

Antifertility activity of Chloroform and Alcoholic flower extracts of *Leucas cephalotes* (Roth.) Spreng. in albino rats

Reena Bhoria¹, Sushma Kainsa^{2*}, Manjusha Chaudhary³

¹Asst. Prof. Maharishi Dayanand University, Rohtak, Haryana, India

²Phd- Research scholar, Maharishi Markandeshwar University, Mullana, Haryana, India

³Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana, India

Abstract

Aim and objective: *Leucas cephalotes* is traditionally used as an emmenagogue. The present aim of the study is to evaluate and explore the antifertility effect of chloroform and alcoholic flowers extract of *Leucas cephalotes*.

Material and method: Using soxhlet, extraction for 48 hrs with solvents chloroform and ethanol, flower extract was prepared. Anti-implantation, estrogenic and anti-estrogenic activities were evaluated using albino female rat animals.

Result and conclusion: The chloroform and alcoholic extract was found to be more potent in causing significant anti-implantation activity at the tested dose of 200 mg/kg and 400 mg/kg body weight. The extracts further showed more significant increase in uterine weight in immature ovariectomised rats. It revealed that administration of extract with ethinyl estradiol causing significant anti-estrogenic activity. Thus it can be concluded that chloroform and alcoholic extract of *Leucas cephalotes* has antifertility activity.

Key words:

Leucas cephalotes, anti-fertility, anti-implantation, estrogenic, anti-estrogenic.

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INTRODUCTION

Population explosion is a major problem for all-round human development in developing countries. In India population growth is as far as concerned from few years of time period. Since population is

*Corresponding author, Mailing address:

Sushma Kainsa

E-Mail: sushma.mehla@gmail.com

increasing dramatically at an alarming rate and affects the social economic growth of the country. So family planning has been promoted through several methods of contraception but severe adverse effects have been produced by the use of synthetic contraceptive agents. Thus there is need to replace these agents by safe and effective agents. Hence there has been a renewed interest in the plant remedies throughout the world. Because in recent time plants have been used in attempt to replace steroidal contraceptives and should be easily available, economic and devoid of deleterious side effects.^[1, 2] Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all indigenous communities possess their distinct cultures throughout history but India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants and is known worldwide for its Ayurveda treatment. *Leucas cephalotes*(Roth.)Spreng. (syn. *Phlomiscephalotes*) is commonly known as Dronapushpi in Sanskrit and Peddatumni in Telgu. ^[3] It is a rainy season weed belonging to family Labiatae/Lamiaceae and grows in almost all parts of India along the roadsides and wastelands. Dronapushpi is widely used as a homeopathic drug in indigenous system of medicine. It has been used for the diagnosis of several disease like edema, diaphoretic, inflammatory and obstinate urinary troubles. Plant is a valuable drug in the treatment of snakebite. Flowers are stimulant, emmenagogue, diaphoretic and expectorant and its syrup sometimes mixed with honey are useful in diagnostic remedies of cough and colds. ^[4, 5] Leaves are used for the treatment of bleeding and itching piles while smoked with tobacco in 1:3 ratio. ^[6] Leaves also used in fever and urinary discharge. ^[7] Pharmacologically plant has been reported to exert multiple biological effects including antifilarial activity ^[8], antibacterial ^[9], antidiabetic^[10], anti-inflammatory ^[11], antioxidant, analgesic, antihelminthic, ^[12]antimicrobial ^[13] and antioxidant. ^[14] The plant is rich to contain triterpenes, oleanolic

acid, sterols and flavones. ^[15] Other constituents like lauric acid, glutaric acid, Labellic acid ^[16] and adipic acid (seed oil) were also reported. ^[17]Literature revealed that there is yet no reported investigation on the antifertility effects of this plant. The objective of the study was to evaluate antifertility activity of chloroform and alcoholic flowers extracts of *Leucas cephalotes*.

