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Anti-fertility Investigation of *Butea monosperma* (Lam.) Kuntze Root in Female Albino Mice

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ABSTRACT

The root of *B. monosperma* was successively extracted by solvents in increment of polarity. The extracts were administered orally at the dose of 200 mg kg^{-1} body weight to adult female mice of proven fertility for evaluation of anti-implantation activity. The extracts showing anti-implantation activity were further evaluated at the similar dose for estrogenic and anti-estrogenic activity in immature female mice. Biological and histological studies were also performed. Of the four extracts i.e., petroleum ether, chloroform, methanol and aqueous extracts, petroleum ether and chloroform extracts showed significant anti-implantation activity at dose 200 mg kg^{-1} body weight. Both these extracts showed anti-estrogenic effect. The weight of ovaries of petroleum ether and chloroform extracts treated animal reduced significantly ($p<0.01$) as compared to control. This was associated with an elevation in the level of cholesterol. Petroleum ether and chloroform extracts inhibited the activity of G-6-PDH to a significant ($p<0.05$, $p<0.01$) extent and thus indicating anti-steroidogenic activity of the extract. It may be concluded that petroleum ether and chloroform extracts possess some estrogen like compounds which may be responsible for anti-estrogenic activity of the extracts.

Key words: Anti-estrogenic, anti-implantation, *Butea monosperma,* histological studies

INTRODUCTION

Population control is the practice of limiting population increase, usually by reducing the birth rate. The population explosion has negative impact on our economic policies and simultaneously misbalances our socio-economic infrastructure. Thus the control of human fertility in the sense of its limitation is the most important and urgent of all-biosocial and medical problem confronting mankind today. Plant products have attracted the attention of many scientists as a primary source of naturally occurring fertility regulating agents. Number of plants has been reported to posses estrogenic properties (Koneri *et al*., 2007; Kumar *et al*., 2007; Shukla and Pandey, 2008; Montaserti *et al*., 2007; Sailani and Moeini, 2007; Shukla and Pandey, 2008; Sharangouda and Patil, 2008; Edwin *et al*., 2009; Thakare *et al*., 2009; Suzuki *et al*., 2008; Garg *et al*., 1978). The genus *Butea* is of immense medicinal importance. The methanol extract of *B. superba* Roxb. root and flowers have shown antifungal activity (Bhatnagar *et al*.,1961). Ethanol extract of stem bark of *B. monosperma* have shown anti-diarrhoeal activity (Gunakkunru *et al*., 2005). Flavonoids from methanol and aqueous extracts of *B. frondosa* Koen ex. Roxb. flowers possess anti-hepatotoxic activity on albino rats (Rane and Grampurohit, 1998). In the present study, investigation was done on anti-implantation and estrogenic and anti-estrogenic activity on successive extracts of *B. monosperma* (Lam.) Kuntze root.

MATERIALS AND METHODS

Plant collection and preparation of extract: The roots of *Butea monosperma* (Lam.) Kuntze (Fabaceae) were collected from Khejuri, Ballia (U.P.), India. The plant was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum Division of National Institute of Science Communication and Information Resources. The voucher specimen has been deposited in the herbarium section of the Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar for future reference.

The shade-dried root (1 kg) was successively extracted with petroleum ether, chloroform, methanol (4 L, each) by continuous soxhlation process and distilled water (5 L, each) for 7 days. The extracts were filtered and concentrated to dryness using a rotary evaporator to obtain 1.8 g of petroleum ether (pale yellow), 2.2 g of chloroform (chocolate brown), 2.5 g of methanol (dark brown) and 2.75 g of aqueous (dark brown) extracts, respectively. Dried extracts were stored at 4°C till further use. The suspensions of the extracts were prepared in 1% acacia before administration to the mice.

Animals: Adult female and male Swiss albino mice weighing between 25-40 g, of were used for the anti-implantation activity and immature female mice, 18 days old were used for estrogenic and anti-estrogenic activity. Mature female Swiss albino mice were used for biochemical estimation. Adult mice of either sex were used for acute toxicity studies. All animals were acclimatized to laboratory conditions before the beginning of the experiments and were maintained under standard laboratory conditions. The Institutional Ethical Committee for Animal Care and Use approved all experimental procedures. The mice were divided into groups and each group consisted of six mice.

