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Research Article

PHARMACOLOGICAL EVALUATION AND ANTI-FERTILITY ACTIVITY OF BARK EXTRACT OF *JATROPHA CARCUS* IN RATS

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ABSTRACT

Aim of present study was to assess the antifertility activity of ethanolic (EtJC) and aqueous (AqJC) leaf extract of *Jatropha carcus* in rats. The anti-fertility activity of the extracts was evaluated using two experimental animal models. Estrogenic activity was carried out in immature female rats using ethinyl estradiol as standard. The evaluation parameters included changes in uterine weight and histopathology of uterus. Anti-implantation and early abortifacient activity was performed in female Wistar rats. The number of implants and resorbtions were compared to vehicle control. Phytochemical analysis of EtJC and AqJC revealed the presence of carbohydrates, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins. In estrogenic activity, the EtJC and AqJC were offered significant estrogen-like activity at 400mgkg^{-1}, p.o. by increasing the uterine weight compared to vehicle control group. In Anti-implantation and early abortifacient activity study, EtJC (400 mgkg^{-1}, p.o.) showed significant effect and it was evident by decrease in the number of implants and increase in the number of resorbtions compared to vehicle control group. The EtJC at 400 mgkg^{-1}, p.o. possess significant estrogenic, anti-implantation and early abortifacient activity, while the AqJC at 400 mgkg^{-1}, p.o. was found to possess significant estrogenic activity and the results are in consistent with the literature reports related to anti-fertility effect of flower extracts of *Jatropha carcus.*

Keywords: Antifertility, Estrogenic activity, Ethinyl estradiol, Anti-implantation.

1. INTRODUCTION

In emerging countries, the population explosion has caused a significant setback in economic progress and overall human development. The current pandemic population surge necessitates the development of new potential contraceptives as soon as possible [1]. Many studies have shown that there is an unmet demand for safe, affordable, and acceptable contraception to prevent unwanted pregnancies and abortions [2]. The search for an oral contraceptive that can limit human reproduction dates back to the beginning of time. Despite the availability of a wide range of synthetic contraceptive drugs [2, 3], these cannot be taken indefinitely because of their significant side effects [4, 5]. As a result, people are reminiscing about their youth. Herbal medications have a long history of use, with few negative effects. The Western Ghat region of India, in particular, contains an abundance of medicinal plants. In our laboratory, we are currently conducting a large-scale study of medicinal plants for their phytochemical, biological, and pharmacological capabilities, including antifertility properties [6, 7]. We present in this work, the antifertility efficacy of the bark of the plant *Jatropha carcus* as part of this research programme. *Jatropha carcus*, a popular tropical garden plant, has long been used as a traditional medicine. Plants are well recognized as a significant source of modern medications. Plants have been used to treat or prevent ailments since the dawn of mankind, leading to the birth of traditional medicine. *Jatropha carcus* is one of the genera used to cure fever, pain, and diarrhoea in Chinese, Ayurvedic, and Thai traditional medicine [1, 2]. Flavonoid lignans, coumarin tannin, phenanthrenes, quiones, phenolic acid, alkaloids, cyanogenic glycosides, and glucosinolates are among the phenolic chemicals. According to a review of the literature, no systematic method to studying the topic has been taken for antifertility activity of leaves of this plant. In the present work, we have investigated the antifertility activity of the ethanolic extract of *Jatropha carcus* bark against Ethinyl estradiol.

2. MATERIAL AND METHODS

2.1. Animals

Female Swiss albino mice (18-22 g), Wistar albino rats (150-200 g) and immature female Wistar albino rats of 21-23 days old (40-60 g) were used in this study. The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C, relative humidity 65±10 % under 12 hour's light/dark cycle. The animals were fed with rodent pellet diet (Gold Mohur Lipton India Ltd.) and water *ad libitum*. Animal ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethical Committee (IAEC). Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any non-specific stress.

2.2. Plant Material

The bark of *Jatropha carcus* were collected from Bhopal, Madhya Pradesh. The authentication was done by Prof. Saba Naaz (Head Department of Botany Safia Science College, Bhopal (M.P.) India.

