



Research Article

ANTIFERTILITY ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *CROCUS SATIVUS* (SAFFRON) ON FEMALE RATS

*Haroon Bashir

Sri Satya Sai University of Technology and Medical Sciences, Sehore - 466001, Madhya Pradesh, India

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ABSTRACT

Ethanollic and Aqueous extract of *Crocus sativus* (Saffron) was study, antifertility activity in proven fertile female Wistar Rats at the doses 50mg/kg b.wt./day for 30 days. Different parameters were studied in female wistar rats including effect of Reproductive outcome, Anti-implantation, Abortifacient study and Estrogenic and Anti-estrogenic activity, Phytochemical were observed. Saffron shown positive test for Alkaloids, Steroid, Flavonoids, Terpene, Carbohydrates and Tannin. The extract of *C. sativus* has anti-fertility effect the control rats showed good number of litters. After 21 days of the extracts free period, the antifertility effect of the extracts was reversed. The extract treatment with saffron, an increase in the percentage of resorption index indicates the failure in development of embryo. The decrement in implantation caused by the extracts may be due to estrogenic or anti-estrogenic activity. Clinical assessment of female antifertility agents should include acceptability, safety and efficacy during and after the treatment. The present study was therefore carried out to evaluate the claimed antifertility effect of saffron using different aspects of reproductive physiology in female wistar rats.

Keywords: Antifertility, Anti-implantation, Abortifacient, Estrogenic, Antiestrogenic.

INTRODUCTION

Fertility control is an issue of global and national public health concern. There is a global need to support individuals in family planning due to the increasing growth rate of the world's population with its negative impact on environment, economic growth and poverty reduction in underdeveloped countries. About 90% of the world's contraceptive users are women. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception in females, progress and possibilities on males are still slow and limited (Thakur *et al.*, 2010). Aware of this responsibility, health organizations and pharmaceutical companies continue to financially support or actively pursue research towards new contraceptive approaches (Ajayi & Akhigbe, 2012; Jain *et al.*, 2013). Current methods of contraception result in an unacceptable rate of unintended pregnancies and many side effects also (Devi *et al.*, 2015). *Crocus sativus* commonly known as saffron crocus, or autumn crocus (Chauhan *et al.*, 2008) is a species of flowering plant of the *Crocus* genus in the iris

family Iridaceae. It is best known for producing the spice saffron from the filaments that grow inside the flower. The term "autumn crocus" is also used for species in the *Colchicum* genus, which strongly resemble crocuses. However, crocuses have 3 stamens and 3 styles, while colchicums have 6 stamens and 1 style, and belong to different family, Colchicaceae. They are also toxic (Ezumi *et al.*, 2006).

MATERIAL AND METHODS

Selection and animals and plants

Albino rats (females) of wistar strain (150 ± 10 g b.w.) and swiss albino female mice (20 ± 10 g b.w.) were used in this study. The rats were placed in plastic cages of 36x35x19 cm in dimensions. Animals were housed under standard husbandry condition of temperature ($24 \pm 2^\circ\text{C}$), light (photocycle of 14 h light and 10 h dark) and relative humidity (60 % to 70 %). The animals were fed on standard pellet diet (Pranav agro industries, New Delhi) and water

*Corresponding Author: Haroon Bashir, Sri Satya Sai University of Technology and Medical Sciences, Sehore, India, Email: phdzooologyssutms@gmail.com

ad libitum. Flower Parts of *Crocus sativus* (saffron) were procured from authenticated Ayurvedic dealer and was identified by the taxonomist, IIIM, Jammu. The flower were washed with distilled water and air-dried before being ground into fine powder with an electrical grinder. The powdered root sample was extracted in distilled water (500g / 1000 ml) for 7 days after which the resulting mixture was filtered through a Whatman filter paper.

RESULTS AND DISCUSSION

The clear filtrate, concentrated, dried in vacuo (yield 12.6% w/v), and the residue stored as stock solution in a refrigerator at 4°C until used. Immature female rats of wistar strain 21-23 days old weighing 40-60 g.m. were used. They were divided in to eight groups of six animals each. The various groups were treated as follows: Group I- Control: (Vehicle Tween 80, 1%, 5 ml/kg.) p.o. Group II- Standard: DES50 mg/kg. Group III-V - ethanolic extract of aerial part of Saffron 50,100 and 200 mg/kg p. o. Group VI-VII - Aqueous extract of aerial part of Saffron 50, 100 and 200 mg/kg p.o.

All the above treatments were given for 7 days. Vagina and the vaginal smears were examined in all the animals in the treated groups for 7 days of treatment and 24 hrs. of last treatment all the animals were sacrificed by decapitation and uteri were dissected out, cleared off the adhesive tissue, blotted on filter paper and weighted quickly on a sensitive

balance. The tissues were fixed in Bouin's fixative for 24 hrs. Dehydrated in alcohol and embedded in paraffin. The paraffin blocks were sectioned at 6 and stained with haemotoxylene-eosin solution (H & E Stain) for histological observations^{100, 102}. The diameter of the uterus, thickness of endometrium, and the height of endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer.

