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Effect of ethanolic extract of *Jatropha curcus* seeds on estrus cycle of female albino rats

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ABSTRACT

Ethanol extract of *Jatropha curcus* seed was evaluated for antifertility activity in adult female rats. Antifertility activity was evaluated by observing estrus cycle, weight of ovaries and biochemical constituents. Ethanol extract arrested the normal estrus cycle at diestrus phase and reduced the weight of ovaries significantly. Cholesterol and ascorbic acid content in ovaries significantly enhanced while glycogen content decreased. Histological, degeneration of ovarian follicle and formation of atretic follicles indicated the fall of ovarian hormone level. Normal estrus cycle and biochemical milieu was restored after withdrawal of treatment with extract on 30 days. Antifertility activities of crude extract were found to be reversible.

Key words: *Jatropha curcus*, Antifertility, Estrus cycle.

INTRODUCTION

Search for an effective, safe and reversible female antifertility agent with minimum side effects remain a challenge. To date many steroidal and non-steroidal substances have been and are being used as contraceptive agents. Although they act as potent antifertility agents but they are not free from marked side effects [1]. The major side effects associated with many potent antifertility drugs are severe and painful uterine contraction. Irregularity in the menstrual cycle for a longer time, mammary and other tissue cancers also occur [2]. Hence, world is looking the solution in herbal medicine. *Jatropha curcus* (Euphorbiaceae) seed is a rich source of hydrocarbon, which is used as petro-crop. Beside this the plant also holds great medicinal value. The drug obtained from *Jatropha* is termed as Dravanti and is reported as bitter, astringent and pungent in taste. It has anthelmintic. It has also been used in chronic dysentery, thirst, urinary discharge, abdominal complains, anaemiata, fistula, ulcer and in heart disease. Leaves of this plant are used as rubefacient and lactagogue. Leaf juice is used as external application for piles. Seed oil is used as external stimulating application in rheumatism and in paralysis. A decoction of leaf is used to relieve cough and as an antiseptic after birth [3]. Goonasekera et al. (1995) [4] reported

pregnancy terminating effect of *Jatropha* seeds in rats. In this study we report the effect of ethanolic extract of *Jatropha curcus* seed on estrus cycle, weight of ovaries and biochemical milieu of ovary.

MATERIALS AND METHODS

Preparation of extract

Jatropha curcus seeds were procured from local suppliers of Bilaspur market and authenticated by Professor T.R. Sahu, Department of Botany, Dr. H.S. Gour University Sagar, India where a voucher specimen is preserved. Seeds were dried coarsely powdered and stored. Five hundred g of seed powder was extracted with 50% ethanol in soxhlet apparatus. The extract was concentrated to dryness under reduced pressure in rotatory evaporator at controlled temperature (50–60 °C). The yield was found to be 4.30 g. Ethanolic extract thus obtained suspended in 1% carboxy methyl cellulose (CMC) to obtain 25 mg/kg and 50mg/kg body weight dose and administered orally with the help of intragastric catheter.

Animals

Adult healthy female albino rats of Wister strain (100 ±10 g) were used for antifertility testing. Albino mice of either sex were used for acute toxicity studies. All the animals were maintained under controlled standard animal house conditions with access to food and water *ad libitum*. The Institutional Ethical Committee for animal cares and use approved all experimental procedure (Reg. No.397/01/ab/CPCSEA). Vaginal smear of each rats were examined daily, only those rat showing 3-4 normal consecutive estrus cycles were selected for the experiment.

Acute toxicity study

The acute toxicity study was performed as described by Turner (1971) [5]. Adult albino mice of either sex were divided into two groups containing six animals in each group. The mice were fasted for 24 h with water *ad libitum*. The suspension prepared as above was administered orally at the dose of 500 mg/kg body weight. Control mice received vehicle only (CMC 0.2ml). The animals were observed for 72 h for behavioral changes and mortality. No mortality was observed, suggested that extract was non toxic up to the dose of 500 mg/kg dose. Therefore, one tenth and one twentieth i.e. 25 and 50 mg/kg body weight doses were selected for experiment.

