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Antisteroidogenic Activity of *Corallocarpus epigaeus* Benth. Ex hook. Tubers in Female Mice Ovaries

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Ethanol (90%) extract of tubers of *Corallocarpus epigaeus* Benth. Ex hook (EECE) (Family Cucurbitaceae) was evaluated for its antifertility activity. The ethanol extracts at the doses of 50 and 75 mg/kg body weight (i.p.) arrested and content in ovarian tissues. The exract 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 tubers of *Corallocarpus epigaeus* Benth. Ex. hook ethanol act as an antisteroidogenic agent.

Key Words: *Corallocarpus epigaeus*, EECE, Δ^5-3β-hydroxy steroid dehydrogenase, Glucose-6-phosphate dehydrogenase, Antisteroidogenic medicinal plant.

INTRODUCTION

Corallocarpus epigaeus (cucurbitaceae) is a glabrous, climbing and monoecious plant, considerably employed by the natives of India as a valuable remedy for snakebite, dysentery, alterative, laxative and syphilitic rheumatism[1]. The tuber of this plant is reported to possess antiinflammatory, analgesic and spasmolytic activities[2]. Sesquiterpenes (citrulline, Ishwarone, Beta Selenine) were reported from this plant[3]. In the present communication we have assessed the *in vitro* antisteroidogenic activity of ethanolic (90%) extract of *Corallocarpus epigaeus* tubers.

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EXPERIMENTAL

The tubers of *Corallocarpus epigaeus* Benth. Ex hook (EECE) were collected in the month of December from Thanjavur, Tamilnadu, India. The plant was identified by the Botanical Survey of India, Shibpur, Howrah. A voucher specimen (GBD-2) has been kept in our laboratory for future reference. The roots were dried under shade, powdered by a mechnical grinder and passed through 40-mesh sieve and stored in an airtight container for future use.

Preparation of extract

The powder of the dried roots was extracted with ethanol in soxhlet extraction apparatus. After the completion of extraction the solvent was completely removed under reduced pressure to yield solid mass (yield 3.2% w/w with respect to dried powder) and stored in a vacuum desiccator for further study. The ethanol extract was subjected to qualitative chemical tests as well as thin layer chromatographic analysis. This indicates the presence of steroids and flavonoids. This extract was dissolved in propylene glycol for further experiment.

Animal

Mature female albino mice of Swiss strain weighing 20–25 g were used for the present study. They were supplied with standard pellet diet (Hindustan Lever) and water *ad libitum*. The experiment was performed under the guidance of Ethics Commitee, Jadavpur University, Kolkata.

Experiment design

The mice showing four consecutive normal estrous cycles were then divided into four groups (n = 10 per group). In their proestrous phase they received normal saline (5 mL/kg body weight), vehicle (propylene glycol 5 mL/kg body weight), ethanol extract of *C. epigaeus* Benth. Ex hook (EECE) tubers (50 and 75 mg/kg body weight) intraperitoneally respectively to each group on every alternate day for 18 d. Body weight was noted and estrous cycles were observed everyday by microscopic examination of vaginal smear. On 19th day mice were sacrificed after 24 h of the final dose and ovaries were dissected out, weighed and kept in ice for biochemical estimation.

Biochemical estimation

Ovaries were homogenized in chloroform : ethanol mixture (2 : 1) and non-polar part was extracted out and total cholesterol content estimated according to the methods of Kingsley and Roscoe[4].

About 5 mg of tissue was homogenized in 2.5 mL ice-cold 5% metaphosphoric acid and centrifuged for 20 min at 3500 rpm. Then, the ascorbic acid content was measured[5].

Ovaries were homogenized with 1 mL of normal saline and 1 mL of in 0.1 M phosphate buffer (pH 7.4) and centrifuged. The activity of Δ^5-3β-HSD was estimated as described by Rabin *et al*[6].

About 3 mg of ovarian tissue was again homogenized in Potter Elvehjem homogenizer using 0.5 M tris-HCL (pH 8.3) and centrifuged to estimate G-6-PD[7].

Protein was estimated with Folin's phenol reagent and activity of enzyme was expressed in unit per mg of protein as described by Lowry *et al*[8].

Statistical analysis

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

RESULTS AND DISCUSSION

Ethanol extract of *Corallocarpus epigaeus* Benth. Ex hook (EECE) tubers arrested normal oestrus cycle at dieostrus phase at the dose of 50 and 75 mg/kg body weight ofter 10 days and 6 days of treatment respectively. It was found that the EECE significantly reduced the wet weight of ovaries ($P < 0.05$ and $P < 0.01$). Both doses of ethanol extract (of 50 and 75 mg/kg body weight) elevated the level of total cholesterol and ascorbic acid contents of ovaries significantly. The activities of Δ^5-3β-HSD were inhibited significantly ($P < 0.05$ by 50 mg and $P < 0.001$ by 75 mg). Similarly, the activities of G-6-PD were inhibited significantly ($P < 0.01$ by 50 mg and $P < 0.001$ by 75 mg) by both doses of ethanol extract of *Corallocarpus epigaeus* Benth. Ex hook (EECE) (Table-1).