Single bolus dose of DES at the dose of 50 mg/kg caused significant rise ($P \leq 0.05$) in the enzymatic activity of acid phosphatase in ovary and uterus, as shown in Table 1. Treatment *C. sativus* significant protection in the hepatic enzymatic activity at 5% level by Tukey's HSD *post hoc* test. AA therapy showed significant decrease in the renal acid phosphatase activity ($P \leq 0.05$). Significant F- variance was noted at 5% level in the acid phosphatase activity (Table 2). Table shows significant fall in the activity of alkaline phosphatase in ovary and uterus after DES administration ($P \leq 0.05$). Therapy with *A. indica* showed significant increase in the alkaline phosphatase activity of ovary ($P \leq 0.05$), and uterus was equally effective in alkaline phosphatase activity, however. Tukey's HSD *post hoc* test showed non-significant seen very clearly with statistical analysis. Results obtained from this study were also compared with the reference drug silymarin (Table 2). Significant decline was observed in the activity of adenosine triphosphatase in ovary and uterus after DES administration as shown in Table 2.

Table 1. Micrometric changes in the uterus due to administration of various extracts of aerial parts of *Crocus sativus*.

Sl. No.	Treatment Extracts/ Drugs	Dose mg/kg	Diameter of Uterus	Thickness of Endometrium	Epithelial cell height
1	Control (Vehicle)	Tween 80 (1%, 5 ml/kg)	641.8 ± 2.190	341.0±1.498	10.00±0.258
2	DES	50 mg/kg	1820.0± 6.802***	641.4±2.255***	17.83±0.2789***
3	Ethanolic extract	50 mg	1114.0±23.56***	576.6±2.318***	15.00±0.2887***
4	Ethanolic extract	100 mg	825.1±4.369**	400.3±3.630**	12.50±0.428**
5	Ethanolic extract	200 mg	890.6±2.939***	431.3±1.521***	13.00±0.2887***
6	Aqueous Extract	50 mg	851.5±2.876**	276.9±4.32**	11.50±0.4655**
7	Aqueous Extract	100 mg	801.81±2.281**	405.6±2.256**	12.42±0.273**
8	Aqueous Extract	200 mg	748.1±2.972**	356.5±1.419**	11.33±0.557**

Table 2. Plant extract showed better protective effect by Tukey's HSD *post hoc* test ($P \leq 0.05$).

Treatments	ACP(IU/L)	ALP(IU/L)	ATP
Control	72.2 ± 5.60	47.0 ± 3.13	214 ± 17.1
DES	222 ± 20.4 [#]	280 ± 17.2 [#]	421 ± 25.5 [#]
DES + CS 50mg/kg	130 ± 10.2*	117 ± 8.67*	284 ± 18.1*
DES+ CS 100mg/kg	(61.4%)	(69.9%)	(66.2%)
DES+ CS 200mg/kg	96.6 ± 7.11* (83.7%)	92.3 ± 6.27*	251 ± 17.1*
	84.3 ± 6.27*	(80.5%)	(82.1%)
	(78.5%)	84 ± 18.1* (61.2%)	275 ± 18.1*(62.2%)
F value	33.2 [@]	110 [@]	20.6 [@]

Post treatment with crude extract and *C. sativus* restored the depleted values and prevented the release of enzyme activity in both organs significantly ($P \leq 0.05$). The initial experiment was to characterize the kinetics of neonatal rat follicle development in vivo and in vitro. At birth, the vast majority of oocytes were not associated with follicles (Figure 1 and 2). In vivo the number of primordial follicles in the ovary rose to about 75%. There was a gradual

increase in primordial to primary follicle transition with the percentage of primary follicles being 25% of all the follicles within 3 d of postnatal development (Figure 2). The data obtained in the present study indicates that 70% ethanolic, aqueous and 95% ethanolic extract of aerial part of *C. sativus* exhibited more significant anti-fertility activity in dose dependent manner. 70% ethanolic, 95% ethanolic, and aqueous extracts at dose of 500 mg/kg b.w.,

