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Antisteroidogenic activity of *Raphanus sativus* seed extract in female albino mice

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SUMMARY

The defatted methanol extract of *Raphanus sativus* Linn. (Cruciferae) seed (MERS) was evaluated for its antisteroidogenic potential in mature female Swiss albino mice. The methanol extract at the doses of 100 and 200 mg/kg body weight significantly elevated the levels of cholesterol and ascorbic acid contents which serve as a precursor for the synthesis of steroid hormones in ovaries. The extract also significantly inhibited glucose-6-phosphate dehydrogenase and Δ^5-3β-hydroxy steroid dehydrogenase, the two key enzymes involved in ovarian steroidogenesis. Hence the extract (MERS) exhibited significant antisteroidogenic activity.

Key words: *Raphanus sativus* seeds; Antisreroidogenic activity; Δ^5-3β-HSD, Glucose-6-phosphate dehydrogenase

INTRODUCTION

Raphanus sativus Linn. (Cruciferae) commonly known as Radish (English), *Mula* (Bengali), is a small erect annual herb, a very common root vegetable grown and consumed world wide. In India the plant is used traditionally for various medicinal purposes. The seed is used as emenagogue, expectorant, diuretic, carminative and in peptic ulcer diseases. The juice of fresh leaves is used as diuretic and laxative whereas the root is used in treatment of piles and gastrodynic pains (Chopra *et al.*, 1958; Nadkarni, 1976; Chatterjee *et al.*, 1992). The phytochemical report of the plant revealed the presence of flavonoids, diterpenes, fixed oil, a sulphuretted volatile oil, starch, albuminoids and carotenoids in the seeds and roots (Nadkarni, 1976). The present study was undertaken to evaluate the antisteroidogenic activity of the defatted methanol extract of seeds from *R. sativus* in mature female mice ovaries.

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MATERIALS AND METHODS

Plant material

The seeds and aerial parts of *Raphanus sativus* Linn. were collected during the month of November 2006 from Hooghly district, West Bengal state, India. The species was identified by the Botanical Survey of India, Shibpur, Howrah, India, and a voucher specimen (No. PKH-17) was kept in our laboratory for future reference. After collection, the seeds were shade dried at temperature 21 - 24°C and ground into a coarse powder.

Preparation of extract

The air dried powdered seed of *R. sativus* (150 g) was successively extracted using petroleum ether (40 - 60°C), and then methanol in a Soxhlet extraction apparatus. The methanol extract was concentrated under reduced pressure and after complete evaporation of methanol a semi solid mass was obtained (MERS, yield 5.2% w/w). The dry extract was then stored in a vacuum desiccator for future use. Preliminary phytochemical screening indicated the presence of steroids, flavonoids and terpenoids in MERS. The extract (MERS) was dissolved in propylene glycol (10% v/v) for use in the study.

Animals

Adult female albino mice of Swiss strain weighing 20 ± 2 g were maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum.* The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the University Animal Ethical Committee, Jadavpur University, Kolkata-700032, India.

Treatment of animals

The mice showing four consecutive regular oestrus cycles were taken and divided into four groups (n = 10).The first group received normal saline (0.9% NaCl, 5 ml/kg b.w., *i.p*). The second group received vehicle (propylene glycol, 10% v/v, 5 ml/kg b.w., *i.p.*). The third and fourth group received methanol extract of *R. sativus* (MERS) in propylene glycol at the dose of 100 and 200 mg/kg b.w., *i.p.* respectively. In this way the mice were treated once a day for 15 days. On the 16th day the mice were sacrificed by cervical dislocation, 24 h after final treatment and after 18 h fasting. The ovaries were dissected out, weighed, and kept on ice for further processing and biochemical estimations.

Biochemical estimations

Ovarian tissues of about 3 mg weight, were carefully homogenized in Potter Elvehjem homogenizer using chloroform: ethanol mixture (2:1) and the homogenate was centrifuged at 8,000 rpm and the non-polar supernatant part was extracted out and total cholesterol content was estimated according to the method of Kingsley and Roscoe (Kingsley *et al.*, 1949).

For estimation of ascorbic acid content, about 5 mg of tissue was homogenized in Potter Elvehjem homogenizer using 2.5 ml ice cold 5% w/v metaphosphoric acid and centrifuged for 20 min at 355 rpm; then ascorbic acid content was estimated by the method of Roe and Keuther (Roe *et al.*, 1943).

About 2 mg of ovarian tissue was homogenized in Potter Elevhjem homogenizer using 1 ml of normal saline (0.9% NaCl) and 1 ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged at 1,000 rpm. The activity of Δ^5-3β-hydroxy steroid dehydrogenase (HSD) was estimated as described by Rabin *et al* (Rabin *et al.*, 1961).

