ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Evaluation of Anti-fertility activity of *Decaschistia crotonifolia* leaves on female wistar rats

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Article History:

Received on: 22.03.2019
Revised on: 05.06.2019
Accepted on: 09.06.2019

Keywords:

Hormonal alteration, Fertility potential, Dechachistia Crotonifolia, Formation of implants

ABSTRACT

Medicinal Plants were used from the ancient to the modern era and has proved in treating and preventing many different types of diseases which are not treatable with other means of treatment. The present study was aimed to prove the anti-fertility activity of *Decaschistia crotonifolia* leaves on female wistar rats. The extracts were mainly estimated for their anti-implantation activity by taking mainly 2 dose levels: 200 & 400 mg/kg, respectively. The extracts were also tested for their hormonal alteration effects on female wistar rats. The reports obtained in this study strongly prove the anti-fertility potential of leaves extracts of *Decaschistia crotonifolia*, as the extracts has shown a potential decline in the formation of implants (100%), and also the increase in uterine weight projects its estrogenic effect in Ovariectomised rats. Hence by considering the above-mentioned results, it may be proved that the leaves extracts of *D. Crotonifolia* poccess strong anti-fertility activity.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i3.1451

Production and Hosted by

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INTRODUCTION

The explosion of the population has brought up the requirement for new, safe and pharmacologically effective contraceptive method. The Indian population is rapidly multiplying and has crossed more than one billion. Fertility control now, therefore, became a major threat for the economy of developing countries. Anti-fertility agents are those which are capable of terminating the pregnancy. Moreover, the synthetic anti-fertility methods available in the market have significant side effects and morbidity rates (Sharma *et al.*, 1983).

Hence it's an alarming time for mankind to think about the natural sources, which has been neglected from the past few decades for their safe and effective means of medical needs. A lot of recent past studies in the field the herbal research has promising results in treating many ailments (Hiremath and Rao, 1990).

D. Crotonifolia is a widely grown plant belonging to the family Malvaceae found in forests of Andhra Pradesh, Karnataka, Tamilnadu and Kerala. Which is a shrub, grows at the height of 2m (Prasad *et al.*, 1986). A large source of wild plants possess a wide range of pharmacological uses, hence there is a need

to explore uses such plants to treat many diseases. The present was an attempt made to investigate the antifertility effect of *D. Crotonifolia* as the investigations on this particular plant were very much limited.

MATERIALS AND METHODS

Plant material

D. Crotonifolia leaves were collected from Thalakona forest, Tirupathi and authenticated by a botanist. After collection, the plant material was thoroughly washed, dried in air for two weeks. Then the leaves were subjected to extraction by using 70% methanol by soxhlation using soxhlet apparatus.

Animals Used

Healthy adult Wistar female rats (150-200g) with proven fertility were marked housed in polypropylene cages under a 24-hour day and night cycle. The experiment was carried out after obtaining institutional ethical committee approval.

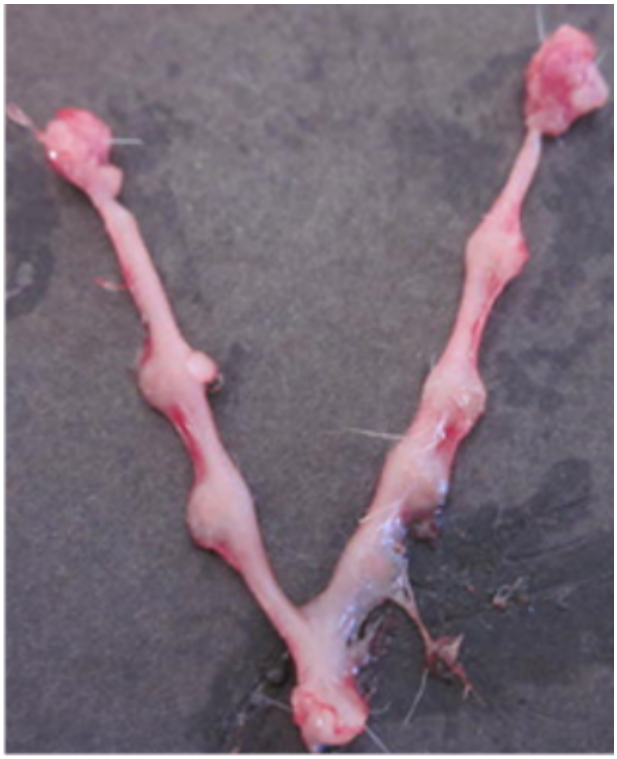

Figure 1: Control group showing 5 implants

Assessment of Anti-implantation activity

Healthy adult fertility proven female Wistar rats (150−200 g) were chosen and kept in the same cage containing healthy male rats in 3:1 ratio for copulation and the animals were examined for the evidence of copulation next day morning. The rats proven positive in pregnancy confirmation kits were selected and separated, and the present day was