MATERIAL AND METHODS

Plant materials

Flowers of *Leucas cephalotes* were collected from the nursery of Sharanpur, Uttar Pradesh, India during September and October 2010 and authenticated by Dr. H.B. Singh, Scientist Faculty and Head, Raw Material Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi-110012 (India). The flowers were cleaned, dried in the shade, then powdered to 40 mesh sizes and stored in an airtight container at 25°C.

Extraction

The coarse powder of *Leucas cephalotes* flowers was subjected to Soxhlet extraction for 48 hrs using chloroform and ethanol. The extract was filtered and extra traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The resulted crude extracts were collected and preserved in airtight glass container at 4°C - 8°C.

Phytochemical studies

Various chemical constituent in the chloroform and alcoholic extracts of plant was determined by preliminary phytochemical screening. ^[18, 19]

Animals

The experimental procedures were approved by the Institutional Animal Ethics Committee of Kurukshetra University, Kurukshetra, Haryana (India) vide approved number 536/02/A/CPCSEA and Chaudhary Charan Singh Agriculture University, Hisar, Haryana (India) vide approved number 235/CPCSEA. Albino Wistar rats (150-200g) and immature female rats (25-40 g) were obtained from

animal house of Chaudhary Charan Singh, Haryana Agriculture University, Hisar, Haryana (India). The animals were housed in Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana (India).

Anti-implantation activity

The anti-implantation study was determined as suggested. [20] The vaginal smear of each female rat was examined daily; only normal estrous cycle rats were selected. The female rats in proestrous phase of cycle were caged overnight with male of proven fertility in 2:1 ratio at the early stage of estrous cycle and examined the vaginal smear from each rats the following morning for evidence of copulation. Consequent day was designated as day 1 of pregnancy. The pregnant female rats were segregated and grouped into five groups containing 6 rats in each groups.

Group 1: received vehicle orally and served as control for one to seven day of pregnancy.

Group 2: received 200 mg/kg of the Chloroform extract from one to seven day of pregnancy.

Group 3: received 400 mg/kg of the Chloroform extract from one to seven day of pregnancy.

Group 4: received 200 mg/kg of the Alcoholic extract from one to seven day of pregnancy.

Group 5: received 400 mg/kg of the Alcoholic extract from one to seven day of pregnancy.

On 10th day, laparotomized was performed on rats under light ether anesthesia. The uteri were examined to determine the number of Implantation sites.

Estrogenic and anti-estrogenic activity

Colony-bred immature female albino rats, 21-23 days old (weighing 35-45g) were bilaterally ovariectomized by dorsolateral approach under light ether anesthesia and sterile conditions. They were divided into ten groups, containing 6 rats each.

Group 1: control and received vehicle Tween-80 (2%) only.

Group 2: received ethinyl estradiol in olive oil (1µg/rat) per day, subcutaneously (s.c.)

Group 3 & Group 5: received chloroform and alcoholic extract at a dose of 200mg/kg body weight respectively.

Group 4 & Group 6: received chloroform and alcoholic extract at a dose of 400 mg/kg body weight respectively.

Group 7 & Group 9: received a test dose of chloroform and alcoholic extract at a dose of 200 mg/kg body weight respectively, along with ethinyl estradiol.

Group 8 & Group 10: received in addition to ethinyl estradiol, a test dose of chloroform and alcoholic extract at 400 mg/kg body weight respectively.

All the above treatments were given for 7 days. On 8th day, the rats were sacrificed by ether anesthesia. The final body weight, vaginal opening and vaginal cornification of all the rats were observed before anaesthesia and uterus of all the animals were weighed quickly on a sensitive balance. Serum was collected from blood of each animal. Further, serum was processed for estimation of biochemical parameters such as: estrogen level, cholesterol contents, triglycerides, LDL and HDL etc. [21, 22]

RESULTS

Phytochemical screening

The following chemical constituents were present in chloroform and alcohol extracts as: alkaloid, steroid, saponin glycosides, carbohydrates, protein, flavonoids, fixed oils and fats, tannins and phenolics.