About 3 mg of ovarian tissue was again homogenized in Potter Elvehjem homogenizer using 0.5 M Tris-HCl (pH 8.3) and centrifuged at 1,000 rpm. The activity of glucose-6-phosphate dehydrogenase (G-6-PD) was estimated as described by Lohr and Waller (Lohr *et al.,* 1974).

Protein content of the ovaries was estimated with Folin's phenol reagent and the activities of enzymes were expressed in unit per mg of protein as described by Lowry et al (Lowry *et al.*, 1951).

Statistical analysis

The results are expressed as mean ± standard error of the mean (S.E.M). Student's '*t*' test was used to verify the statistical significance.

RESULTS

The effects of methanol extract from *R. sativus* seeds (MERS) on ovarian steroidogenesis of mature female mice are shown in Table 1. It was

Table 1. Influence of methanol extract of *Raphanus sativus* seeds on cholesterol and ascorbic acid content and the activities of Δ^5-3β-HSD and G-6-PD enzymes in ovarian tissues of mature female mice

Treatment	Dose	Wet wt. of ovary (mg) ± S.E.M.	Cholesterol (mg/mg of ovary tissue) ± S.E.M.	Ascorbic acid (mg/mg of ovary tissue) ± S.E.M.	Δ^5-3β-HSD (U/mg of protein) ± S.E.M.	G-6-PD (U/mg of protein) ± S.E.M.
Control (normal saline)	5 ml/kg	6.1 ± 0.2	17.7 ± 0.4	7.3 ± 0.2	1.59 ± 0.01	4.75 ± 0.03
Control (propylene glycol, 10 % v/v)	5 ml/kg	6.3 ± 0.1	17.4 ± 0.7	6.9 ± 0.3	1.61 ± 0.06	4.86 ± 0.02
MERS	100 mg/kg	5.5 ± 0.4*	19.1 ± 0.5*	9.8 ± 0.2**	1.2 ± 0.08*	1.5 ± 0.01***
MERS	200 mg/kg	5.3 ±0.9**	21.5 ± 1.2***	11.7 ± 0.6***	1.0 ± 0.04**	0.94 ± 0.07***

Number of animals per group (n) = 10. *P < 0.05, **P < 0.01, ***P < 0.001, compared to control (propylene glycol) group. Degree of significant activity was assessed by student's '*t*' test.

found that the MERS significantly reduced the wet weight of ovaries in a dose dependent manner (P < 0.05 by 100 mg and P < 0.01 by 200 mg). The MERS at 100 and 200 mg/kg body weight significantly increased the level of total cholesterol and ascorbic acid contents of ovaries in treated mice. The activities of Δ^5-3β-HSD were inhibited significantly (P < 0.05 by 100 mg and P < 0.01 by 200 mg). Similarly, the activities of G-6-PD were inhibited significantly (P < 0.001 by both 100 and 200 mg) by MERS.

DISCUSSION

Cholesterol acts as precursor for the synthesis of steroidal hormones in ovaries. Significant elevation of ovarian cholesterol level suggests that it was not utilized for the synthesis of steroid hormones and thereby indicates decreased steroidogenesis (Tamooki *et al.*, 1961). Administration of leutinizing hormone and simultaneous increase in ovarian steroidogenesis are associated with depletion of ovarian cholesterol. Ovarian stimulation by chorionic gonadotrophins, leutinizing hormones and interstitial cell stimulating hormone results in depletion of ascorbic acid in the glands. Thus accumulation of ascorbic acid in the ovaries of the treated mice gives additional support of the depressed steroidogenic activity (Baille *et al.*, 1960; Tamooki *et al.*, 1961; Guillemen *et al.*, 1963; Krum *et al.*, 1964). The steroidogenesis is under the physiological control of two key enzymes i.e. glucose-6-phosphate dehydrogenase (G-6-PD) and Δ^5-3β-hydroxy steroid dehydrogenase (HSD) (Armstrong, 1982; Suzuki *et al.*, 1984). Decrease in these enzyme activities indicated impaired steroidogenesis by MERS.

The methanol extract from *R. sativus* seeds (MERS) elevated the ascorbic acid and cholesterol content and significantly reduced the activities of Δ^5-3β-hydroxy steroid dehydrogenase (Δ^5-3β-HSD) and glucose-6-phosphate dehydrogenase (G-6-PD) in a dose dependent manner. These results revealed that MERS produced ovarian malfunction by altering substrate and enzyme activities. Preliminary phytochemical screening indicated the presence of flavonoids in the methanol extract of seeds of *R. sativus*. Since various flavonoids have been reported to possess antifertility activity, the antisteriodogenic property of the methanol extract of seeds of *R. sativus* might be due to the presence of such compounds (Qureshi *et al*., 2006; Nandakishore *et al*., 2007).

From the present investigation it can be concluded that the methanol extract of seeds of *Raphanus sativus* demonstrated significant suppression of gonadal steriodogenesis in mature female mice and hence, may produce a significant antifertility effect in female mice.

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