tannins, flavonoids, triterpenoids and Paeoniflorin in the methanolic extract. According to the literatures, flavonoids and Paeoniflorin,are known to exhibit antifertility activity. Themethanolic extractof*Paeonialactiflora* atthedoseof250&500mg/kgshowed a significant effect on rat estrous cycle, increasedALPandcholesterollevelsandamildhyperce llularityofleydigcellsinthehistopathologicalobservati on. The prolongation in the diestrous phase explains the remote possibility of the rats getting pregnancy. The reversible nature of the antifertility activity of the extract is explained through the observation that there was no significant change in the diestrous and the estrous cycle after withdrawing the extract from those of the control. As a result, the extracts provoked inhibition of the ovulation with consequent reduction of the cyclicity. Estrous cycle and the shift in different stages are mainly governed by the synthesis of ovarian estrogen, which, in turn, is controlled by the secretion of pituitary gonadotropins and hypothalamic-releasing factor.

CONCLUSION

The results of the present study indicate that the methanolic extracts of *Paeonia lactiflora*roots have significant antifertility activity. The roots of this plant could be used to induce abortion. The extracts of this plant can be further explored for contraceptive use.

Competing interests

The author's declare that they have no competing interests

Author's contributions

Agarwal SK performed overall supervision of the research project; Swapna D carried out pharmacological and experimental research work, statistical,analysis and interpretation of data;Kiranmai.G carried out the pharmacognostical studies and performed acquisition of data and manuscript drafting. All authors read and approved the final manuscript.

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