Post-Implantation changes in the uterus of rats: Response to *Thespesia populnea* bark extracts

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Abstract: The isolated pure principles from successive extracts of Ethanol, Ethylacetate and Aqueous extract of *Thespesia populnea* L (bark) has been studied on post-implantation stages of the uterus of rats so as to elucidate its antifertility mode of action. The crude extracts were tested for possible antiimplantation activity in wistar albino rats of normal proestrus cycle after overnight cohabitation with males of proven fertility. The day when spermatozoa were detected in vaginal smear was treated as 1st day to 7th day of pregnancy at the dose level of 14.3mg/kg/b.wt/day. On the 8th day, the rats were laprotomized under light anesthesia and the numbers of implantation sites and corpora lutea were noted. The biochemical estimation results showed that successive increase in the Acid phosphate, Alkaline phosphate level (One way ANOVA; $p<0.005$) and the decrease in protein and cholesterol level (One way ANOVA; $p<0.0.05$). The aqueous extract of *T. populnea* was administered there was a significant reduction in all these biochemical constituents except ACP level when compared to their respective control groups. The role of these biochemical transformations has been discussed in relation to anti-implantation action of the extract. The results, it is concluded that all extracts of *T. populnea* bark exhibited female antifertility activity by reducing implants. It was found that aqueous extract and ethyl acetate extract of *T. populnea* bark were more effective than the ethanol extract.

Keywords: Anti-implantation, *Thespesia populnea*, Biochemical Parameter, Uterus, Implantation site.

1. INTRODUCTION

Thespesia populnea bark, leaves, flower and fruits are useful in cutaneous infection such as scabies, psoriasis, eczema, ring worm and guinea worm. The decoction of the bark is commonly used for the treatment of skin and liver diseases. A compound oil of bark and capsules is useful in urethritis and gonorrhoea.

The barks were used in dysentery, cholera and haemorrhoids (The wealth of India, 1995). The bark and flowers posses astringent, hepatoprotective, antioxidant and anti-inflammatory activities in rats (Manivasudevan *et al*., 2007) and also supposed to improve the memory (Vasudevan and Parle, 2006). 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 ethanol, ethylacetate, aqueous extract on the biochemical constituents of the uterus during post-implanting stages in rats so as to pin-point its antifertility mode of action.

2. MATERIALS AND METHODS

Bark of *T. populnea* L. were collected from the local areas of Puthanampatti village. They were ground well and its ethanol, ethylacetate and aqueous extracts were prepared as described earlier (Shukla *et al*., 1987).

Mature healthy female albino rats of Wistar albino rats (150±10 g) were selected for the present study. These animals were kept under uniform husbandary conditions and were given "Sai Durga" Pelleted diet and water *ad libitum.*

The vaginal smear of each adult female rat was examined daily to identify the stage and when he animals showed proestrus stage, these were caged with nature healthy adult male rats of proven fertility (2 females: 1 male). The mating was further confirmed by the presence of vaginal plug and spermatozoa in the vaginal smear next morning and the day was considered as day 1 of pregnancy (Prakash and Mathur, 1976). The rats were randomly divided into four groups, control and experimental groups. A dose of 14.3mg/kg body weight of ethanol, ethylacetate and aqueous extract was fed orally for different days of pregnancy to post implantation periods and animals were killed after 8th of last treatment. Control pregnant rats for each set were maintained simultaneously and received vehicle only. At autopsy both the uteri were excised, freed from adhering tissue, weighed and processed for biochemical estimation of local proteins (Lowry *et al*., 1951) and activity of acid and alkaline phosphatase (Hawk *et al*., 1954). The

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results were analyzed statistically using spss software 16 version.

3. RESULTS

The changes in the biochemical constituents in the post implanting uterus of control and treated animals are shown in table 1. It was found that the protein level was higher in control rats than *T. populnea* extracts treated rats. One way ANOVA ($f_{3,12}$= 95.85; $p<0.005$) showed significant difference among the groups. SNK multiple test showed that three subsets with heavy reduction in aqueous extract treated rats ($p<0.05$), and significant reduction in the ethyal acetate and ethanol extract treated rats compared to control rats ($p<0.05$).