2.3. Preparation of extracts

The bark of *Jatropha carcus* were air dried under shade, pulverized by a mechanical grinder and passed through a 40 mesh and then stored in airtight containers. The powdered leaves were extracted with petroleum ether, ethanol (80% w/v) and distilled water using soxhlet extractor. This extracts were concentrated to dryness under reduced pressure and controlled temperature to yield solid masses that were completely free from solvents. The percentage yield of petroleum ether, ethanolic and aqueous extracts was found to be 2.8%, 48% and 6% *W/W* respectively.

2.4. Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on petroleum ether, ethanol and aqueous extracts of bark of *Jatropha carcus* for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods described in practical pharmacognosy by C. K. Kokate [8] and K. R. Khandelwal [9].

2.5. Acute toxicity study

The acute toxicity for EtJC and AqJC was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment and fixed dose method was adopted as per OECD Guideline No. 420 - Fixed dose method [10].

2.6. Anti-fertility activity

2.6.1. Estrogenic activity on immature female rats

Immature female rats of Wistar strain 21-23 days old weighing 40-60 g were used. They were divided into six groups of six animals each. The various groups were treated as follows.

Group I - Control (Saline solution) p.o.
Group II - Reference standard (Ethinyl estradiol 0.02 $mgkg^{-1}$, p.o.)
Group III - Ethanolic leaves extract of JC (200$mg.kg^{-1}$, p.o.)
Group IV - Ethanolic Leaves extract of JC (400$mg.kg^{-1}$, p.o.)
Group V - Aqueous leaves extract of JC (200$mg.kg^{-1}$, p.o.)
Group VI - Aqueous Leaves extract of JC (400$mg.kg^{-1}$, p.o.)

The treatment was given for six days, 24 h after the last treatment, all the animals were sacrificed by decapitation and uterus were dissected out, cleared off the adhesive tissue, blotted on filter paper and weighed quickly on a sensitive balance. The tissues were fixed in Bouin's fixative for 24 h, dehydrated in alcohol and embedded in paraffin. The paraffin blocks were sectioned at 6μ and stained with haemotoxylene-eosin solution (H and E Stain) for histological observations [11].

2.6.2. Anti-implantation activity

Female rats of proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence of copulation. The animal which showed thick clumps of spermatozoa in vaginal smear was separated from the male partner. Only the rats with normal oestrous cycles were selected for the experiment. The animals were divided into six groups of six animals each. The various groups were treated as follows.

Group I - Control (Saline solution) p.o.
Group II - Reference standard (Ethinyl estradiol 0.02 $mgkg^{-1}$, p.o.)
Group III - Ethanolic leaves extract of JC (200 $mgkg^{-1}$, p.o.)

Group IV - Ethanolic Leaves extract of JC (400 $mgkg^{-1}$, p.o.)
Group V - Aqueous leaves extract of JC (200 $mgkg^{-1}$, p.o.)
Group VI - Aqueous Leaves extract of JC (400 $mgkg^{-1}$, p.o.

The extracts were administered orally from day 1 to day 7 of gestation. On the 10th day, laparotomy was carried out under light ether anesthesia in sterile conditions. The uteri were examined to determine the number of implantation sites; the numbers of corpora lutea in ovaries were recorded. The abdomen was sutured and the animals were left in cages. The drugs were administered orally again for 3 days (day 14-16). On the 18^{th} day laprotomy was carried out again for evaluating the early abortifacient activity. The percentages of anti-implantation and early abortifacient activities were calculated using formula given in Equation 1 and 2. The sum total of anti-implantation and early abortifacient activity gives percentage anti-fertility activity of the extract (Equation 3).

Equation 1

% Anti implantation activity = 100-(No.of Implantations/ No.of Corpora lutea) × 100

Equation 2

% Anti implantation activity = 100-(No.of Implantations/ No.of Corpora lutea) × 100

Equation 3

% Anti fertility activity = %Anti - implantation activity + %Abortifacient activity

2.7. Statistical analysis

Values were expressed as $x \pm s$ from 6 animals. Statistical difference in the mean will be analyzed using one-way ANOVA followed by Turkey's multiple comparison tests $P<0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

Preliminary phytochemical analysis of extracts revealed the presence of carbohydrates, steroids, glycosides, flavonoids, tannins and alkaloids in both ethanolic and aqueous extract.