Estrus cycle study

To Study the effect of ethanol extract on estrus cycle, the above selected animals were divided into three groups containing ten animals in each group, the treatment was started when the animals were in the estrus phase [6]. The group I received vehicle only (CMC, 0.2ml) and served as control. Group II and III received ethanol extract at the doses of 25 mg /kg and 50 mg/kg body weight, respectively. The treatment was given for 20 days to cover five regular estrus cycles. Vaginal Smear from the experimental animal was observed every morning. On day 21, after 24 h the last dose, half of the animals from each group were sacrificed, ovaries were dissected out freed from adhering tissues and weighed. One ovary from each animal was processed for biochemical analysis of glycogen [7] cholesterol [8] and ascorbic acid [9]. Tissues from either side were fixed in Bouin's fluid for histological studies. Paraffin sections were made and stained with hematoxylin and eosin. The remaining animals from each group were used for the post treatment studies. The observation of vaginal smears of these animals continued for 30 days after withdrawal of the treatment and allowed for colony breeding.

Statistical method

Statistical analysis was carried out using Student's *t* – test. Results were judged significant if $p < 0.05$.

RESULTS

Results are tabulated in table 1 to 3 and photomicrograph 1 to 3.

Table 1. Effect of ethanolic extract of *J. curcus* on estrus cycle of female albino rats (n = 5).

GP	Treatment and dose mg/kg	Phases of Estrus Cycle (in days)			
		Estrus	Proestrus	Metaestrus	Diestrus
I	Control CMC 0.2ml	2.1±0.03	1.01±0.01	1.2±0.02	4.6±1.10
II	EE 25	2.3±0.01	1.00±0.02	1.1±0.01	3.6±1.23
III	EE 50	2.7±0.09	1.01±0.05	1.0±0.07	2.1±0.06*

* = $p < 0.05$ when compared to control, *p. o.* = Per Oral, CMC = Carboxy Methyl Cellulose, EE = Ethanolic extract.

Table 2. Effect of ethanolic extract of *J. curcus* on weight of ovaries, content of glycogen, ascorbic acid, and cholesterol in female rat ovary (n = 5)

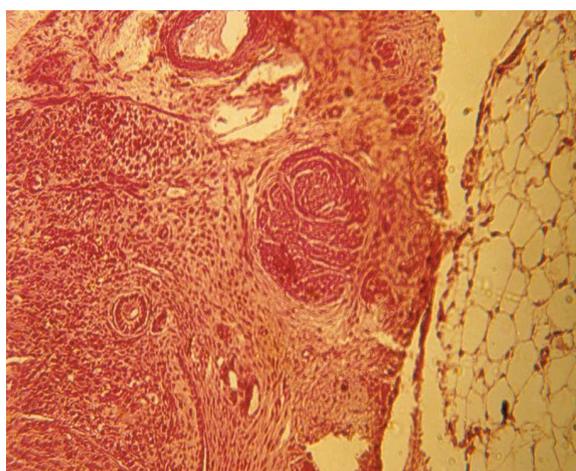
GP	Treatment and dose (p o)	Ovary weight (mg/100 g)	Glycogen Units/mg tissue weight	Ascorbic acid µg/mg of ovary	Cholesterol µg/mg of ovary
I	CMC 0.2ml	16.23±1.41	140.01±7.56	63.21±4.13	32.12±3.14
II	EE 25 mg/kg	13.11±1.14	129.22±5.41	70.34±11.83	39.25±1.14
III	EE 50 mg/kg	8.12±0.32*	73.23±7.11*	91.23±13.01*	65.22±12.13*

*: $p < 0.001$ CMC = Carboxy methylCellulose, EE = Ethanolic Extract, *p o* = Per Oral

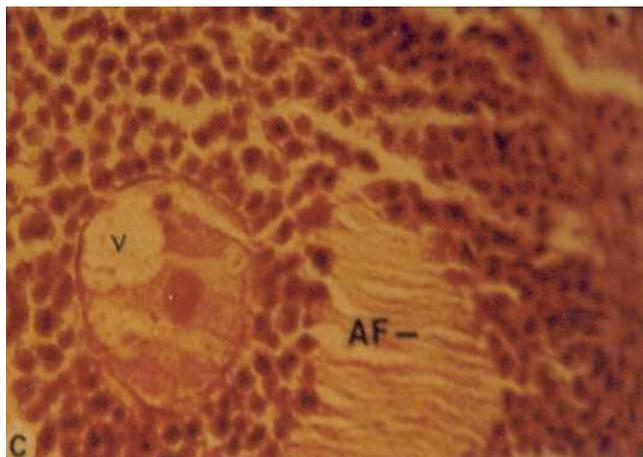
Table 3 Effect of ethanolic extract of *J. curcus* on weight of ovaries, content of glycogen, ascorbic acid, and cholesterol in female rat ovary (n = 5) After 30 days

