
Studies of Antifertility Activity of *Marsilea minuta* Linn.

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The antifertility activity of methanol extract of *Marsilea minuta* (F. Marsileaceae) was studied on female Swiss albino mice. The extract was found to produce significant elevation of the level of total cholesterol and ascorbic acid content of the ovaries of the treated mice. It also produced significant reduction in the activities of glucose-6-phosphate dehydrogenase and Δ^5 -3- β -hydroxysteroid dehydrogenase enzymes in mice. These observations indicate that the methanol extract produced antifertility activity in mice, which may be due to inhibition of gonadal steroidogenesis.

Rapid population growth has caused serious problem in the economic growth and human development in the developing countries. In India, family planning has been promoted through several methods of contraception but due to serious side effects, 1,2 produced by synthetic drugs, attention has now been focused on indigenous plants for birth control.

Marsilea minuta Linn³ is an aquatic herb found almost all over India. The leaves and the whole plant are used as a sedative in insomnia⁴. It finds applications as an antifertility agent^{5,6} among the Santhals of West Bengal and the Kharis of Orissa. It is also used in the treatment of epilepsy and behavioral disorders. The plant was purchased from a local supplier, United Chemicals and Allied Products, Kolkata during the months of November – December and extracted with methanol in a Soxhlet apparatus. The extract was found to contain flavonoids and tannins. The median lethal dose⁷ (LD₅₀) value of the dried extract was found to be greater than 1 g/kg i.p. Mature female Swiss mice weighing between 18-22 g were maintained under standard laboratory conditions with standard commercial diet and water given *ad libitum*. The mice were observed for regular oestrus cycle. The mice showing a natural oestrus cycle for three consecutive cycles were chosen. They were divided into three groups, each consisting of six animals and were given the following treatments. Group 1 was administered normal

saline 10 ml/kg i.p., which served the control. Vehicle (1:1 mixture of water and propylene glycol) 10 ml/kg i.p. and methanol extract of *Marsilea minuta* (MEMM) dissolved in the vehicle 250 mg/kg i.p. were administered to groups II and III, respectively, once every week for six weeks. Changes in the oestrus cycle were observed twice a day by the examination of vaginal smears. At the end of the treatment period, the animals were sacrificed after 24 h of the last dose. Ovaries were dissected out and biochemical estimations were performed on the ovarian tissues for cholesterol, ascorbic acid, protein, Δ^5 -3- β -hydroxysteroid dehydrogenase (HSD) and glucose-6-phosphate dehydrogenase (G6PD). The cholesterol content of ovary was measured by the method of Schoenheimer and Sperry⁸. For ascorbic acid content, tissue extract was prepared in a mixture of 5% w/v metaphosphoric acid, - 10% glacial acetic acid. Ascorbic acid in the tissue extract was oxidized to dehydroascorbic acid by the addition of bromine water. The dehydroascorbic acid formed was estimated by the method of Roe and Keuther⁹ at 540 nm against a reagent blank. The activity of HSD enzyme was measured by the method of Hubener¹⁰. Ovary tissues were extracted with 1 ml of 0.1 M phosphate buffer, to this NAD (6 mg in 2 ml of 0.1 M PBS, pH 7.4) and dihydroepiandrosterone sulfate (5 mg/ml) were added. After 90 min incubation at 37°, it was acidified with 3 M acetate buffer. The increase in absorption of NADH was measured at 340 nm. G6PD was assayed by the method of Lohr and Waller¹¹. The tissue was extracted with 0.5 M tris-HCl buffer

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(pH 8.3) and centrifuged at 1000 rpm for 5 min. To the supernatant 20 mM of NADP solution and 100 mM substrate were added. The formation of NADPH was monitored at 340 nm for 2 min at 30 s interval. The protein content of ovary was measured using the method of Folin and Lowry¹². The colour formed by the addition of Folin Ciocalteu's reagent was measured at 660 nm against reagent blank.