In uterus, the cholesterol level was 481.5±7.68mg/100g of tissue in control rats while it was reduced in *T. populnea* extracts treated rats. A significant difference was observed in cholesterol level among different groups (one way ANOVA; $f_{3,12}$= 824.74; $p<0.005$). SNK test showed two subsets with significant reduction of cholesterol in the extracts treated rats compared to control ($p<0.05$).

In uterus, the ACP level was about 1.5±0.29µm/g in the control rats, while it was significantly increased in *T. populnea* extracts treated rats followed by ethanol extract treated rats (one way ANOVA; $f_{3,12}$= 11.42; $p<0.005$). SNK multiple comparison post hoc test showed that two subsets with significant increase of ACP level in extracts treated rats compared to control rats.

In uterus, the ALP level was drastically increased after administration of *T. populnea* extracts compared to control rats. The ANOVA results revealed that there was significant difference ($f_{3,12}$= 8.24; $p<0.05$) among the groups. The ALP levels of extracts treated rats was significantly (SNK test) differed from control rats.

4. DISCUSSION

An mammals, the genital tract undergoes cyclic alterations in their morphological and physiological aspect with respect to various reproductive phases. After fertilization, the uterus encounters many biochemical changes in order to prepare itself for the reception of fertilized eggs (Nordquist,1970; O'Grady *et al*., 1970), and all these transformations depend upon the action of estrogen and progesterone (Psychoyos, 1968). Protein is considered to be the building material and is involved in the alteration of almost every physiological function. Cellular functions are changes when new proteins are formed. Total proteins and the pattern of nucleic acid synthesis is generally changes during the period of pre and post implantation stages as these are urgently required for the survival of developing foetus (Heald and O'Grady, 1969).

During early stages of implantation the activity of acid phosphatase is considerably changes. It has been shown histochemically that during first four days after fertilization, the activity of acid phosphatase is remarkably increased (Lobel *et al*., 1967). Additionally on 5th day of implantation when deciduoma makes its appearance beneath the sites where the blastocyst is lined, he associated cells show strong acid phosphatase reaction (Lobel *et al*., 1967).

An increase in activity of alkaline phosphatase in the uterus at the time of implantation and deciduoma formation has been reported by a number of workers (Finn and Hinchliff, 1964 and Finn and Mc Laren, 1967). There are other evidences which clearly indicate that during early days of pregnancy there is disintegration of uterine epithelial cells which is brought about by the observed changes in the lysosomes including the acid phosphatase (Abraham *et al*., 1970). The exact function of alkaline phosphatase during post implantation stages of the uterus has been reported to be associated with the turnover of DNA within the nucleus (Danielli, 1953), the differentiation of the tissues and in the formation of deciduoma (Moog, 1994).

CONCLUSION

It is thus concluded that due to the low enzymic activity, the aqueous extract fails to trigger the action of these physiological transformation to induce the formation of deciduoma and the endometrial bed. The anti-implantation activity in the aqueous extract of *Thespesia populnea* Lin as observed earlier (9) can therefore, be explained on the facts that the administration of aqueous extract to pregnant rat does not induce any biochemical modifications in the uterus so as to prepare itself for providing suitable milieu to welcome the fertilized eggs. Consequently, the unprepared or non-receptive uterus results in the insult of the blastocysts.

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Table 1: Student-Newman-Keuls (SNK) post hoc test to know the effect of *T. populnea* extracts on levels of protein, cholesterol, ACP and ALP of *R. norvegicus* uterus under anti-implantation study.

Parameter	(Subset for alpha = 0.05) Groups			
Protein (mg/100g) (One way ANOVA; $f_{3,12}$= 59.48; p<0.05)	95.6 (Group IV)	144.5 (Group II)	165.4 (Group III)	210.9 (Group I)
Cholesterol (mg/100g) (One way ANOVA; $f_{3,12}$= 483.88; p<0.05)	161.3 (Group IV)	177.9 (Group II)	178.5 (Group III)	472.75 (Group I)
ACP (µM/g) (One way ANOVA; $f_{3,12}$= 11.42; p<0.05)	1.5 (Group I)	4.9 (Group II)	7.6 (Group III)	9.9 (Group IV)
ALP (µM/g) (One way ANOVA; $f_{3,12}$= 105.39; p<0.05)	11.1 (Group I)	11.1 (Group III)	22.4 (Group IV)	23.6 (Group II)

