



Received on 26 November 2019; received in revised form, 30 January 2020; accepted, 14 March 2020; published 01 December 2020

EVALUATION OF ANTI-IMPLANTATION ACTIVITY OF *THESPESIA POPULNEA* (L.) IN WISTAR ALBINO RATS (*RATTUS NORVEGICUS*)

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Keywords:

Anti-implantation, Hormonal changes and *Thespesia populnea*

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ABSTRACT: The present study describes the anti-implantation activity of *Thespesia populnea* bark extracts (ethanol, ethyl acetate, and aqueous) on female albino rats. Extracts were tested for possible anti-implantation activity in albino female rats of the proestrus cycle after overnight cohabitation with males of proven fertility. The drugs were administered to pregnant female rats from day 1 to 7th day of pregnancy. On the 8th day, the rats were laparotomies under light anesthesia, and the numbers of implantation sites were counted, and the reproductive hormonal levels were assessed. The present results revealed that the short term treatment of *T. populnea* bark extracts reduced the number of implantation sites in the uterus. It also significantly altered the reproductive hormonal level by increasing FSH and LH level and decreasing progesterone, prolactin, and estradiol level. Thus, it is concluded that *T. populnea* extracts possess significant anti-implantation activity.

INTRODUCTION: Current methods of contraception result in an unacceptable rate of unwanted pregnancies and having side effects also. Thus there is a need to replace these agents by safe and effective agents such as plant-based contraceptive agents. Many plants/plant extracts have been used as antifertility agents in folklore and traditional medicines without producing apparent toxic effects^{1,2}. There are numerous ways in which herbs are used to disrupt fertility. Some herbal preparations impress upon the uterus or sometimes block and even disturb the production of hormones.

Some plants have also been reported to exhibit a contraceptive property and inhibition of the implantation activity due to a disturbance in estrogen- progesterone balance^{3,4}. *Thespesia populnea* is a small evergreen tree; the bark is corrugated with scaly twigs. Its bark possesses female antifertility effect⁵.

Although it's antifertility profile has been reported, but nothing is known about its mechanism of action in pregnant animals. Therefore, present findings deal with the effect of its extracts on implantation and reproductive hormones in female albino rats so as to pin-point its antifertility mode of action.

MATERIALS AND METHODS:

Extraction of Plant Material: *T. populnea* bark was cut out from old trees in and around villages of Puthanampatti. Bark pieces were dried under room temperature and powdered with the help of mechanical grinder.

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.11(12).6161-65</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(12).6161-65</p>
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Animal Model: The healthy female albino rats *Rattus norvegicus* (150 to 200 g body weight) with a normal estrous cycle were used for the study. The rats were obtained from Tamil Nadu Veterinary and Animal Science University, Chennai, and brought to the laboratory and maintained under a controlled environment. The rats were maintained as per animal care (Ethical Committee's Approval No.BDU/IAEC/2014/NE/32/dt.18.03.2014) were followed throughout the experimental period. All animals were fed with standard pellet feed (Sai Durga Feeds and Foods, Bangalore).

Experimental Design: Female rats of the proestrus phase were kept with male rats for mating to proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence of copulation. After confirmation of mating, mated female rats were selected and grouped into 4 groups.

Rats were divided into 4 groups. In each group consisted of 4 rats.

Group I: Control;

Group II: Ethanol extract of *T. populnea* bark treated rats (15mg/kg bwt/day);

Group III: Ethyl acetate extract of *T. populnea* bark treated rats (15mg/kg bwt/day) and

Group IV: Aqueous extract of *T. populnea* bark treated rats (15mg/kg bwt/day).

Extract Administration: Extracts were orally administered through oral gavage feeding needle. Group-I mated female rats were considered as control and provided normal laboratory feed and

water throughout the experimental period. Group-II mated female rats were treated with ethanol extract of *T. populnea* bark powder at 15mg/kg/day for 7 days. Group-III mated female rats were treated with ethyl acetate extract of *T. populnea* bark powder at 15mg/kg/day for 7 days. Group-IV mated female rats were treated with aqueous extract of *T. populnea* bark powder at 15mg/kg/day for 7 days.

