

Antisteroidogenic Activity of Ethanol Extract of *Ammania baccifera* (L.) Whole Plant in Female Albino Mice Ovaries

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ABSTRACT

Ethanol (90%) extract of *Ammania baccifera* (L.) whole plant (EEAB) was evaluated for possible antisteroidogenic activity in mature female mice ovaries. The ethanol extract at the doses of 100, 200 and 400 mg/kg body weight (i.p) arrested the normal estrus cycle at dioestrus phase and significantly decreased weight of ovaries. The cholesterol and ascorbic acid content in ovaries were significantly elevated in treated mice. The extract also significantly inhibited the activity of Δ^5 - 3β -hydroxy steroid dehydrogenase (Δ^5 - 3β -HSD) and Glucose-6-phosphate dehydrogenase (G-6-PD), the two key enzymes involved in ovarian steroidogenesis. Results of this study suggested that the ethanol extract of whole plant of *Ammania baccifera* (L.) acts as an antisteroidogenic agent.

Keywords: *Ammania baccifera*, Ovarian steroidogenesis, Δ^5 - 3β -HSD, G-6-PD, Medicinal plant

Ammonia baccifera Linn (Lythraceae) is a glabrous, erect branching herb, found as weed in rice-fields and marshy localities throughout India. The leaves are acrid and used in the treatment of rheumatic pain, as laxative, rubifacient and external remedy for ring worm [1]. This plant was found to possess hypothermic, hypertensive, antiurolithiasis, antibacterial and CNS depressant activities [2-4]. Steroids triterpenes, coumarins, flavonol and tannins were previously isolated from various parts of the plant [5, 6]. It has come to our notice that the rural people of Tamilnadu use this plant to produce sterility in animals. In the present study, we evaluated the antisteroidogenic activity of the ethanol extract of *Ammania baccifera* (L.) whole plant in female mice ovaries.

MATERIALS AND METHODS

Plant Material

The whole plant of *Ammonia baccifera* (L.) was collected from Trichy, Tamilnadu, India and was identified and authenticated by Prof. Sri. Ganesh, Botanist Madura College, Madurai, Tamilnadu. A voucher specimen MG-3 has been kept in our laboratory for future reference. The whole plant was dried under shade, powdered by a mechanical grinder and was passed through 40-

mesh sieve and stored in airtight container for further use.

Preparation of Extract

About 1 kg of the powdered plant material was successively extracted using petroleum ether (40-60°C), chloroform, and then ethanol (90%) in a Soxhlet extraction apparatus. The various extracts were concentrated and the traces of the solvent were completely removed under reduced pressure and were stored in a vacuum desiccator for further use. The yield was found to be petroleum ether extract (0.9%); chloroform extract (1.7%) and ethanol extract (3.6%) w/w with respect to dried powder. Preliminary qualitative chemical tests indicated the presence of steroids, triterpenoids, flavonoids and tannins. Further investigation was carried out using the ethanol extract.

Animals

Adult female albino mice of Swiss strain 20 ± 2 g were acclimatized to normal environmental condition in the laboratory for one week and given a standard pellet diet (Hindustan Lever) and water *ad libitum*.

The experiment was performed under the guidance of the Ethical Committee, Javadpur University, Kolkata-32.

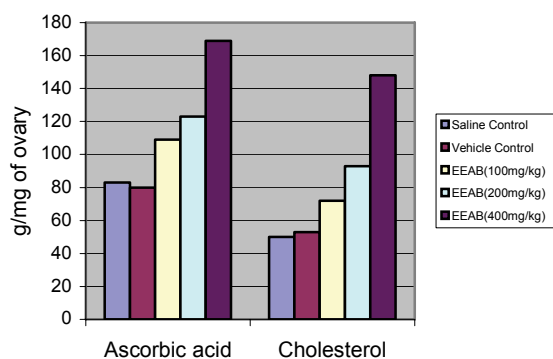


Fig 1. Effect of ethanol extract of whole plant of *Ammania baccifera* (L.) (EEAB) on content of ascorbic acid and cholesterol in female mice ovaries.

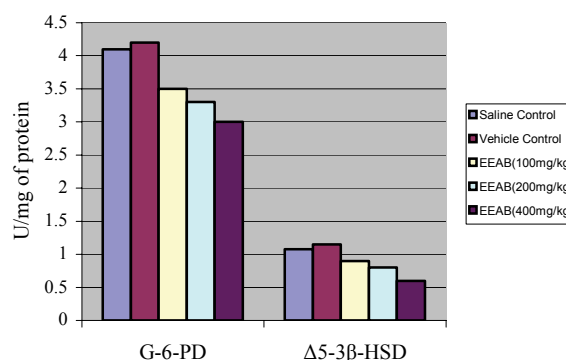


Fig 2. Effect of ethanol extract of whole plant of *Ammania baccifera* (L.) (EEAB) on the activities of Δ^5 -3 β -HSD in matured female mice ovarian tissues.

Experimental Design

Treatment of Animals. The mice showing four consecutive normal oestrus cycle were then divided into five groups (n=10). In their proestrus phase the first group received normal saline (5 ml/kg/day) whereas group 2, group 3, group 4, and group 5, received vehicle (Propylene glycol 5 ml/kg body weight), ethanol extract of whole plant of *Ammania baccifera* (L.) (EEAB) (100, 200 and 400 mg/kg/body weight) intraperitoneally respectively for every alternate days for 18 days. Oestrus cycle was observed everyday by microscopic examination of vaginal smear. On the 19th day the mice were killed by cervical location, 24 hours after the final does and after 18 hours fasting. Ovaries were dissected out, weighed and kept on ice for biochemical estimations.