Acute toxicity studies: Acute toxicity was carried out as described by Turner (1971). Adult mice of either sex were divided into five groups. The mice were fasted for 18 h with water *ad libitum*. The suspensions prepared as above were administered orally at the dose of 2000 mg kg^{-1} body weight, respectively to different groups of mice separately. Control mice received the vehicle (acacia 1% p.o.) only. The animals were observed for 72 h for behavioral changes and mortality.

Anti-implantation activity: Mice with normal oestrus cycle were selected for anti-implantation activity. The vaginal smears of the mice were monitored daily and the mice found in proestrus stage of the cycle were caged with males of proven fertility and examined in the morning for the presence of spermatozoa in the vagina. Mice exhibiting thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy. These mice were divided into groups. Group I (control) received the vehicle (acacia 1% p.o.). Group II, III, IV and V (treated groups) were administered petroleum ether, chloroform, methanol and aqueous extract at the doses of 200 mg kg^{-1} body weight orally from day 1 to 7 of pregnancy, respectively. Laparotomy was performed on the 10th day under light ether anesthesia and sterile conditions. The uteri were examined to determine the number of implantation sites. The abdomen was sutured to continue pregnancy (Kamboj and Dhawan, 1982).

Estrogenic and anti-estrogenic activity: Estrogenic and anti-estrogenic activity of petroleum ether and chloroform at 200 mg kg^{-1} body weight was carried out since at this dose these extracts showed significant anti-implantation activity. Immature 18 days old mice were used for the

experiment. The animals are randomly grouped. The control group (group I) received vehicle only (acacia 1% p.o.). The standard group (group II) received ethinyl estradiol in olive oil, 1 μg/mice per day, subcutaneously. Group III received petroleum ether and group IV received chloroform extract at a dose of 200 mg kg^{-1} body weight. Group V received ethinyl estradiol (1 μg/mice) along with petroleum ether (200 mg kg^{-1}) extract. Group VI received ethinyl estradiol (1 μg/mice) along with chloroform (200 mg kg^{-1}) extract. On the 8th day, the mice were sacrificed by decapitation, the uteri dissected out and surrounding tissues removed. The uteri were blotted on filter papers and weighed quickly on a sensitive balance and fixed in Bouin's fluid for 24 h. The tissues were dehydrated and embedded in paraffin. The paraffin sections were cut at 6 μm and stained with haematoxylin-eosin for histological observation. The diameter of the uteri, thickness of the endometrium and height of epithelial cells were measured in 20 randomly selected sections using an ocular micrometer.

Biochemical estimation: Mature female mice showing normal oestrus cycle for a period of 2 weeks were divided into different groups. The control group (group I) received vehicle only (acacia 1% p.o.). The treated groups (group II and III) received petroleum ether and chloroform extracts, respectively, at a dose (200 mg kg^{-1}) possessing anti-implantation activity. The doses were administered on alternate days for 17 days. On the 18th day, after 18 h of fasting mice were sacrificed by cervical dislocation. The ovaries were dissected out carefully, cleared from adherent tissues and weighed to the nearest mg. Quantitative estimation of cholesterol, glucose-6-phosphate dehydrogenase (G-6-PDH) in the ovary was done (Gupta *et al.*, 2003).

Statistical analysis: Data are expressed as Mean±SEM and analysis for statistical significance by using one way analysis of variance (ANOVA) followed by Dunnett's test. Results were considered significant at $p<0.05$.

RESULTS

Acute toxicity studies: No mortality and changes in the behavior were observed in all treated and control groups of the mice up to the dose of 2000 mg kg^{-1} body weight. Hence one-tenth of the doses were used for anti-fertility testing.

Anti-implantation activity: The anti-implantation activity is expressed as the percentage of animals showing absence of implantations in uteri when laprotomised on day 10 of pregnancy (Table 1). The methanol and aqueous extract of *B. monosperma* root at 200 mg kg^{-1} body weight did not show significant anti-implantation effects. However, the petroleum ether and chloroform extracts of *B. monosperma* root at 200 mg kg^{-1} body weight exhibited significant ($p<0.01$) anti-implantation activity. All the extracts reduced the number of implantation sites. This may be

Table 1: Effect of successive extracts of *B. monosperma* on implantation when fed orally for 1 to 7 day of pregnancy

