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Evaluation of antifertility activity of the ethanol extract of *Ammania baccifera* (L) whole plant in male albino rats

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Abstract
The 90% ethanol extract of *Ammania baccifera* (L) whole plant (EEAB) was administered to mature male rats at different doses in alternate days for 18 days. After the final dose and 18 hours fasting rats were sacrificed to evaluate antifertility activity. Results showed that the weight of the testis and epididymis decreased significantly whereas there was no significant change in body weight. Sperm density and motility was reduced significantly and content of fructose in the seminal vesicles reduced significantly in extract-treated rats. Levels of cholesterol and ascorbic acid were significantly elevated. Also the activities Δ^5-3β -hydroxy steroid dehydrogenase (Δ^5-3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-PD), the two key enzymes involved in androgen biogenesis, were significantly inhibited in extract-treated rats. This study suggests that *A. baccifera* (L.) whole plant extract arrests spermatogenesis and inhibits steroidogenesis thereby acting as antifertility agent in male rats

Keywords: *Ammania baccifera*; Δ^5-3β-Hydroxy steroid dehydrogenase; Glucose-6-phosphate dehydrogenase; Antisteroidogenic; Spermatogenesis

Introduction

Ammania baccifera Linn (Family: 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 (Kirtikar and Basu, 1972). This plant was found to possess hypothermic, hypertensive, antiurolithiasis, antibacterial and CNS depressant activities (Al-Sharma and Mitschar, 1979; Bharathi and Srinivasan, 1994; Dhar *et al.*, 1973). Steroid, triterpenes, coumarins, flavonoids and tannins were previously isolated from various parts of the plant (Atal *et al.*, 1978; Thakkar *et al.*, 1986). It has come to our notice that the rural people of Tamilnadu use this plant for producing sterility in animals. In our earlier investigation, we found that the ethanol extract of whole plant exhibited antifertility (anti-steroidogenic) activity in mature female mice (communicated). Further investigation was therefore carried out to assess the effect

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of the ethanol extract of the whole plant on spermatogenesis and steroidogenesis of sexually mature male rats and is being reported herein.

Experimental

Plant material. The whole plant of *A. baccifera* (L) was collected from Trichy, Tamilnadu, India and was identified and authenticated by Prof. Sri Ganesh, Botanist Madura College, Madurai, Tamilnadu, India. A voucher specimen GD-1 has been kept in out 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°C-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 yields were 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 rests indicates the presence of steroids, triterpenoids flavonoids and tannins. The further investigation was carried out using ethanol extract.

Animals. Mature albino male rats (Wistar) weighing 180-195g were used for the study. They were supplied with standard pellet diet (Hindustan Lever) and water *ad libitum.* The Experiment was performed under the guidance of the Ethics Committee, Jadavpur University, Kolkata, India.

Design of experiment. Thirty healthy rats were selected and divided into five groups containing (n=6). The first group received normal saline (5 ml/kg body weight) and group 2, Group 3, group 4 and group 5 received Propylene glycol (5 ml / kg body weight) ethanol extract of whole plant of *A. baccifera* (L) (100, 200 and 400 mg/kg body weight) intraperitoneally respectively on alternate days for 18 days. The rats were weighed before and after the commencement of the experiment. On the 19th day rats were kept fasting for 18 hours and sacrificed by cervical dislocation and reproductive organs were immediately dissected out, trimmed off from adherent fat, weighed and kept in cold 0.2 M sucrose buffer at 4°C for further processing.

Body weight, sperm density & sperm motility. Body weight was recorded throughout the experiment; the sperm density and motility were assessed in cauda epididymis according to the method of Prasad *et al.*, (1972). Biochemical estimation was carried out in the testis and epididymis.