3.2. Acute toxicity study

No morbidity and mortality were detected till 2000 $mgkg^{-1}$, p.o. for both EtJC and AqJC, hence EtJC and AqJC were considered to be safe till 2000 $mgkg^{-1}$, p.o.

3.3. Anti-fertility activity

3.3.1. Estrogenic activity on immature female rats

Treatement with EtJC (200 and 400 $mgkg^{-1}$, p.o.) and AqJC (200 and 400 $mgkg^{-1}$, p.o.) had showed significant increase in uterine weight in a dose-dependent manner compared to vehicle control. The estrogenic effect of AqJC at 400 $mgkg^{-1}$, p.o. was comparable with reference standard ethynil estradiol (0.02 mgkg-1, p.o.). Furthermore, the EtJC at 400$mgkg^{-1}$ offered more potent estrogenic activity than the reference standard ethynil estradiol. The extract significantly increased the weights of uteri (Table 1) and results obtained were also correlated and supported by the histopathological findings, where the EtJC (400 $mgkg^{-1}$, p.o.) showed significant increase in the height of luminal epithelium, loose and edemators stroma with stimulated uterine glands; while the AqJC (400 $mgkg^{-1}$, p.o.) showed moderate increase in the height of luminal epithelium with stimulated uterine glands (Fig. 1 to 6).

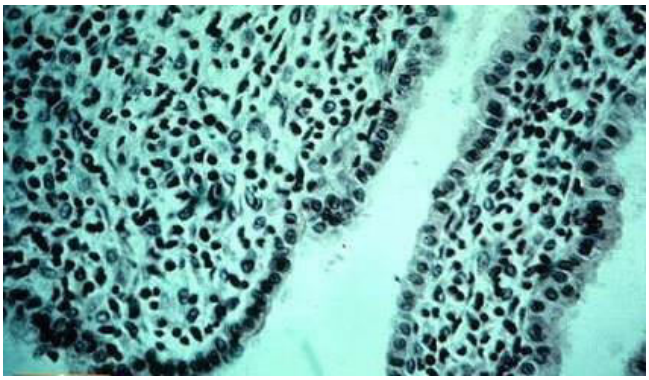

Fig. 1: Photomicrograph showing section of uterus indicating surface epithelium with no secretory activity (Control group) HE 300×

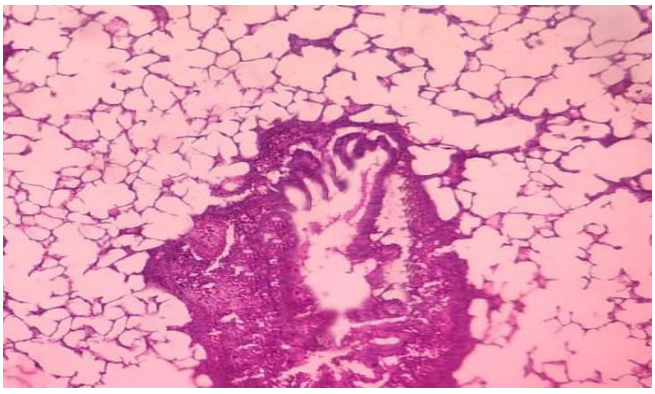

Fig. 2: Photomicrograph showing section of uterus indicating increasing height of luminal epithelium (Ethinyl Estradiol) HE 300×

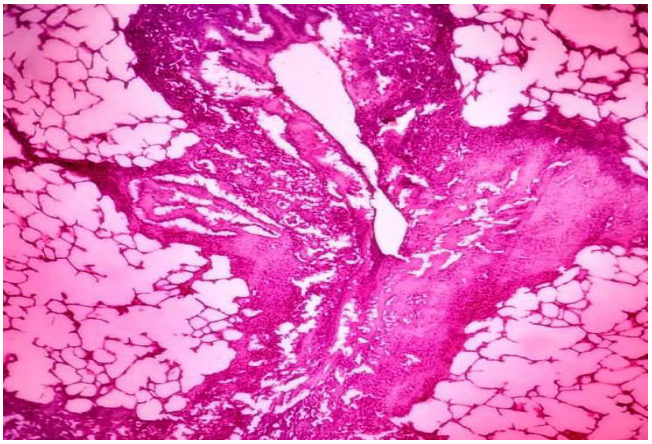

Fig. 3: Photomicrograph showing section of uterus indicating increase in height of luminal epithelium (EtJC 200 mgkg-1) HE 300×