Mean values are arranged in ascending order. Horizontal lines connect similar means.
Group I : Control; Group II : Ethanol extract of *T. populnea* treated rats (14.3 mg/kg bwt/day)
Group III : Ethylacetate extract of *T. populnea* treated rats (14.3 mg/kg bwt/day)

Group I V : Aqueous extract of *T. populnea* treated rats (14.3 mg/kg bwt/day)

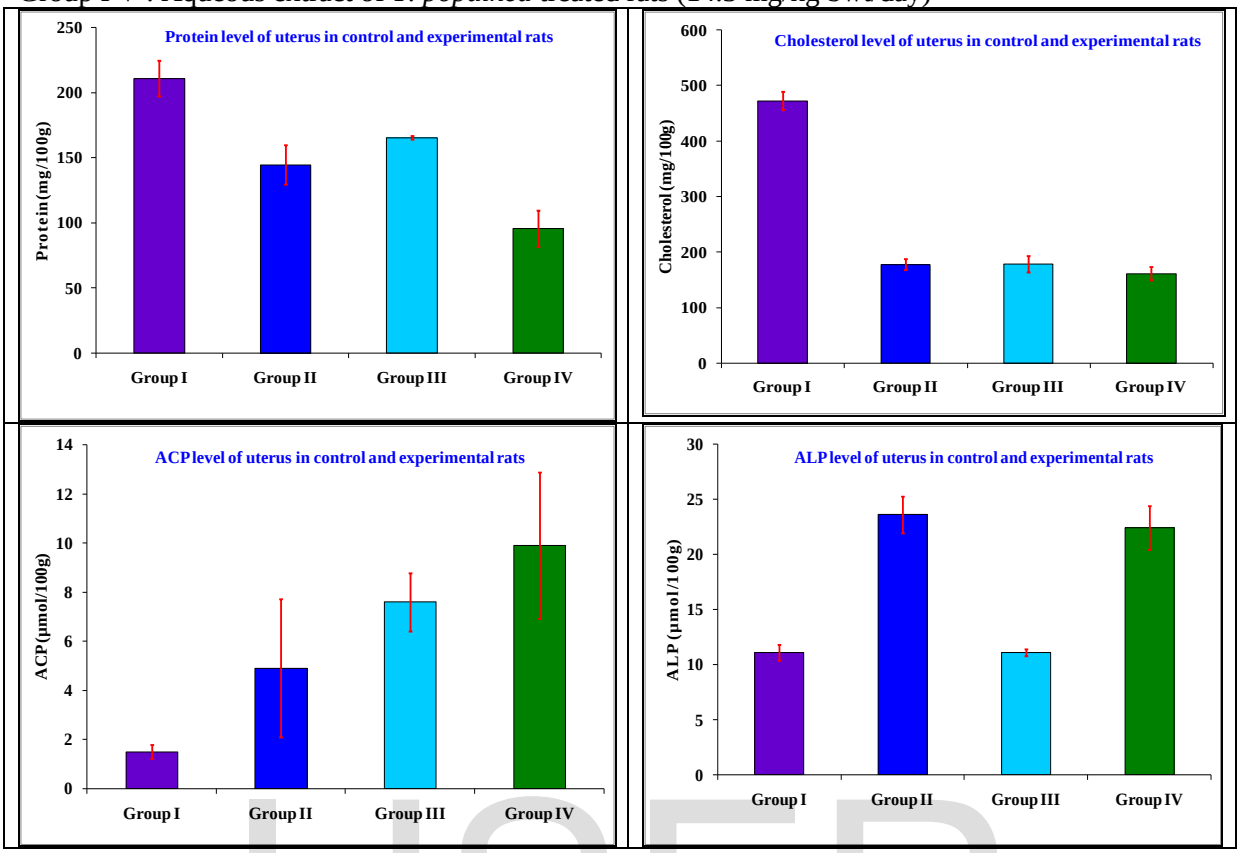

Figure1: Post implantation changes in the uterus of *T. populnea* extracts treated female albino rats. Group I: Control; Group II: Ethanol extract of *T. populnea* treated rats (14.3 mg/kg bwt/day), Group III: Ethylacetate extract of *T. populnea* treated rats (14.3 mg/kg bwt/day), Group IV: Aqueous extract of *T. populnea* treated rats (14.3 mg/kg bwt/day).