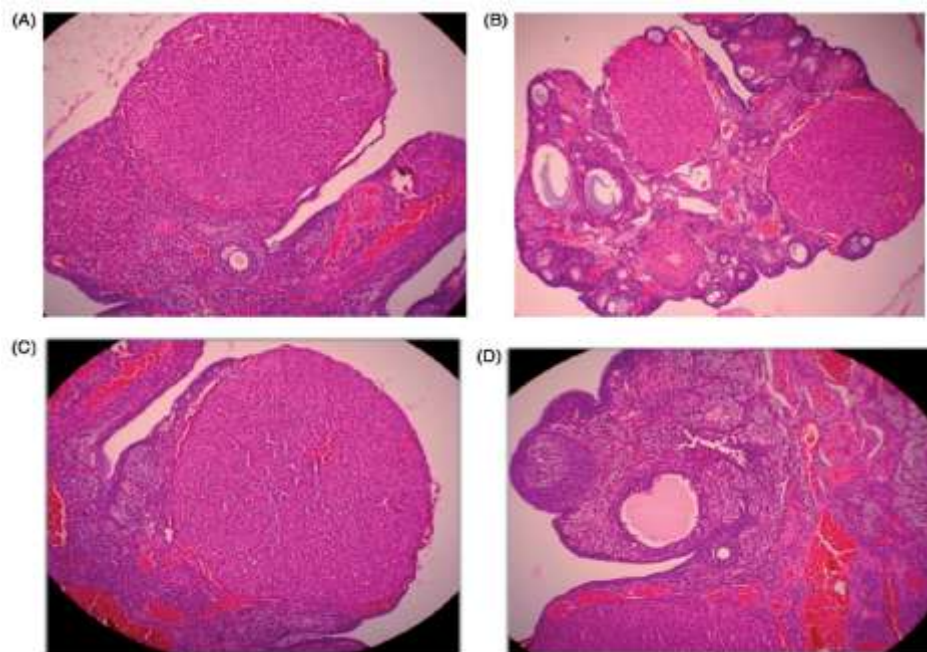


Figure 1. Histological changes in the ovary: (A) normal rat ovary; (B) cystic ovary after PCOS induction; (C-D) ovary treated with Saffron, nearly normal.

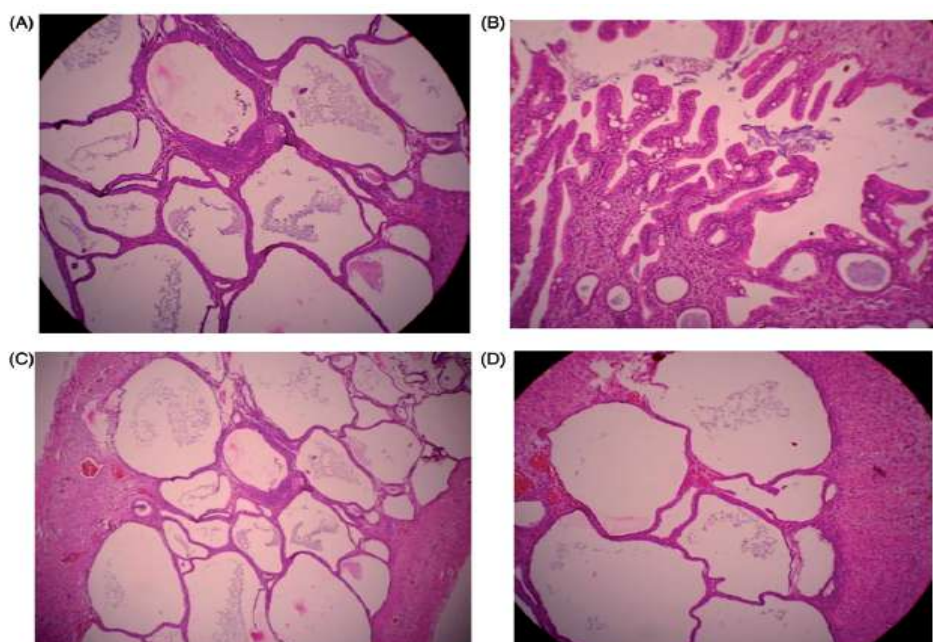


Figure 2. Histological changes in the uterus: (A) normal rat uterus; (B) diseased uterus after PCOS induction; (C-D) rat uterus treated with Saffron.

250 mg/kg b.w. were found to possess highly significant estrogenic activity as indicated by increase in uterine weight, vaginal cornification and uterotrophic responses. In immature female rats, when compared to control, but not significantly greater than standard in dose dependent manner (Li *et al.*, 2018). Estrogenic activity is shared by many steroidal and non-steroidal compounds. The three principal native forms of known endogenous estrogens are 17- estradiolestrone and estriol (Li *et al.*, 2018). The most potent biologic form is 17-estradiol, which is used as a component of oral contraceptives for inhibiting gonadotropin secretion (Dorn *et al.*, 2006). One of the first non-steroidal estrogen is diethylstilbestrol, which is structurally similar to estradiol. The non-steroidal compounds with estrogenic activity including flavonoids (flavones, flavonones and isoflavonoids) alkaloids, phenolics, occur in variety of plants are well documented as anti-fertility agents (Mary *et al.*, 2003).

CONCLUSION

The present findings inferred that the gathering treated with the most noteworthy convergence of plant concentrate indicated great come about as that of the standard medication and was underpinned by histopathological investigations of the antifertility activity on female Wistar rats. Antifertility activity of plant extracts was evaluated with the help of reproductive outcome, antiimplantation, abortifacient, estrogenic and anti-estrogenic study was also performed, which further supported by the hormonal analysis. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility. In our study clearly demonstrates that Extract of saffron, the control rats showed good number of litters. Treatment of animal with different extracts resulted a significant.

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