Treatment (200 mg kg^{-1}) extract	No. of mice having no implantation sites on day 10	Mean No. of implants±SEM	Percentage of mice having on day 10 no implantation sites
Control	Nil	6.33±0.61	-
Petroleum ether	5	1.67±0.83**	83.33
Chloroform	4	2.00±0.86**	66.67
Methanol	2	5.83±0.98	33.33
Aqueous	2	5.17±1.17	33.33

**$p<0.01$ compared with control group

Table 2: Effect of petroleum ether and chloroform extract on the vaginal cornification and weight of uterus

Treatment	Initial body weight (g)	Final body weight (g)	Uterine weight (mg/100 g body weight)	Vaginal cornification
Control (1% acacia)	16.5±2.5	26.8±2.8	63.50±3.90	NIL
Ethinyl estradiol (1 µg/mouse)	17.5±3.6	25.7±1.5	118.00±4.89**	+++
Petroleum ether (200 mg kg^{-1})	18.6±2.3	27.9±1.9	89.10±10.61**°	+++
Chloroform (200 mg kg^{-1})	16.6±1.9	24.3±2.1	74.54±14.47**°°	+++
Ethinyl estradiol (1 µg/mouse)+ petroleum ether (200 mg kg^{-1})	18.7±2.4	29.9±2.6	108.66±7.31**°	+++
Ethinyl estradiol (1 µg/mouse)+chloroform (200 mg kg^{-1})	17.9±1.6	27.8±3.5	100.04±14.34**°	+++

**p<0.01 compared with control group, °p<0.05, °°p<0.01 compared with standard group

Table 3: Histological changes in the uterus and endometrium after treatment with petroleum ether and chloroform extract of *B. monosperma* root

Treatment	Diameter of uterus (mm)	Thickness of endometrium (µm)	Height of epithelial cells (µm)
Control (1% acacia)	55.20±0.66	180.00±0.89	19.98±2.35
Ethinyl estradiol (1 µg/mouse)	74.80±0.47**	300.00±1.91**	43.55±2.43**
Petroleum ether (200 mg kg^{-1})	64.10±1.18**°°	240.90±1.46**°	33.21±1.05*°
Chloroform (200 mg kg^{-1})	60.80±1.21**°°	230.50±0.73*°°	29.56±1.09**°°
Ethinyl estradiol (1 µg/mouse)+petroleum ether (200 mg kg^{-1})	68.70±2.73**°°	264.50±1.46*°°	39.54±1.78**°
Ethinyl estradiol (1 µg/mouse)+chloroform (200 mg kg^{-1})	64.50±0.67**°°	250.00±1.04**°°	35.67±1.56**°°

*p<0.05, **p<0.01 compared with control group, °p<0.05, °°p<0.01 compared with standard group

due to resorption of the implantation sites after day 10 or due to abortion. However no vaginal bleeding was observed. Laparotomy of these mice showed the resorption of implantation sites.

Estrogenic and anti-estrogenic activity: Estrogenic and anti-estrogenic activity was carried on petroleum ether and chloroform extracts that showed significant anti-implantation effects at dose 200 mg kg^{-1} body weight. Oral administration of the petroleum ether and chloroform extract at 200 mg kg^{-1} body weight caused a significant increase (verus control $p<0.01$) in uterine weight in immature mice (Table 2). The uterotrophic changes such as diameter of the uterus, thickness of the endometrium and height of epithelial cells were significantly increased when compared with control mice (Table 3).

The uteri of these mice were inflated and fluid filled, resembling the proestrous/estrous uterus. The epithelium of the endometrium consisted of spindle shaped cells with basal nuclei. The stroma consisted of fibroblast type cells, loose and oedematous (Fig. 1a-f). The petroleum ether and chloroform extract also induced vaginal opening and the smear showed proestrous or estrous conditions, while all the control mice had closed vaginas. Simultaneous administration of ethinyl estradiol along with the petroleum ether and chloroform extract caused a highly significant increase (versus control, $p<0.01$) in uterine weight but the extent of the uterotrophic response was less than that produced by ethinyl estradiol alone (Table 2). It also caused a significant increase in uterine diameter, thickness of the endometrium and height of epithelial cells compared with control mice (Table 3). Therefore, the petroleum ether and chloroform extract showed estrogenic activity when given alone but exhibited slight anti-estrogenic activity when given along with ethinyl estradiol. There was no significant difference between the body weights in the control and treated animals.