About 10 mg of testis was weighed carefully and homogenized in Potter Elvahjem homogenizer using chloroform:ethanol (2:1) mixture. The non-polar part was extracted out and total cholesterol content was estimated according to the method of Kingsley and Roscoe (1949). About 15 mg of testis was weighed carefully and homogenized in Potter Elvahjem r.p.m. The ascorbic acid content was then measured (Omaye *et al.*, 1979). About 20 mg of testis was weighed carefully and homogenized in Potter Elvahjem homogenizer using 1 ml of normal saline and of 0.1 M phosphate buffer (pH 7.4) and centrifuged. The activity of Δ^5-3β – hydroxysteroid dehydrogenase (HSD) was estimated as described by Rabin *et al.*, (1961). About 20 mg of tissue was weighed carefully and homogenized in Potter Elvahjem homogenizer. The activity of glucose-6-phosphate dehydrogenase (G-6-PD) was determined by the method of Mann (1964). Protein was estimated with Folin's phenol reagent and the activity of enzymes expressed

in unit per mg of protein as described by Lowry *et al.*, (1951).

Statistical Analysis. Statistical analysis was by Student's t-test.

Results

The results are summarized in the Tables 1,2 and 3.

Body weight, sperm dynamics. During the period of experiment, the rats were kept healthy. The body weight gain of drug-treated rats was similar to that of control animals. The weights of testis, epididymis reduced in case of drug-treated animals in comparison to vehicle-treated animals (Table 1). Sperm motility was decreased in the treated animals and sperm density was significantly reduced (Table 2).

Biochemical findings. Fructose content in the seminal vesicles decreased significantly in a dose dependent manner after the treatment of ethanol extract of whole plant of *A. baccifera* (L.) (100, 200 and 400 mg/kg) in comparison with that of the vehicle control (Table 2). Cholesterol and ascorbic acid level in testes were elevated significantly. The activities of Δ^5-3β-HSD and G-6-PD, the two key steroidogenic enzymes were significantly inhibited at all the doses of ethanol extract of whole plant of *A. baccifera* (L.) in comparison to vehicle-treated rats (Table 3).

Table 1: Effect of ethanol extract of whole plant of *Ammania baccifera* (L) (EEAB) on body weight, weight of testis and epididymis in mature male rats.

Treatment	Body weight before treatment (g)	Body weight after treatment (g)	Weight of testis (mg)	Weight of epididymis (mg)
Saline 5ml/kg b.w. (i.p.)	165 ± 4.2	170 ±5.5	144±3.2	170 ±2.5
Vehicle (PG) 5ml/kg b.w. (i.p.)	170 ± 3.3	176 ± 4.8	145 ±1.8	173 ±1.4
EEAB 100mg/kg b.w. (i.p.)	169 ± 4.2	174 ± 45	131±1.4*	150 ± 4.2*
EEAB 200mg/kg b.w. (i.p.)	16 2 ± 2.4	168 ± 3.3	124 ± 3.5*	138 ±3.1**
EEAB 400mg/kg b.w. (i.p.)	165 ± 4.28	171±4.2	98 ± 2.18***	108 ±1.86***

EEAB= Ethanol extract *A. baccifera* (L). PG=Propylene glycol, i.p- intraperitoneal. Treatment period = 18days P<0.05, **P<0/01, ***P<0.001 significantly different from vehicle control. Values are mean±S.E. of 6 rats.

Table-2: Effect of ethanol extract of whole plant of *Ammania baccifera* (L) (EEAB) on sperm density, sperm motility and seminal vesicle fructose content in mature male rats.

Treatment	Sperm density (Millions/ml)	Sperm motility (%)	Seminal vesicle fructose (mg/gm)
Saline 5ml/kg b.w (i.p)	53.6±2.1	67.3±1.6	4.2±0.3
Vehicle (PG) 5ml/kg b.w (i.p)	54.5±2.6	66.4±1.7	4.3±0.2
EEAB 100mg/kg b.w (i.p)	38.5±2.4*	54.6±2.2*	4.0±0.1*
EEAB 200mg/kg b.w (i.p)	28.2±2.6**	39.4±2.7**	3.2±0.2**
EEAB 400mg/kg b.w (i.p)	18.3±3.8***	24.4±1.8***	2.4±0.1***

EEAB= Ethanol extract *A. baccifera* (L) whole plant. PG=Propylene glycol, i.p- intraperitoneal. Treatment period = 18days. P<0.05, **P<0/01, ***P<0.001 significantly different from vehicle control. Values are mean±S.E of 6 rats.