Antiimplantation activity of the *T. populnea* bark extracts was evaluated in female rats with the 8th day of pregnancy. They were sacrificed by light chloroform anesthesia and counting the number of implant sites. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy. Bodyweight, food intake, and water intake were measured every morning. Blood was collected by cardiac puncher and used for hormonal estimations.

Hormonal Analysis: The quantitative determination of hormones viz., FSH, LH, Progesterone, Prolactin, and Estradiol was done by Enzyme Immunoassay (EIA) method.

Statistical Analysis: Values were represented as Mean \pm Standard deviation. Analysis of Variances (one way ANOVA) and post hoc multiple comparisons test (Student-Newman-Keuls test) were used to know the impact of herbal treatment on female reproduction. All the statistical analyses were made using windows based SPSS software⁶.

RESULTS: Anti-implantation activity of *T. populnea* was evaluated by studying changes in implantation sites and reproductive hormonal levels, and results are given in **Fig. 1** and **Table 1**.

TABLE 1: STUDENT-NEWMAN-KEULS (SNK) POST HOC TEST TO KNOW THE EFFECT OF *T. POPULNEA* EXTRACTS ON IMPLANTATION AND HORMONAL LEVEL OF FEMALE *R. NORVEGICUS*

Parameter	(Subset for alpha = 0.05) Group			
	0.5	4.5	5.0	8.0
Number of implantation (one-way ANOVA; $f_{3,12} = 76.00$; $p < 0.05$)	0.5 (Group IV)	4.5 (Group III)	5.0 (Group II)	8.0 (Group I)
FSH (mIU/ml) (one-way ANOVA; $f_{3,12} = 19.00$; $p < 0.05$)	3.7 (Group IV)	4.0 (Group I)	5.1 (Group II)	5.5 (Group III)
LH (mIU/ml) (one-way ANOVA; $f_{3,12} = 17.18$; $p < 0.05$)	2.0 (Group I)	3.3 (Group II)	4.4 (Group IV)	4.6 (Group III)
Progesterone (ng/ml) (one-way ANOVA; $f_{3,12} =$ 306.77 ; $p < 0.05$)	13.2 (Group IV)	29.7 (Group II)	32.4 (Group III)	44.3 (Group I)
Estradiol (pg/ml) (one-way ANOVA; $f_{3,12} =$ 138.91 ; $p < 0.05$)	5.6 (Group IV)	23.6 (Group III)	38.4 (Group II)	47.5 (Group I)
Prolactin (ng/ml) (one-way ANOVA; $f_{3,12} =$ 186.36 ; $p < 0.05$)	0.3 (Group IV)	22.0 (Group III)	31.9 (Group II)	59.6 (Group I)

Mean values are arranged in ascending order. Horizontal lines connect similar means