Biochemical estimation: The weight of ovaries of petroleum ether and chloroform extracts treated animal was reduced significantly ($p<0.01$) as compared to control (Table 4). This was

Table 4: Effect of petroleum ether and chloroform extract of *B. monosperma* on content of glucose-6-phosphate dehydrogenase and cholesterol on ovary tissues after 17 days of treatment

Treatment (200 mg kg^{-1}) extract	Weight of ovaries	Glucose-6-Phosphate Dehydrogenase (U mg^{-1} of protein)	Cholesterol µg mg^{-1} ovary
Control	9.5±2.1	588.87±20.01	32.31±1.05
Petroleum ether	4.7±2.3**	442.76±11.83*	40.31±1.32**
Chloroform	4.4±1.9**	465.83±38.46**	43.89±1.62**

*p<0.05, **p<0.01 compared with control group

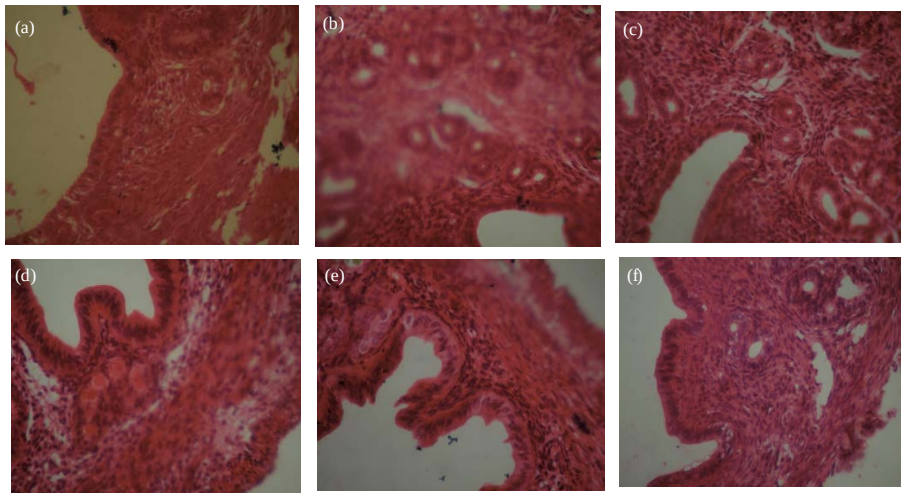

Fig. 1(a-f): Histological changes in the uterus of the mice receiving various extracts of *B. monosperma*. (a) Control mice, (b) Mice receiving petroleum ether extract, (c) Mice receiving chloroform extract, (d) Mice receiving ethinyl estradiol, (e) Mice receiving petroleum ether extract and ethinyl estradiol and (f) Mice receiving chloroform extract ethinyl estradiol

associated with an elevation in the level of cholesterol. Petroleum ether and chloroform extracts inhibited the activity of G-6-PDH to a significant ($p<0.05$, $p<0.01$) extent and thus indicating anti-steroidogenic activity of the extracts (Table 4).

DISCUSSION

In the present findings, the petroleum and chloroform extracts showed significant anti-implantation activity. These extracts showed estrogenic activity when given alone and anti-estrogenic activity when given along with ethinyl estradiol in immature mice as revealed by significant increase in uterine weight and remarkable histological stimulation in endometrium epithelium. The estrogenic nature of these fractions may be responsible for their anti-implantation activity, as estrogens are known to increase the uterine contractility and thus expel the ova or blastocysts from the reproductive tract of pregnant animals. The strong estrogenic nature of the extracts also disturbed the normal estrogen and progesterone level equilibrium of uterus, thus creating an unfavorable hormonal milieu in the uterus to prevent implantation (Greenwald, 1961; Banik and Pincus, 1964; Pincus *et al.*, 1964; Bennett *et al.*, 1966; Moghissi and Hafez, 1972). The

decrease in the wet weight of the ovary in the extract treated animals compared to the control animals may indicate inhibition of ovulation through suppression of follicular stimulating hormone. This is also supported by the elevation of cholesterol level of the ovaries which serves as the precursor for the synthesis of steroid hormones in ovaries suggesting thereby that cholesterol was not utilized. There was significant decrease in G-6-PDH, a key enzyme involved in steroidogenesis, indicating the anti-steroidogenic activity of petroleum ether and chloroform extracts (Gebrie *et al.*, 2005).

It may be concluded that petroleum ether and chloroform extracts possess some estrogen like compounds which may be responsible for anti-estrogenic activity of the extracts.

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