Figure 2: Control group showing 6 implants

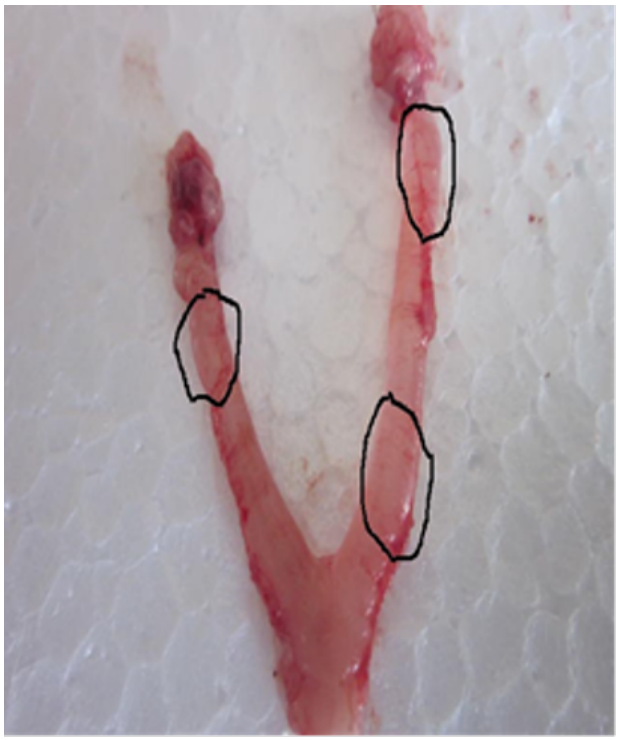

Figure 3: MEDC-Igroup showing 3 implants

Table 1: Anti-implantation activity of *Decaschistia Crotonifolia* leaves

S. No	Dose mg/kg	Treatment (in days)	No. of rats without implants on day 11/no. of rats used	No. of implants on day 11	% Anti fertility activity
1	Control (CMC-0.2 ML)	10	0/6	6.4 ± 0.46	0%
2	MEDC-I 200 mg	10	3/6	1.6 ± 0.61***	50%
3	MEDC-II 400 mg	10	6/6	0 ± 0	100%

*** P < 0.05 vs control; mean ±SEM, n = 6

Table 2: Whole body and reproductive organ weights of control and *Decaschistia Crotonifolia*

Treatmen	Body weight/g		vital organ weight/g						Entire repro-ductive	Relative reproductive
	Initial	Final	Ovaries	Uterus	Liver	Kidney	Heart	Spleen	organs weight	organ
Control (0.2 mL CMC)	126.3± 1.94	121.2± 4.46	0.036± 0.01	0.073± 0.01	4.26± 0.11	1.23± 0.04	0.53± 0.05	0.260± 0.01	0.25±0.03	0.22/121.2 = 0.0018
MEDC (200MG/KG)	153.5± 2.07	146.8± 5.00	0.053± 0.02	0.117± 0.03	5.33± 0.45	1.16± 0.05	0.46± 0.08	0.281± 0.02	0.31±0.06	0.29/14.= 0.001
MEDC (400MG/KG)	151.8± 7.27	154.3± 11.46	0.071± 0.03	0.136± 0.03	6.51± 0.63	1.46± 0.20	0.63± 0.07	0.36± 0.02	0.41±0.17	0.41/151.3= 0.0027

Treated animals (mean ±SEM, n = 6); P value Non significant

Table 3: Hormonal effects (Estrogenic) of MEDC in Ovariectomised female rats

Treatment	Dose	Uterine weight	Vaginal cornification
Standard (MALA D)	0.03 mg + 0.15 mg	0.125 ± 0.004***	+++
MEDC	300 mg/kg	0.066 ± 0.004***	+
MEDC	600 mg/kg	0.103 ± 0.004*	++

***P < 0.001;*P< 0.05 vs control, +: nucleated epithelial cells; ++: nucleated and cornified cells; +++: cornified cells; mean ±SEM, n = 6

assumed as the first day of pregnancy.

The fertile rats were then grouped into 3 groups of 6 rats in each group. I Group was administered with vehicle (CMC (1%) suspension of 10 mL/kg), which was grouped as a control group. II & III Groups were administered with MEDC (Methanolic extract of *Decaschistia Crotonifolia*) at 200 and 400 mg/kg b.w., of dose respectively. Treatment was continued for up to 10^{th} day of pregnancy.