Anti-implantation activity

Chloroform extract of *Leucas cephalotes* [CELC] and alcohol extract of *Leucas cephalotes* [AELC] at a dose of 200 mg/kg and 400 mg/kg were found having significant response for inhibited pregnancy in all the six rats with respect to control. However both the extracts dose of 200 mg/kg body weight were found to be significant ($p < 0.05$), whereas 400 mg/kg body weight dose of both extract was found more significant ($p < 0.01$). The body weight gain of the treated animals was unaffected and slightly decreased at particular dose of extracts. In female rats both the extracts show percentage of inhibition of implantation in treated groups of female rats, 34.33%, 81.23%, 24.95%, 53.09% at a doses of 200

mg/kg and 400 mg/kg body weight, respectively in comparison to control rats. No toxic effects were observed either by gross visual examination or in the weight of the animals. The anti-implantation activity results were shown below. (Table 1)

Estrogenic and anti-estrogenic activity

The anti-estrogenic effects of the alcoholic and chloroform extract are shown in table 2 and 3. Administration of chloroform and alcohol extract caused a significant increase in uterine weight at 400 mg/kg body weight dose in immature rats as comparable to control ($p < 0.01$). The uterotrophic potency of alcoholic extract is 43% and chloroform extract is 50% as that of ethinyl estradiol. Simultaneously administration of the ethinyl estradiol and prepared extract of dose 400mg/kg body weight also caused increase in uterine weight ($p < 0.001$) when compared to control but less than that produced by ethinyl estradiol ($p < 0.001$) when compared with standard drug. It appears that chloroform and alcohol extract have shown anti-estrogenic activity at 400 mg/kg body weight dose.

DISCUSSION AND CONCLUSION

In this study, the *Leucas cephalotes* flower extracts was tested for its anti-implantation and estrogenic or anti-estrogenic properties in rats. Among these the two extracts tested, at the 200 and 400 mg/kg body weight. However the chloroform and alcohol extract at higher 400 mg/kg body weight dose were more potent in their anti-implantation activity when compared no. of implantation with control female rats.

The loss of implantation at higher dose of prepared extract may be due to the antizygotic, blastocytotoxic or anti-implantation activity. It was concluded that chloroform and alcohol extract may exhibited significant estrogenic activity as shown by the significant increase in uterine weight when given alone.

For implantation it is well known that exact estrogen and progesterone equilibrium is necessary and any disturbance in the level of these hormones cause infertility. [23] The hormonal values of compound disturbs hormonal milieu in the uterus and provokes the infertility effects. [24]

Simultaneously administration of chloroform extract with ethinyl estradiol caused a significant increase in uterine weight when compared with control immature rats, the degree of uterotrophic potency was decreased than that produced by ethinyl estradiol alone ($p < 0.01$). Further administration of standard drug with alcohol extract also caused increase in uterine weight when compared to control but less than that produced by the ethinyl estradiol alone. Thus it appears that chloroform and ethanol extracts have significant estrogenic activity when given alone and shown anti-estrogenic activity when given along with standard ethinyl estradiol dose.

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Table 1: Anti-implantation activity of prepared extracts on female albino rats

Treatment dose	Body weight gain (g) (Mean \pm S.E.M)	No. of implantation sites (Mean \pm S.E.M)	% inhibition of implantation sites on day 10
Control (Tween-80, 2% v/v)	48.00 \pm 1.22	10.67 \pm 0.33	Nil
Chloroform extract (200 mg/kg)	37.80 \pm 1.16**	7.00 \pm 0.00**	34.33
Chloroform extract (400 mg/kg)	43.80 \pm 1.59ns	2.00 \pm 1.00**	81.23
Alcoholic extract (200 mg/kg)	44.20 \pm 0.58ns	8.00 \pm 0.57*	24.95
Alcoholic extract (400 mg/kg)	40.40 \pm 1.63**	4.66 \pm 0.33**	53.09

N = 6; ** Significant as compared to control at $p < 0.01$; * Significance as compared to control at $p < 0.05$

Table 2: Estrogenic activity and anti-estrogenic activity of different doses of CELC and AELC; effect on the body weight and uterine weight in ovariectomized rats.