Table-3: Effect of ethanol *of Ammania baccifera* (L) whole plant on content of cholesterol, ascorbic acid and G-6-PD and Δ^5-3β-HSD activities in testis of mature male rats.

Design of treatment	Cholesterol (μg/mg of tissue)	Ascorbic acid (μg/mg of tissue)	G-6-PD (U/mg of protein)	Δ^5-3β-HSD (U/mg of protein)
Saline 5ml/kg b.w (i.p)	73.3 ± 0.8	114.3 ±0.8	4.2±0.8	170 ±0.06
Vehicle (PG) 5ml/kg b.w (i.p)	70.6 ±1.1	110.2 ± 1.6	4.0±0.12	1.1 ±0.06
EEAB 100mg/kg b.w (i.p)	109.2 ±2.62**	138.4 ±2.1*	3.7±0.05	0.9 ± 0.04*
EEAB 200mg/kg b.w (i.p)	119.3 ±2.5***	153.3 ± 2.6**	3.1 ± 0.04***	0.7 ±0.02***
EEAB 400mg/kg b.w (i.p)	167 ± 4.6***	194±6.6***	2.2 ± 0.02***	0.5 ±0.008***

EEAB= Ethanol extract *A. baccifera* (L) whole plant. PG=Propylene glycol, i.p- intraperitoneal.
Treatment period = 18days P<0.05, **P<0/01, ***P<0.001 significantly different from vehicle control.
Values are mean±S.E of 6 rats.

Discussion

Sperm motility and sperm density were reduced in aluminum chloride treated mice due to lack of fructose (which is the source of energy for sperm motility) in the seminal vesicles of mice (Chinoy and Bhattacharya, 1997). Cholesterol is involved in steroidogenesis in testes and it is an important precursor in the synthesis of steroid hormones. Its level is therefore related to the fertility of individuals (Eik-Nes and Hall, 1962). Increased level of cholesterol may be due to decreased androgen production, which resulted in accumulation of cholesterol in testes, resulting in impaired spermatogenesis (Bedwal *et al.*, 1994) and may suggest non-utilization of lipid towards testosterone biosynthesis (Krum *et al.*, 1964). Steroidogenesis in testes is under the control of two enzymes namely Δ^5-3β -HSD and glucose-6-phosphate dehydrogenase. Decrease in activities of these enzymes indicates antisteroidogenic effect (Krum *et al.*, 1964).

The ethanol extract of whole plant of *A. baccifera* (L.) (EEAB) at the tested doses reduced the sperm motility, sperm density and fructose content and also elevated the ascorbic acid and cholesterol content the extract also reduced the activity of Δ^5-3β -hydroxy steroid dehydrogenase (Δ^5-3β HSD) and Glucos-6-phosphate dehydrogenase (G-6-PD), the two key steroidogenic enzymes in a dose dependent manner. These results reveal that they inhibited spermatogenesis by reducing sperm motility, sperm density and fructose content, inhibited testicular steroidogenesis by increasing the elevation of substrate level and reduction of the two enzymes. Previously quercitin has been isolated from this plant (Thakkar *et al.*, 1986). Quercitin was found to possess antigonadotrophic activity (Harborne and Baxter (1983). The presence of this constituent may be responsible for its antifertility effect.

On the basis of above findings and experimental data, it could be concluded that the ethanol extract of whole plant of *Ammania baccifera* L. (EEAB) exhibited inhibition of spermatogenesis and testicular steroidogenesis in male rats thereby acting as an antifertility agent.

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