Results are summarized in Tables 1 and 2, respectively and the statistical significance is determined by performing the Student's t test¹³. From Table 1, it is evident that the oestrus cycle becomes irregular after administration of the fourth dose of the plant extract (250 mg/kg.) The cycle was arrested at the dioestrus stage after five weeks of treatment while it was regular in the mice which received normal saline and vehicle i.p. The level of total cholesterol and ascorbic acid contents in the MEMM treated mice ovaries were

elevated significantly. The activities of G6PD and HSD were decreased significantly in the ovaries and the adrenal glands of the treated animals. Leutinizing hormone administration and simultaneous increase in ovarian steroidogenesis are associated with depletion of ovarian cholesterol¹⁴. Ovarian stimulation by chorionic gonadotropins, leutinizing hormone and interstitial cell stimulating hormone results in depletion of ascorbic acid from the glands¹⁵. From Table 2, it was observed that there was an increase in the level of total cholesterol and ascorbic acid along with a reduction in the activity of the two enzymes, G6PD and HSD. The considerable increase in total cholesterol content of ovarian tissues of the treated mice suggests impaired utilization of cholesterol in the synthesis of the steroid hormones resulting in decreased steroidogenesis in drug-treated mice. The accumulation of ascorbic acid in the ovaries of the drug-treated mice gives additional support of the depressed steroidogenic

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *MARSILEA MINUTA* STEM AND LEAVES ON OESTRUS CYCLE OF MICE.

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Normal Saline (10 ml /kg)	P/O/M/D	P/O/M/D	P/O/M/D	P/O/M/D	P/O/M/D	P/O/M/D
Vehicle (5 ml/kg)	P/O/M/D	P/O/M/D	P/O/M/D	P/O/M/D	P/O/M/D	P/O/M/D
MEMM (250 mg/kg)	P/O/M/D	P/O/M/D	P/O/M/D	P/O/M/D	D/D/D/D	D/D/D/D

P indicates prooestrus stage, O indicates oestrus stage, M indicates metoestrus stage and D indicates dioestrus stage in the oestrus cycle.

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *MARSILEA MINUTA* STEM AND LEAVES ON THE MICE OVARIES.

Treatment	Weight of Ovary (mg)	Cholesterol ($\mu\text{g}/\text{mg}$ of ovary tissue)	Ascorbic acid ($\mu\text{g}/\text{mg}$ of ovary tissue)	HSD (U ^a /mg of protein)	G6PD (U/mg of protein)
Normal Saline (10 ml /kg)	6.1 \pm 0.2	17.7 \pm 0.4	6.6 \pm 0.2	1.59 \pm 0.1	4.75 \pm 0.3
Vehicle (5 ml/kg)	6.0 \pm 0.7	17.4 \pm 0.3	7.3 \pm 0.3	1.61 \pm 0.1	4.86 \pm 0.5
MEMM (250 mg/kg)	5.4 \pm 0.6	19.5 \pm 0.2**	11.9 \pm 0.5**	1.2 \pm 0.2	0.94 \pm 0.2**

U^a represent one enzyme unit, All p values are calculated with respect to vehicle control. **Denotes Statistical significance at $P \leq 0.05$.

activity. The decreased steriodogenic activity due to the treatment with the plant extract is further established by the decrease in the activity of two enzymes, G6PD and HSD, which are related to the synthesis of steroid hormones.

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An Improved HPLC Method for Estimation of Sennosides in Senna

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A simple high performance liquid chromatographic (HPLC) method for estimation of sennosides A and B in senna has been established. The aqueous extract of senna was directly injected and separated using reverse phase Shim pack CLC-CN column with UV detector monitoring at 220 nm. Compounds were well resolved when mixture of 20 mM sodium citrate buffer (pH 4.5) and acetonitrile in the ratio of 9:1 was used as mobile phase at the flow rate of 1.5 ml/min. The minimum detectable limit was 0.05 µg. The chemical analysis method supported the result. The present method is more suitable than the earlier reported HPLC method for routine analysis of sennosides.

Senna (Cassia augustifolia Vahl), locally known as *sonamukhi* has been found very much suitable for cultiva-

tion in arid region¹. It has been widely accepted by the farmers of the region because of its drought tolerance, perennial nature, non-palatability by animals, low cost of cultivation and yield stability. Because of its purgative action, it is widely

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