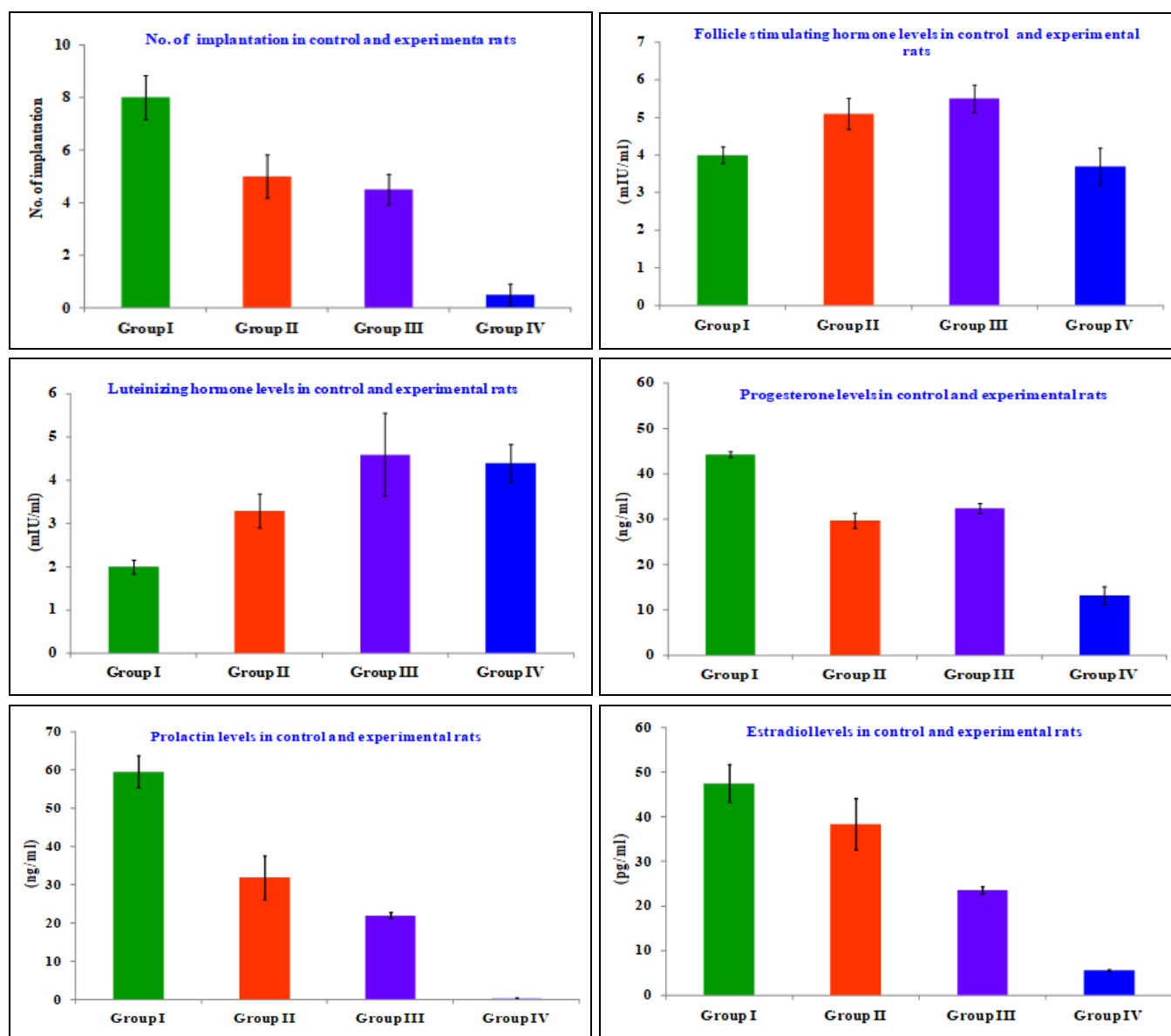


FIG. 1: CHANGES IN NUMBER OF IMPLANTATION AND LEVELS OF REPRODUCTIVE HORMONES IN THE *T. POPULNEA* EXTRACTS TREATED FEMALE ALBINO RATS. Group I: Control, Group II: Ethanol extract of *T. populnea* treated rats (14.3mg/kg bwt/day), Group III: Ethylacetate extract of *T. populnea* treated rats (14.3mg/kg bwt/day), Group IV: Aqueous extract of *T. populnea* treated rats (14.3 mg/kg bwt/day).

Effect of *T. populnea* Extracts on Implantation Loss: One way ANOVA ($f_{3,12} = 76.00$; $p < 0.005$) results revealed that there was a significant difference in the mean number of implantation among different groups of rats. SNK test revealed that the number of implantation was significantly reduced in extracts treated rats compared to control rats ($p < 0.05$). Thus, the order of reduction in implantation was arranged as; aqueous extract treated rats $<$ ethyl acetate extract-treated rats \approx ethanol extract treated rats $<$ control rats. The results revealed that the *T. populnea* extracts treatment amazingly reduced the number of implantation in the uterus especially aqueous extract treatment yielded almost zero.