GP	Treatment and dose (p o)	Ovary weight (mg/100 g)	Glycogen Units/mg tissue weight	Ascorbic acid µg/mg of ovary	Cholesterol µg/mg of ovary
I	CMC 0.2ml	16.21±1.21	143.09±7.16	63.10±3.18	32.22±1.34
II	EE 25 mg/kg	17.19±1.09	142.11±5.31	62.14±11.52	32.62±2.07
III	EE 50 mg/kg	17.15±0.12	142.21±7.20	61.11±14.11	33.19±12.18

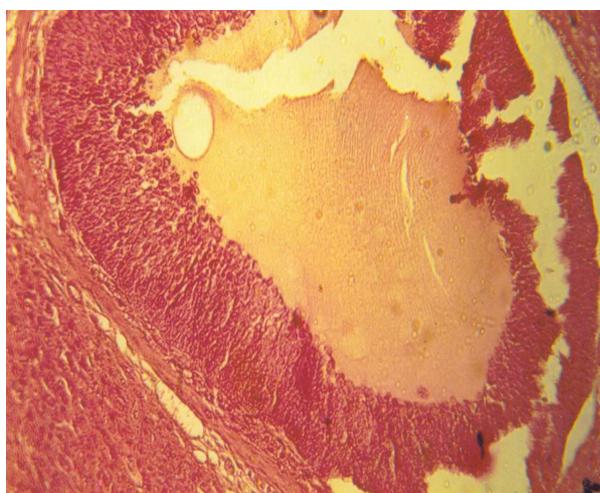
CMC = Carboxy methylCellulose, EE = Ethanolic Extract, *p o* = Per Oral



Photomicrograph 1. Section of the ovary of a rat of group I (Control) showing all the normal conditions of ovary including stroma (S), mature and developing follicles (MF, DF) (x100)



Photomicrograph 2. Section of the ovary of a rat of group II showing atretic follicles (AF) (x100)



Photomicrograph 3. Section of the ovary of a rat of group III showing degeneration of ovarian follicles (OF) (x100)

DISCUSSION

Synthesis of ovarian hormones governs the stages of the estrus cycle and their inter-conversion are under control of ovarian hormones estrogen and progesterone secreted by the cells of membrana granulosa of the matured follicles and corpus luteum, which are in turn controlled by the secretion of pituitary gonadotropins and hypothalamic-releasing factors [10]. Vaginal cornification is due to the estrogen in the adult rats and also induces cornified cells in spayed female mice, rats and immature rats. Inhibition of cornification of vaginal epithelium is a most important criterion to detect the antiestrogenic nature of a compound [11]. Oral administration of an antiestrogenic compound to cyclic rats resulted in the cessation of estrus cycle in diestrus stage and decreased the cornified cells in the vaginal smear [12]. In our study ethanol extract arrested normal estrus cycle at diestrus phase (Table 1) and reduced the wet weight of ovaries (Table 2) where minimum activity of steroids was reported [13]. This was associated with an elevation in the level of cholesterol (Table 3), which is the precursor for the synthesis of steroid hormones in ovaries suggesting thereby that cholesterol was not utilized [14]. The high accumulation of cholesterol in ovaries may suggest non-utilization of lipids towards hormonal biosynthesis in the ovaries of extract treated rats. Ascorbic acid, an easily diffusible water-soluble reductant found abundantly in ovaries where it plays an important role in ovarian steroidogenesis [15]. Its deficiency leads to malfunction of ovaries along with the elevation of

ascorbic acid levels. In the present study ethanol extract caused significant elevation of ascorbic acid (Table 3) levels due to malfunction of ovaries.

Glycogen mobilization in different parts of the female reproductive tract is apparently brought about by the ovarian hormones estrogen and progesterone. Estrogen is known to increase the glycogen content in ovary and uterus of adult and spayed rats [16]. Decrease the glycogen content (Table 3) may be due to non-availability of estrogen which is correlated with histological features by fibrillar structure of the antral fluid (Photomicrograph 2) and structural degeneration of ovarian follicles (Photomicrograph 3) characterized by vacuolation of ovum. On the basis of these findings we concluded that this plant required further investigation for detailed antifertility activities especially to identify their active constituents.

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