TABLE-1
EFFECT OF ETHANOL EXTRACT OF TUBERS OF *CORALLOCARPUS EPIGAEUS* BENTH. EX HOOK (EECE) ON WEIGHT OF OVARIES, CONTENT OF ASCORBIC ACID, CHOLESTEROL AND THE ACTIVITIES OF G-6-PD AND Δ^5-3β-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^5-3β-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
EECE (50 mg b.w., i.p.)	18	10.1 ± 0.8*	105 ± 2.8*	76 ± 5.4*	3.6 ± 0.06†	0.9 ± 0.02*
EECE (75 mg b.w., i.p.)	18	9.3 ± 0.4†	137 ± 2.4†	108 ± 9.2‡	3.2 ± 0.03‡	0.8 ± 0.03‡

PG = Propylene glycol, b.w. = body weight, i.p. = intraperitoneal, EECE = Ethanol extract of *Corallocarpus epigaeus* Benth. Ex hook tubers.

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ significantly different from vehicle control.

In dioestrus stage minimum activity of steroid hormones has been reported[9-11]. This was associated with an elevation in the level of cholestrol as well as ascorbic acid content which serves as a precursor for the synthesis of steroid hormones in ovaries suggesting thereby that both cholestrol and ascorbic acid were not utilized[12-15]. The steroidogenesis is under the physiological control of two

enzymes namely, Δ^5-3β-HSD and G-6-PD, and decreases in enzyme activities indicate antisteroidogenic effect[16, 17].

The ethanol extract of *Corallocarpus epigaeus* Benth. Ex hook (EECE) tubers at both doses arrested normal oestrus cycle at dioestrus phase and also elevated the ascorbic acid and cholestrol content and significantly inhibited the activities 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, thereby suggesting antisteroidogenic activity. These results reveal that they produced ovarian malfunction by altering substrate and enzyme activities. This could be the possible mechanism of action towards inhibition of fertility.

From the present investigation, we confirm that the ethanol extracts of tubers *Corallocarpus epigaeus* Benth. Ex hook act as an antisteriodogenic agents.

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REFERENCES

1. K.R. Kirtikar and B.D. Basu, *Indian Med. Plants*, **2**, 1166 (1991).
2. S.R. Sarkar, B.N. Sinha and E. Sukumar, *Fitoterapia*, **64**, 171 (1993).
3. P.M. Dunhill and L. Fowden, *Phytochemistry*, **4**, 933 (1965).
4. G.R. Kingsley and R.S. Roscoe, *J. Biol. Chem.*, **180**, 315 (1949).
5. S.T. Omye, J.D. Turnbull and H.E. Souberlich, Methods in Enzymology, Academic Press, New York, pp. 3–47 (1979).
6. B.L. Rabin, G. Leipsner and H.W. Deane, *Endocrinology*, **68**, 619 (1961).
7. L.C. Lohr and H.D. Waller, Glucose-6-Phosphate Dehydrogenase, in: H.U. Bergmeyer (Ed.), Methods of Enzymatic Analysis, Verlag Chemie, Florida, Vol. 2, pp. 636–646 (1974).
8. O.H. Lowry, M.J. Rosenbrough, A.L. Farm and R.J. Randell, *J. Biol. Chem.*, **193**, 365 (1951).
9. W.G. Wiest, R.B. Wilcox and H.T. Kirschbuan, *Endocrinology*, **70**, 588 (1963).
10. B.D. Ramirez and S.M. McCamm, *Endocrinology*, **71**, 814 (1964).
11. R.R. Anderson and W.H. MsShan, *Endocrinology*, **73**, 976 (1966).
12. B. Tamooki and G. Pincus, *Endocrinology*, **68**, 527 (1961).
13. R. Guillemin and E. Sakiz, *Endocrinology*, **70**, 813 (1963).
14. A.A. Krum, M.D. Morris and L.L. Bennet, *Endocrinology*, **71**, 543 (1964).
15. A.H. Bailey, M.M. Ferguson and M.C. Hardt, Developments in Steroid Biochemistry, Academic Press, New York, pp. 10–25 (1960).
16. D.G. Armstrong, *J. Endocrin.*, **93**, 415 (1982).
17. S. Suzuki, Y. Endo, S. Tanaka and R. Iizuka, *Am. J. Obstetr. Gynec.*, **148**, 76 (1984).

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