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RESEARCH ARTICLE

Anti-Fertility Activity of Methanol Extract of *Bassia latifolia and Cajanus cajan* in Female Albino Mice Ovaries

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ABSTRACT

Bassia latifolia bud and *Cajanus cajan* seed were evaluated for anti-fertility activity in mature female mice. Anti-fertility activity was evaluated by observing the estrus cycle, body weight, wet weight of ovaries, steroidogenic enzymes, and substrates. *Bassia latifolia* bud and *Cajanus cajan* seed extract arrested normal oestrous cycle at dioestrous stage. The decrease in weight of ovary and uterus after administration of methanol extracts of *Bassia latifolia* bud and *Cajanus cajan* seed in mice is related to inhibition of ovarian steroidogenesis in mature female mice. The accumulation of total cholesterol and ascorbic acid in the ovaries of the mature female mice also indicates the inhibition of ovarian steroidogenesis due to the reduced activity of Glucose-6-Phosphate Dehydrogenase and Δ^5 –3β–Hydroxysteroid Dehydrogenase. The oestrus cycle of the extract-treated mice resumes to normal after 28 and 42 days respectively for both plants. Thus, the anti-fertility activities were found to be reversible.

Keywords: *Bassia latifolia, Cajanus cajan, Δ^5-3β-HSD; G6PD, Anti-Fertility*

Anti-fertility agents are those which will prevent ovulation or fertilization and ultimately intercept pregnancy [1]. Various experimental parameters, for the investigation of antifertility activity in females, were reported earlier [2,3]. Currently, the most effective method to prevent conception is use of steroids to inhibit or modify the cyclic changes in endogenous production of hormones. *Bassia latifolia* [4] (Family: Sapotaceae) commonly known as Madhuka, Indian Butter Tree, Mahua etc. It grows well in hot and dry, moist climate of central, western and eastern India. Fresh corollas of *Bassia latifolia* are used as contraceptive. Its seeds are used as abortifacient. Its fresh root is used as an abortifacient agent. *Cajanus cajan* [5,6] (Family: Fabaceae) is commonly known as Arhar. It is cultivated in West Bengal and all over India. The plant is used in the treatment of cough, fever, inflammation, pain, ulcer, wound, diabetes etc. and the seed of *Cajanus cajan* is a traditional tribal medicine for birth control. This work is aimed to study the anti-fertility effects of these plants.

MATERIALS AND METHODS

Preparation of extract

The bud of *Bassia latifolia* and seed of *Cajanas cajan* were collected from West Bengal and were authenticated by the division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata. Shade-dried, powdered, and sieved in 40 × mesh. The plant material was soxhelet extracted first with petroleum ether and then with methanol. The methanol extract was evaporated to dryness. The trace amount of methanol which might be present within the solid mass of methanol extract was removed by vacuum pressure. For pharmacological testing, methanol extract (ME) of *Bassia latifolia* bud and *Cajanas cajan* seed were dissolved in propylene glycol (PG). The yield of methanol extracts were 7.1% and 12.5% for *Bassia latifolia* and *Cajanas cajan* respectively on dry weight basis.

Table 1. Effects of methanol extract of *Bassia latifolia* bud (MEBL) on the oestrous cycle in mice

Treatment	Dose (i.p.)	Different phases of oestrous cycle in subsequent 7 days						
		1st	2nd	3rd	4th	5th	6th	7th
Saline (0.9% NaCl, w/v)	5 ml/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P
Vehicle (PG)	5 ml/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P
MEBL	55 mg/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	D	D	D
MEBL	75 mg/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	D	D	D	D
MEBL	110 mg/kg body weight	O/M/D/P	O/M/D/P	D	D	D	D	D

n= 6, i.p.- intraperitoneal, PG- Propylene Glycol, M: Metoestrus phase, D: Dioestrus phase, P: Prooestrus phase, O: Oestrous phase

Table 2. Effects of methanol extracts of *Cajanus Cajan* seed (MECC) on the oestrous cycle in mice

Treatment	Dose (i.p.)	Different phases of oestrous cycle in subsequent 7 days						
		1st	2nd	3rd	4th	5th	6th	7th
Saline (0.9% NaCl, w/v)	5 ml/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P
Vehicle (PG)	5 ml/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P	O/M/D/P
MECC	65 mg/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	D	D	D	D
MECC	90 mg/kg body weight	O/M/D/P	O/M/D/P	O/M/D/P	D	D	D	D
MECC	130 mg/kg body weight	O/M/D/P	O/M/D/P	D	D	D	D	D

n= 6, i.p.- intraperitoneal, PG- Propylene Glycol, M: Metoestrus phase, D: Dioestrus phase, P: Prooestrus phase, O: Oestrous phase.