Treatment (dose) mg/kg	Initial Body weight (g)	Final Body weight (g)	Body weight gain (g)	Uterine weight (mg)
Control (Tween-80, 2% v/v) (orally)	33.43 ± 1.50	41.51 ± 1.36	8.33 ± 2.36	24.52 ± 1.91
Ethinyl Estradiol (s.c)	39.43 ± 1.14	84.41 ± 1.97	47.98 ± 1.46**	75.38 ± 2.04**
Chloroform extract (200 mg/kg)	37.63 ± 1.04	48.62 ± 12.10	10.98 ± 1.19*	34.40 ± 0.51 ^{ns}
Chloroform extract (400 mg/kg)	37.47 ± 0.61	75.40 ± 1.23	37.93 ± 1.42**	45.33 ± 1.52**
Chloroform extract (200 mg/kg) + Ethinyl estradiol (1µg/rat per day)	34.33 ± 1.79	64.38 ± 1.58	30.05 ± 2.38**	55.56 ± 1.64**
Chloroform extract (400 mg/kg) + Ethinyl estradiol (1µg/rat per day)	40.53 ± 1.39	85.69 ± 0.75	41.16 ± 2.09*	68.37 ± 1.43**
Alcoholic extract (200 mg/kg)	35.47 ± 0.82	51.57 ± 1.13	16.09 ± 1.60*	30.45 ± 1.84**
Alcoholic extract (400 mg/kg)	35.76 ± 0.60	55.59 ± 1.28	19.83 ± 1.07**	42.63 ± 1.85 ^{ns}
Alcoholic extract (200 mg/kg) + Ethinyl estradiol (1µg/rat per day)	32.42 ± 1.74	45.49 ± 1.76	27.06 ± 3.03*	51.40 ± 0.79**
Alcoholic extract (400 mg/kg) + Ethinyl estradiol (1µg/rat per day)	37.37 ± 1.12	71.45 ± 1.83	35.87 ± 2.18**	62.49 ± 0.69*

N = 6. ns: Non significant as compared to control: $p > 0.05$; ** Significance as compared to control: $p < 0.01$.

Table 3: Estrogenic / anti-estrogenic activity of CELC and AELC in ovariectomized rats

Treatment (dose) mg/kg	Estrogen (pg/ml)	Cholestrol (mg/dl)
Control (Tween-80, 2% v/v) (orally)	148.52 ± 0.10	57.16±1.85
Ethinyl Estradiol (s.c)	462.44 ± 0.241	77.63±0.27
Chloroform extract (200 mg/kg)	174.33 ± 1.21**	48.39±0.39*
Chloroform extract (400 mg/kg)	213.53 ±1.68 **	52.42±0.17**
Chloroform extract (200 mg/kg) + Ethinyl estradiol (1µg/rat per day)	286.39 ± 0.17**#	47.71±0.97 **#
Chloroform extract (400 mg/kg) + Ethinyl estradiol (1µg/rat per day)	298.21 ± 1.09**#	25.93±0.45*#
Alcoholic extract (200 mg/kg)	152.27 ± 0.208**	39.17±0.56**
Alcoholic extract (400 mg/kg)	179.14 ± 1.32**	45.15±0.59**
Alcoholic extract (200 mg/kg) + Ethinyl estradiol (1µg/rat per day)	217.57 ± 0.06*#	66.54±1.34*#
Alcoholic extract (400 mg/kg) + Ethinyl estradiol (1µg/rat per day)	252.58 ± 0.15**#	52.33±1.79**#

N = 6. Ethinyl estradiol was taken as standard; * significant to control: $p < 0.05$;

** Significant as compared to control: $p < 0.01$; # Significant as compared to standard: $p < 0.01$

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