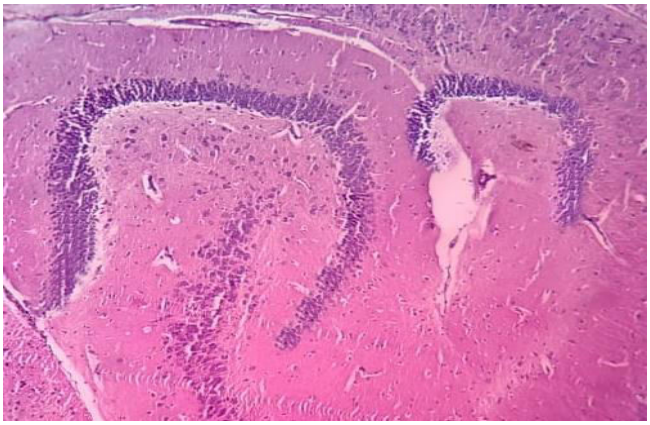

Fig. 4: Photomicrograph showing section of uterus indicating increase in height of luminal epithelium, loose and edematous stroma with stimulated uterine glands (EtJC 400 mgkg-1) HE 300×

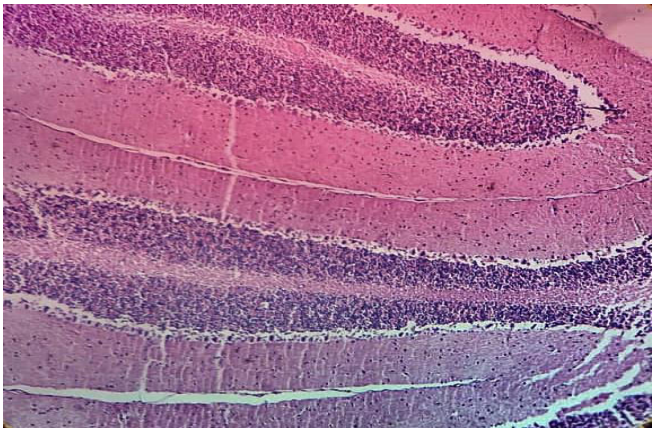

Fig. 5: Photomicrograph showing section of uterus indicating moderate increase in height of luminal epithelium with moderate stimulation of uterine weight (AqJC 200 mgkg-1) HE 3 00×

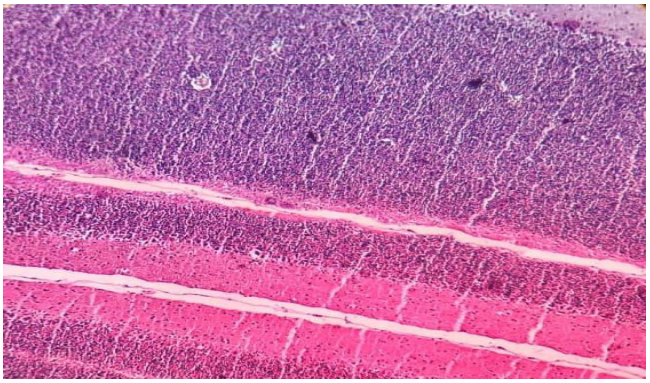

Fig. 6: Photomicrograph showing section of uterus indicating moderate increase in height of luminal epithelium with stimulated uterine glands (AqJC 400 mgkg^{-1}) HE 300×

Table 1: Effect of bark of *Jatropha carcus* extracts on uterine weight of immature female rats

Group	Extracts / Drug	Dose (mg.kg^{-1})	Uterine weight (mg)
I	Control (vehicle)	--	214.25 ± 22.79
II	Ethinylestradiol (Standard)	0.02	281.13 ± 21.50*
III	Ethanolic Extract	200	326.21 ± 08.38**
IV	Ethanolic Extract	400	359.27 ± 13.42**
V	Aqueous Extract	200	281.12 ± 23.50*
VI	Aqueous Extract	400	296.2 ± 16.10*