Biochemical Estimation. Ovarian tissues about 3 mg weight, were carefully homogenized in Potter Elvehjem homogenizer using chloroform: ethanol mixture (2:1) and non-polar part was extracted out and total cholesterol content was estimated according to the method of Kingsley and Roscoe [7].

About 5 mg of tissue was homogenized in Potter Elvehjem homogenizer using 2.5 ml ice cold 5% metaphosphoric acid and centrifuged for 20 min at 355 rpm then ascorbic acid content was calculated [8].

About 2 mg of ovarian 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 -3 β -HSD was estimated as described by Rabin et al [9].

About 3 mg of ovarian tissue was again homogenized in Potter Elvehjem homogenizer using 0.5M Tris-HCL (pH 8.3) and centrifuged. The activity of G-6-PD was estimated as described Iohr 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].

Statistical Analysis

Statistical analysis was done by using Student' t-test.

RESULTS

Ethanol extract of whole plant of *Ammania baccifera* (L.) (EEAB) arrested normal spelling cycle at dioestrus phase at doses of 100, 200 and 400 mg/kg body weight after 13,10 and 6 days of treatment respectively. It was found that the EEAB significantly reduced the wet weight of ovaries in a dose dependent manner ($p < 0.05$ by 100mg and $p < 0.01$ by both 200 and 400 mg). The ethanol extract of whole plant of *Ammania baccifera* (L.) (EEAB) at 100, 200 and 400 mg/kg body weight significantly increased the level of total cholesterol and ascorbic acid contents of ovaries in treated mice. The activities of Δ^5 -3 β -HSD were inhibited significantly ($p < 0.05$ by 100 mg and $p < 0.001$ by both

Table 1. Effect of ethanol extract of whole plant of *Ammania baccifera* (L.) (EEAB) on weight of ovaries, content of ascorbic acid, cholesterol and the activities of G-6-PD and Δ^5 -3 β -HSD in matured female mice ovarian tissues.

Design of Treatment	No. of days	Weight of ovary (mg)	Ascorbic acid (g/mg of ovary)	Cholesterol (g/mg of ovary)	G-6-PD (U/mg of protein)	Δ^5 -3 β -HSD (U/mg of protein)
Saline (5 ml/kg b.w, i.p)	18	14 ± 1.2	83 ± 2.2	50 ± 1.7	4.1 ± 0.05	1.08 ± 0.04
Vehicle (PG) (5 ml/kg b.w, i.p)	18	13 ± 1.8	80 ± 3.8	53 ± 0.4	4.2 ± 0.02	1.15 ± 0.06
EEAB (100 mg b.w, i.p)	18	10.0 ± 0.4*	109 ± 2.6*	72 ± 5.7*	3.5 ± 0.06**	0.9 ± 0.03*
EEAB (200 mg b.w, i.p)	18	9.3 ± 0.3**	123 ± 3.4**	93 ± 8.3***	3.3 ± 0.03***	0.8 ± 0.04***
EEAB (400 mg b.w, i.p)	18	7.3 ± 0.3**	169 ± 5.4**	148 ± 6.6***	3.0 ± 0.04***	0.6 ± 0.02***

PG=Propylene glycol, b.w = body weight, i.p = intraperitoneal, EEAB=Ethanol extract of *Ammania baccifera* (L.) whole plant.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significantly different from vehicle control.

200 and 400 mg). Similarly, the activities of G-6-PD were inhibited significantly ($p < 0.01$ by 100 mg and $p < 0.001$ by both 200 and 400 mg) by all the doses of whole plant of *Ammania baccifera* (L.) (EEAB). (Table 1, Fig 1 and Fig 2).

DISCUSSION AND CONCLUSION

The minimum activity of steroid hormones has been reported to occur in the dioestrus stage. [12-14]. This was associated with an elevation in the level of cholesterol as well as ascorbic acid content which serve as precursor for the synthesis of steroid hormones in ovaries, suggesting, that cholesterol and ascorbic acid were not utilized [15-**Error! Reference source not found.**]. The steroidogenesis is under the physiological control of two enzymes [19, 20].

The ethanol extract of whole plant of *Ammania baccifera* (L.) (EEAB) at treated doses arrested normal oestrus cycle at dioestrus phase and also elevated the ascorbic acid and cholesterol content and significantly reduced the activity of Δ^5 -3 β -hydroxy steroid dehydrogenase (Δ^5 -3 β -HSD) and Glucose-6-phosphate dehydrogenase (G-6-PD) in a dose dependent manner. These results revealed that EEAB produced ovarian malfunction by altering substrate and enzyme activities. Preliminary phytochemical tests indicated the presence of flavonoids in the ethanol extract of whole plant of *Ammania baccifera* (L.) Since various flavonoids have been reported [21, 22] to possess antifertility activity, the antisteroidogenic property of the ethanol extract of whole plant of *Ammania baccifera* (L.) might be due to the presence of such compounds.

From the present investigation it may be concluded that the ethanol extract of whole plant of *Ammania baccifera* (L.) may be considered as an antisteroidogenic agent.

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