Effect of *T. populnea* Bark Extracts on Reproductive Hormonal Levels:

FSH: One way ANOVA results showed a significant difference in the FSH level among the different groups of rats ($f_{3,12} = 19.00$; $p < 0.005$). SNK test results revealed that significant increases in the FSH level ($p < 0.05$) of ethanol extracts treated rats and ethyl acetate extracts treated rats compared to control rats. However, aqueous extract treatment exhibited no difference in the FSH level from the normal level ($p > 0.05$). Thus, the order of the FSH level was arranged as; aqueous extract treated rats \approx control rats $<$ ethanol extract treated rats \approx ethyl acetate extract treated rats.

LH: A significant difference (One way ANOVA; $p < 0.005$) was observed in the LH level among the different groups of rats. SNK test result showed that the aqueous and ethyl acetate extract-treated rats significantly increased LH level compared to that of ethanol extract treated rats. However, the LH level of ethanol extract treated rats was significantly higher than control rats ($p < 0.05$).

Thus, the order of LH level was arranged as; control rats < ethanol extract treated rats < aqueous extract treated rats \approx ethyl acetate extract treated rats.

Progesterone: It showed a significant difference (One way ANOVA; $p < 0.005$) among the different groups of rats. SNK post hoc test revealed that the extracts of *T. populnea* treated rats significantly decreased progesterone level compared to control rats ($p < 0.05$). Thus, the order of progesterone level was arranged as; aqueous extract treated rats < ethanol extract treated rats < ethyl acetate treated rats < control rats.

Prolactin: One way ANOVA results revealed that there was a significant difference among the prolactin level of different groups ($p < 0.005$). SNK test results showed that ethanol and ethyl acetate extracts of *T. populnea* treatment significantly decreased the prolactin level and highly decreased in aqueous extract treated rats ($p < 0.05$) compared to that of control rats. Thus, the level of prolactin was arranged as; aqueous extract treated rats < ethyl acetate treated rats < ethanol extract treated rats < control rats.

Estradiol: One way ANOVA ($p < 0.005$) showed a significant difference among the different groups of rats. SNK test showed that a significant reduction in the estradiol level of extract-treated rats compared to control rats ($p < 0.05$). Thus, the level of prolactin was arranged as; aqueous extract treated rats < ethyl acetate treated rats < ethanol extract treated rats < control rats. The present results revealed that the short term treatment of *T. populnea* bark extracts produced a significant increase in the FSH level (except aqueous extract) and LH level, a significant decrease in the progesterone, prolactin, and estradiol.

DISCUSSION: The anti-implantation activity of the *T. populnea* bark extracts may be due to the

failure of the egg to develop up to the required blastocystic stage for implantation. These must have caused the imbalance in the required hormonal microenvironment for endometrial interaction. The loss of implantation may be due to their anti-zygotic, blastocytotoxic, anti-implantation, or by early abortifacient effect⁷ of plant extracts. Sanaula and Konadawar⁸ have described the failure of the implantation process due to the administration of plant extracts is because of imbalanced hormonal environment.

T. populnea extracts treated rats (at the post-coital stage) showed an abnormal level of reproductive hormonal level. The short term treatment of *T. populnea* bark extracts produced a significant increase in the FSH level (except aqueous extract) and LH level and a significant decrease in the progesterone, prolactin, and estradiol level. The presence of flavonoids, saponin, tannin, and alkaloids may be responsible for the anti-implantation activity exhibited by the *T. populnea* extracts. A similar activity is reported in many plants⁹. This is also possible that the administration of the extracts might have caused the contractions of uterine smooth muscles, which has resulted in the expulsion of ova from the uterus. Estrogen causes an increase in protein synthesis, uterine weight, water uptake, and retention of fluid, leading to a ballooning of the uterus¹⁰. In addition, estrogen also induces uterotrophic changes such as an increase in diameter of the uterus, the thickness of endometrium, the height of endometrial epithelium, providing non-receptive conditions for implantation¹¹.