Animal experiments

Adult female albino mice of Swiss strain were acclimatized to normal laboratory conditions in the laboratory (25-30°C, 75-80 % relative humidity, 12 hr light/dark cycle) for one week. There were given pellet diet (Hindustan lever limited, India) and water *ad libitum*. The experiment was performed under the guidance of The Institutional Ethical Committee, Jadavpur University, Kolkata.

The study was divided into 2 parts: Experiment A and B.

Experiment A

The mice showing a normal oestrus cycle for a period of 2 weeks were then divided into 8 groups, each group containing 6 mice as follows:

Group I - Normal saline as control (5 ml/kg, b.w., 0.9% NaCl w/v, i.p.)

GroupII - Propylene glycol as vehicle control (PG, 5 ml/kg, b.w., i.p.)

Group III - Methanol extract of *Bassia latifolia* dissolved in Propylene glycol (55 mg/kg, b.w., i.p.)

Group IV - Methanol extract of *Bassia latifolia* dissolved in Propylene glycol (75 mg/kg, b.w., i.p.)

Group V - Methanol extract of *Bassia latifolia* dissolved in Propylene glycol (110 mg/kg, b.w., i.p.)

Group VI - Methanol extract of *Cajanus cajan* dissolved in Propylene glycol (65 mg/kg, b.w., i.p.)

GroupVII - Methanol extract of *Cajanus cajan* dissolved in Propylene glycol (90 mg/kg, b.w., i.p.)

Group VIII - Methanol extract of *Cajanus cajan* dissolved in Propylene glycol (130 mg/kg, b.w., i.p.)

The LD_{50} values for *Bassia latifolia* and *Cajanas cajan* were 451.88 mg/Kg. body weight and 530.88 mg/Kg body weight respectively. Low, medium and high dose of the extracts were approximately 1/8th, 1/6th and 1/4th of the LD_{50} value. Normal saline (0.9 % w/v), propylene glycol (5 ml/kg, b.w.) and Methanol extract (low, medium and high dose) were given intraperitoneally in alternate days for 14 days for all the groups.

Observation of oestrous cycle

A drop of normal saline was pipetted in and out of the vagina and placed on a slide. The smear was stained with methylene blue and examined under the microscope (40 × 15) twice daily at an interval of 12 hours. On the 15th day, after 24 hours of the last injection and 18 hours of fasting, mice were sacrificed by cervical dislocation. Normal saline- and PG-treated groups were sacrificed in the same oestrus phase of the ME-treated groups (dioestrus phase). Ovaries and uterus were dissected out, weighed and kept on ice for biochemical estimation.

Biochemical estimations

Ovarian tissues about 3 mg weight, were carefully homogenized in Potter Elvehjem homogenizer using chloroform:ethanol mixture and non-polar part was extracted out and total cholesterol content was estimated according to method of Kingsley and Roscoe [7]. Ascorbic acid content was estimated according to method of Omye and Turnbull [8].

Table 3. Effest of methanol extracts of *Bassia latifolia* bud (MEBL) on the weights of ovary and uterus in mature female mice

Treatment	Dose (i.p.)	Initial body weight (gm)	Final body weight (gm)	Weight of ovaries (both sides) (mg)	Weight of uterus (both sides) (mg)
Saline (0.9% NaCl, w/v)	5 ml/kg body weight	20.3 ± 2.3	21.2 ± 2.1	9.3 ± 1.4	46.5 ± 0.8
Vehicle (PG)	5 ml/kg body weight	20.6 ± 1.7	21.1 ± 1.1	9.1 ± 0.9	46.4 ± 0.2
MEBL	55 mg/kg body weight	20.0 ± 1.1*	21.0 ± 1.4	5.8 ± 0.4	37.2 ± 1.2*
MEBL	75 mg/kg body weight	20.5 ± 1.2*	20.9 ± 1.5*	5.6 ± 1.0	36.1 ± 1.0*
MEBL	110 mg/kg body weight	20.1 ± 1.3	20.8 ± 0.7*	5.2 ± 0.4	35.5 ± 1.4

Values are mean ± SEM, 6 mice in each group; n= 6, *$p < 0.05$ as compared with vehicle control, i.p.: intraperitoneal, PG: Propylene Glycol.