At the 11^{th} day, the animals were euthanized by using anesthesia, and the animals were examined for the presence of implantation (Sandhya *et al.*, 2006).

Assessment of Estrogenic activity

The estrogenic potential of MEDC was investigated by using Ovariectomised immature female rats (Ciganda and Laborde, 2003). Ovariectomized 21 to 23 days old female rats of 30-40 gm weight was selected and grouped into 4 groups consisting of 6 animals in each group. I Group was administered with vehicle CMC (1%) suspension of 10 ml/kg, which was grouped as control. II Group was administered with marketed synthetic hormonal preparation (MALA D) and was served as the standard in

Figure 4: MEDC-II group showing 0 implants

olive oil, subcutaneously. III Group was administered with methanolic extract of *D. Crotonifolia* 200 mg/kg, and IV Group was given with extract of *D. Crotonifolia* 400 mg/kg orally. Treatment was given for consecutive one week (7 days), and the next day (8^{th} day) of dosing, the animals were euthanized under anesthesia and uterus were separated and weighed by using the sensitive balance.

Statistical analysis

The present study was subject to statistical analysis for accurate results by using one way ANOVA test using prism graph pad and the data was tabulated as Mean $\pm$ SEM. Significance was taken as $P < 0.05$.

RESULTS AND DISCUSSION

Dose Selection

The acute toxicity data of plant extract *D. Crotonifolia* was obtained as 2 g/kg according to earlier work done on the plant. Hence $1/10^{th}$ of the actual dose, i.e., 200 mg/kg, was fixed as a test I dose, and 400 mg/kg was fixed as teat II dose.

Effect on implants formation

Results of anti implantation activity stated and confirmed that the methanolic plant extract showed significant ($P < 0.05$) concentrated dependant implants inhibition, on the other hand, the test II dose has shown 100% inhibition information of implants, whereas the test I extract has shown 50% inhibition information of implants as shown in Table 1. Furthermore, the extracts also proved to bring change (increase) in all the important and the organs of reproduction, respectively, compared to control group animals, data provided in Table 2. The images (Figures 1, 2, 3 and 4) proves the above-mentioned data.

Effects on hormonal profile

The results of plants crude extracts on female rat uterus is as shown in Table 3. The plant extract at both doses significantly increased reproductive organ like uterus weight.

One of the major quests of drastically growing countries is the fast and rapidly growing population. Moreover, India's foremost hindrance is pollution, and overpopulation, hence it has become mandatory to use active herbal substances or anti-fertility products of plants which process natural patterns of reproduction (Shweta *et al.*, 2011).

Plants that have proven fertility manipulating effects are expected to removal of the fertilized ovum, furthermore due to hormonal manipulation most of the plant extracts may lead to decrease information of implants and to lead to abortion (Noumi and Tchakonang, 2001; Nivsarkar *et al.*, 2005; Schwarz *et al.*, 2008).

The present study revealed that the plant extracts of *D. Crotonifolia* has a strong effect on the formation of implants as the results indicated decreased observational implants.

In the estrogenic activity on the *D. Crotonifolia* extracts revealed that it contains the properties as similar to that of the estrogen as it increased the weights of female reproductive organs.

As one of the earlier works reported that Hibiscus leaves extracts of caused diminished and altered implants formation in mice (Emmens, 1970). The implantation plays a major role in various species like mice, rats and humans because it is dependent on estrogen/progesterone balance (Farnsworth *et al.*, 1975), Furthermore, according to literature decreased hormonal synthesis may also lead to diminished implants formation (Sadik *et al.*, 2001).

After the administration plant extracts (*D. Crotonifolia* leaves) at both low and high dose, the extract at test II shown significant diminished implants formation, as shown in Table 1.

This result is no more different with that of the findings of earlier studies, which stated the Pergularia leaf extract shown diminished implant formation (Montanari and Bevilacqua, 2002). Similar finding were also stated in an earlier study, which

stated that *Maytenus* leaf extract showed diminished implants formation (Freitas *et al.*, 2005).

From the above observations of the reduced formation of implants and increased uterine weight becomes evident, which confirms the anti-fertility portentials of methanol extract of *Decaschistia crotonifolia leaves*.

CONCLUSION

In conclusion, the results obtained in the study revealed strong hormonal alteration and decreased implants formation after dosing the animals with methanolic extract of *D. Crotonifolia.* Hence, this observation clearly demonstrates the strong antifertility effect of *D. Crotonifolia.*

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