*Values are mean±SEM (n=6). *P < 0.05, **P < 0.01, ***P < 0.001 as compare to control group*

3.3.2. Anti-implantation activity

On day 10 of pregnancy, the anti-implantation activity is expressed as a percentage decrease in the number of implantations in the uterus, and the number of resorbed implants from the total number of implants is recorded on day 18 to assess the early abortifacient activity. When compared to the vehicle control, the EtJC and AqJC showed strong anti-implantation and early abortifacient activity by reducing the number of implantation sites and showing significant resorption of

existing implants. The EtJC at 400 $mgkg^{-1}$, p.o. showed 74.27 % anti-fertility activity and it was found to be more potent than AqJC, at 400 $mgkg^{-1}$, p.o. AqJC (400 $mgkg^{-1}$, p.o.) offered 46.78 % anti-fertility activity. The results are shown in Table 2.

Histological architecture confirmed the results obtained with the extracts on uterine weight (Table 1) of immature female rats. Histopathological investigations were carried out to see what alterations happened as a result of the extract treatment. The normal anatomy of the uterus may be seen in a section of the control group. It denotes surface epithelial cells with little secretory activity. Ethanolic extract treatment resulted in an increase in luminal epithelium height, a loose and edematous stroma, and activated uterine glands in the treated groups.

Table 2: Effect of bark of *Jatropha carcus* extracts on anti-implantation and early abortifacient activity in rats ($x \pm s$, $n = 6$)

Treatment	% Antiimplantation activity	% Early Abortifacient activity	% Anti-fertility activity
Vehicle control	0	0	0
AqJC 200 $mgkg^{-1}$, p.o.	24.35 ± 0.58	2.85 ± 0.59**	26.47 ± 0.65
AqJC 400 $mgkg^{-1}$, p.o.	42.98 ± 0.40	5.71 ± 2.54***	46.78 ± 0.29
EtJC 200 $mgkg^{-1}$, p.o.	29.47 ± 0.39	3.03 ± 0.42**	33.31 ± 0.16
EtJC 200 $mgkg^{-1}$, p.o.	63.70 ± 0.34	10.00 ± 4.15***	74.27 ± 0.28

*** P < 0.01, ***P < 0.001 vs vehicle control*

The considerable increase in the diameter of the uterus, height of the endometrial epithelium, and thickness of the endometrium in extract treated mice compared to control animals suggests that ethanolic extract has estrogenic activity at a dose of 400 $mgkg^{-1}$ body weights. The rats given AqJC (400 $mgkg^{-1}$, p.o.) had enhanced luminal epithelium height as well as activated uterine glands. There was no anti-estrogenic action in these extracts. The right balance of oestrogen and progesterone is necessary for implantation, and any changes in these hormone levels can impact fertility [12].

As a result, the EtJC and AqJC's anti-fertility effect may be mostly owing to their estrogenic activity. Non-steroidal phytoestrogens include phytochemical elements such as isoflavones, coumentans (also flavonoids), and lignans, which cause infertility in animals [7]. Furthermore, by virtue of their ability to bind and activate nuclear oestrogen receptors, numerous widely occurring flavonoids have been shown to mimic the pharmacological actions of 17-estradiol [13].

Jatropha carcus is a plant that has been used for family planning in the past [14]. It was discovered that this plant contains a wide range of phytochemical elements with a wide range of pharmacological effects. The presence of carbohydrates, steroids, glycosides, flavonoids, alkaloids, and tannins in ethanolic extract is revealed in this early phytochemical study on leaf extracts. In comparison to the control group of rats, the ethanolic extract of *Jatropha carcus* showed more pronounced estrogenic action with an increase in uterine weight.

4. CONCLUSION

The results of the present study provide the evidence for the anti-fertility activity of *Jatropha carcus* as claimed in the tradition use. The terpenoids, phytosterols and flavanoids present in the extracts may be responsible for their activity. Further studies are going on this laboratory to find out the active principal and the exact mechanism of action. With these preliminary results, we can conclude that the EtJC and AqJC showed significant anti-fertility activity by means of potent estrogenic, anti-implantation and early abortifacient activities in a dose-dependent manner.

Conflict of interest

None declare

5. REFERENCES

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