Table 4. Effects of methanol extracts of *Cajanus Cajan* seed (MECC) on the weights of ovary and uterus in mature female mice

Treatment	Dose (i.p.)	Initial body weight (gm)	Final body weight (gm)	Weight of ovaries (both sides) (mg)	Weight of uterus (both sides) (mg)
Saline (0.9% NaCl, w/v)	5 ml/kg body weight	20.1 ± 2.2	21.3 ± 2.1	9.5 ± 1.3	46.3 ± 0.9
Vehicle (PG)	5 ml/kg body weight	20.3 ± 2.0	21.2 ± 1.5	9.3 ± 1.1	46.1 ± 1.2
MECC	65 mg/kg body weight	20.4 ± 0.8	21.2 ± 1.3*	7.3 ± 0.2*	38.9 ± 0.9
MECC	90 mg/kg body weight	20.3 ± 1.0*	21.2 ± 0.4	6.2 ± 0.5*	36.3 ± 0.7*
MECC	130 mg/kg body weight	20.4 ± 0.9*	21.4 ± 1.0*	5.8 ± 0.3*	36.1 ± 1.6*

Values are mean ± SEM, 6 mice in each group, n= 6, *$p < 0.05$ as compared with vehicle control, i.p.: intraperitoneal, PG: Propylene Glycol.

About 5 mg of tissue was homogenized in Potter Elvehjem homogenizer using 1 ml of normal saline and 1 ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged. The activity of Δ^5-3β-HSD (hydroxy steroid dehydrogenase) was estimated as described by Rabin et al [9]. About 3 mg of ovarian tissue was again homogenized in Potter Elvehjem homogenizer using 0.5 M Tris-HCL (pH 8.3) and centrifuged. The activity of G6PD (glucose 6-phosphate dehydrogenase) was estimated as described by Lohr and Waller [10]. Protein was estimated with Folin's phenol reagent and the activities of enzymes were expressed in unit per mg of protein as described by Lower et al [11].

Experiment-B

Another set of animals was treated in the same manner but was not sacrificed after the treatment. They were kept till their estrous cycle revived completely. This study was aimed to investigate whether the methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed causes temporary or permanent contraception.

Statistical analysis

The experimental results were expressed as the Mean ± SEM (Standard Error Mean). The One way ANOVA followed by Student's t-test was used to make a statistical comparison between the groups [12].

RESULTS

Results are summarized in Tables 1-6. Normal cyclic changes of the vaginal smear were examined all animals throughout the treatment period. From the Tables 1 and 2, it is evident that *Bassia latifolia* bud and *Cajanus cajan* seed extract arrested normal oestrous cycle at dioestrous stage after the 4th, 3rd, 3rd & 3rd, 3rd, 2nd dose of treatment in low, medium & high dose level respectively. Though the cyclical changes were seen arrested in the dioestrous stage, it was regular in saline and vehicle treated mice.

From the experiment II, it was found that mice treated with methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed plant were revived completely to normal estrous cycle after 28 and 42 days respectively.

The wet weight of ovaries and uterus was reduced significantly whereas there was no significant change in their body weight (Tables 3-4). As depicted in Tables 5-6, the crude extract of *Bassia latifolia* bud and *Cajanus cajan* seed significantly elevated the level of total cholesterol and ascorbic acid contents in a dose-dependent manner. Weekly low, moderate and high doses of *Bassia latifolia* bud and *Cajanus cajan* seed increased the total cholesterol and ascorbic acid content (125.53%, 254.55%, 364.22% & 14.06%, 26.42%, 41.27% respectively). Weekly low, moderate and high doses of *Cajanus cajan* seed increased the total cholesterol and ascorbic acid content (117.20%, 151.79%, 215.05% & 18.97%, 30.04%, 40.68% respectively).

Table 5. Effects of methanol extracts of *Bassia latifolia* bud (MEBL) on the content of ascorbic acis, cholesterol and the activities of G6PDH and HSD in mouse ovary

Treatment	Dose (i.p.)	Ascorbic acid (µg/mg of ovary)	Cholesterol (µg/mg of ovary)	G-6-PDH (Unit/mg protein)	Δ^5–3β–HSD (Unit/mg protein)
Saline (0.9% NaCl, w/v)	5 ml/kg body weight	88.2 ± 0.41	51.7 ± 0.81	4.1 ± 0.02	1.1 ± 0.007
Vehicle (PG)	5 ml/kg body weight	90.4 ± 0.79	53.4 ± 0.57	4.0 ± 0.03	0.9 ± 0.008
MEBL	55 mg/kg body weight	100.6 ± 0.68	116.6 ± 0.38	2.3 ± 0.01*	0.7 ± 0.006*
MEBL	75 mg/kg body weight	111.5 ± 0.39*	183.3 ± 0.78*	1.2 ± 0.01*	0.4 ± 0.006
MEBL	110 mg/kg body weight	124.6 ± 0.39*	240.0 ± 0.76*	0.7 ± 0.01	0.2 ± 0.007

Values are mean ± SEM, 6 mice in each group; n= 6, *$p < 0.05$ as compared with vehicle control, i.p.: intraperitoneal, PG: Propylene Glycol.