Estradiol stimulates the growth of the uterine lining, causing it to thicken during the pre-ovulatory phase of the cycle. It is well established that estradiol is directly responsible for the growth and development of reproductive organs. In synergy with FSH, estradiol stimulates granulosa cell proliferation during follicle development. Therefore the pregnancy interceptor effect of extracts of *T. populnea* bark might be due to the inhibition of circulating estrogen-progesterone balance which creates a non-receptive stage in the uterus by changing the reproductive biochemical milieu especially uterine environment which is directly involved in the implantation of eggs^{11, 12} and thus produce significant antifertility effect.

CONCLUSION: Results of the current study revealed that the treatment of *T. populnea* bark extracts to the adult mated female rats reduced the number of implantation sites in the uterus. It drastically and unfavorably altered the reproductive hormonal level by increasing FSH and LH level and decreasing progesterone, prolactin, and estradiol level. Thus, it is concluded that *T. populnea* extracts possess significant anti-implantation activity, and it may be recommended as a female antifertility agent.

ACKNOWLEDGEMENT: The authors would like to thank the Management, Nehru Memorial College for partial financial support and also thank the Principal and Head of the Department of Zoology for providing necessary facilities to do this research work successfully. The first author thanks the Tamil Nadu Educational Trust, Chennai, for financial Support.

CONFLICTS OF INTEREST: The authors confirm that this research article content has no conflicts of interest.

REFERENCES:

1. Singh A and Singh SK: Evaluation of antifertility potential of brahmi in male mouse. *Contraception* 2009; 79: 71-79.
2. Singh R, Kakar S, Shah M and Jain R: Some medicinal plants with anti-fertility potential: a current status. *Journal of Basic and Clinical Reproductive Sciences* 2019; 7: 7-19.
3. Noumi NYC and Tchakonang C: Plants used as abortifacients. *Journal of Ethnopharmacology* 2001; 76: 263-68.
4. Ciganda C and Laborde A: Herbal infusions used for induced abortion. *Journal of Toxicology and Clinical Toxicology* 2003; 41: 235-39.
5. Priya G and Saravanan K: Post-Implantation changes in the uterus of rats: Response to *Thespesia populnea* bark extracts. *International Journal of Scientific & Engineering Research* 2018; 9: 40-43.
6. Sokal RH and Rohlf FJ: *Biometry*. W.H. Freeman and Company, New York 1987.
7. Hafez ESE: *Reproduction and breeding techniques for laboratory animals*. Lea and Febiger. Philadelphia 1970.
8. Sanaula T and Konadawar MS: Anti-implantation activity of the leaf extract of *Ailanthus ecelsa* Roxb. *International Journal of Pharmacy and Pharmaceutical Sciences* 2013; 5: 128-29.
9. Yakubu MT and Bukoye BB: Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Contraception* 2009; 80: 308-31.
10. Koneri R, Balaraman R and Saraswati CD: Antiovolatory and abortifacient potential of the ethanolic extracts of roots of *Momordica cymbalaria* Fenzl in rats. *Indian Journal of Pharmacology* 2006; 38: 111-14.
11. Koneri R, Saraswati CD, Balaraman R and Ajeesha EA: Antiimplantation activity of the ethanolic root extract of *Momordica cymbalaria* Fenzl in rats. *Indian Journal of Pharmacology* 2007; 39: 90-96.
12. Vasudeva N and Sharma SK: Post-coital antifertility activity of *Hibiscus rosasinensis* Linn. roots. *Evidence-Based Complementary and Alternative Medicine* 2007; 5: 91-94.

How to cite this article:

Priya G and Saravanan K: Evaluation of anti-implantation activity of *Thespesia populnea* (L.) in Wistar albino rats (*Rattus norvegicus*). *Int J Pharm Sci & Res* 2020; 11(12): 6161-65. doi: 10.13040/IJPSR.0975-8232.11(12).6161-65.

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