Table 6. Effects of methanol extracts of *Cajanus Cajan* seed (MECC) on content of ascorbic acid, cholesterol and the activities of G6PDH and HSD in mouse ovary

Treatment	Dose (i.p.)	Ascorbic acid (µg /mg of ovary)	Cholesterol (µg /mg of ovary)	G-6-PDH Unit/mg protein	Δ^5–3β–HSD Unit/mg protein
Saline (0.9% NaCl, w/v)	5 ml/kg body weight	91.2 ± 0.59	55.8 ± 0.92	3.8 ± 0.05	1.2 ± 0.05
Vehicle (PG)	5 ml/kg body weight	91.5 ± 0.74	56.1 ± 0.53	4.0 ± 0.03	1.0 ± 0.009
MECC	65 mg/kg body weight	108.5 ± 0.67*	121.2 ± 0.54	1.8 ± 0.01	0.9 ± 0.009
MECC	90 mg/kg body weight	118.6 ± 0.67*	140.5 ± 0.30*	1.1 ± 0.006	0.7 ± 0.01*
MECC	130 mg/kg body weight	128.3 ± 0.86	175.8 ± 0.32*	0.8 ± 0.01	0.4 ± 0.003

Values are mean ± SEM of 6 mice in each group; n= 6, *$p < 0.05$ as compared with vehicle control, i.p.: intraperitoneal, PG: Propylene Glycol.

The activities of two key steroidogenic enzymes G6PDH and HSD (Tables 5-6) were inhibited significantly by crude extract of *Bassia latifolia* bud and *Cajanus cajan* seed respectively. Weekly low, moderate and high doses of *Bassia latifolia* bud decreased the G6PDH and HSD level (43.90%, 70.73%, 82.93% & 36.36%, 63.64%, 81.82% respectively). *Cajanus cajan* seed extract decreased G6PDH and HSD level (52.63%, 71.05%, 78.94% & 25%, 41.66%, 66.66%) respectively.

DISCUSSION

The sequential changes of the vaginal smear in different phases of the estrous cycle are closely associated with simultaneous secretary patterns of gonadal steroids [13]. Ovarian hypofunction and anoestrous vaginal smears appear to be due to the absence or decrease of circulating gonadotropins [14]. The methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed treated doses reduced the wet weight of ovaries and arrested the estrous cycle at dioestrous stage where minimum activity of steroids has been reported [15-17]. This disturbance in the reproductive cycle and the decrease in the weight of the ovary and uterus may be related with the diminution of ovarian steroidogenesis.

The role of cholesterol as an obligatory precursor in progestin biosynthesis in rat, rabbit and bovine luteal tissues has been reported earlier [15,18]. Thus, in present investigation, the significant elevation in cholesterol content of ovarian tissue of extract-treated mice suggest the non utilization of cholesterol towards biosynthesis of hormone in ovaries. Thereby it results the hypofunctioning of steroidogenic activity of the ovary of the extract treated mice [19-21].Ascorbic acid, an easily diffusible water soluble reductant is found abundantly in ovaries where it plays an important role in ovarian steroidogenesis [22]. The accumulation of ascorbic acid in the ovaries of treated animals gives additional support to the inhibition of steroidogenic activity. The steroidogenesis in ovaries is under the physiological control of two dehydrogenases namely Glucose-6-phosphate dehydrogenase (G-6-PDH) and Δ^5–3β-hydroxysteroid dehydrogenase . Δ^5–3β–HSD) [23]. Both extracts inhibited the activity of two enzymes significantly in dose-dependent manner.

The oestrus cycle of the extract treated mice resumes to normal after 28 and 42 days respectively for both plants. Thus from initial investigation it can be deduced that the contraceptive action of both the extract is not permanent and the ovarian activities return few days after the withdrawal of the extract. It may be concluded that the methanol extract of *Bassia latifolia* bud and *Cajanus cajan* seed produce anti-fertility activity on mature female mice.

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