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Research Article

ANTI -FERTILITY AND WITHDRAWAL EFFECT OF THESPESIA POPULNEA (L.)SOLEX CORREA. IN FEMALE MICE

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ABSTRACT

Thespesia populnea Corr. Floral and bark extract has been shown to have anti-steroidogenic effect. Bark extract of *Thespesia populnea* has no toxicity as per the earlier reports of traditional medicine used by the tribal people of Indira Gandhi wild life sanctuary, Pollachi, South India. In the present study, anti-fertility effect of *Thespesia populnea* (Malvaceae) bark was evaluated in adult female mice (*Mus musculus*). Methanolic extract of *Thespesia populnea* bark (METP) was administered orally at the doses of 100, 250, 400 mg/kg body weight for a duration of 60 days, and normal saline (0.9%) to control group. There was significant ($P < 0.05$) reduction in uterine implantation in the METP treated dose depended cyclic female mice mated with male mice. Administration of METP in mice did not show any variation in the haematological and bio-chemical parameters. It is concluded that METP could be due to its anti- fertility effect and resulted in present study revealed the significant reduction in uterine implantation found in METP treated medium and high doses of female mice.

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INTRODUCTION

Many medicinal anti-fertility plant in particular, are widely used by people in developing countries. The family malvacea particularly *Thespesia populnea*, is well known for medicinal effect (Kritkar, 1935). This is a common plant of coastal strands worldwide tropics. The bark has been prescribed for its traditional use as antifertility agent among tribal villagers. The bark methanolic extract of *Thespesia populnea* have anti-steroidogenic effect of cyclic female mice (Chandru and Jajakumar, 2016). The abortifecient activity of some herbs affect the embryogenesis in the early stage of pregnancy. Hence, the search for a suitable product from medicinal plants have been tested for their anti-fertility and abortifacient type of antifertility effect can be produced by compounds in mice model (Farnsworth *et al.*, 1976; Desta, 1994). An abortifecient activity of an agent that can disrupt early pregnancy could be of obvious interest in human fertility control and oral administration of *Jatropha curcas* between 6th and 8th day of pregnancy showed abortifacient effect (Goonasekara *et al.*, 1995). So the present study was designed for evaluation of the dose dependent anti-fertility effect of methanolic extract of

Thespesia populnea (METP) in pregnancy test of adult cyclic female mice.

MATERIALS AND METHODS

Plant materials

Thespesia populnea family (Malvaceae) were collected from Nagapattinam district (Tamil nadu) south India. It was dried in shade, powdered and extracted with methanol (1:3 w/v) using soxhlet apparatus. The extracted residue powder was stored in a labelled sterile screw capped bottle until we use.

Animal used

Healthy mature cyclic female swiss mice, *Mus musculus*, with the body weight of 26-27g were procured from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, Annamalai University, Annamalai nagar, India. They were maintained at $27 \pm 2^\circ\text{C}$ with food and water ad libitum. The mice were closed by grid and placed in the department of animal house for acclimatization (Behringer, 1973). All the experiments were carried out with the approved of institutional animal ethical committee.

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Toxicity study

LD₅₀ was determined by the method of Litchfield and Wilcoxon (1949). The LD₅₀ of METP was found to be 3 g per kilogram body weight, in mice by oral administration.

Animal experiments

The, female mice were divided into three groups of 8 each. Normal saline 0.9%, 0.5 ml/kg per mouse per day was administered orally by an intragastric catheter in group 1 for 60 days. METP at the doses of 100, 250 and 400 mg/kg per day were given to groups 2 and 3 for a duration of 60 days. Group 3 after 60 days allowed to free from without treatment of METP for a duration of 30 days (60+30) for withdrawal effect of METP. Body weight was noted and oestrous cycle was observed every day by microscopic examination of vaginal smear.

Hematological parameters

Hemoglobin was estimated by the method of (Drabkin and Austin, 1932) Estimation of RBC and WBC count (Arthur, 1980)

Biochemical estimation

Serum biochemical parameter

Serum was used for the estimation of serum cholesterol (Zlatkis *et al.*, 1953), estimation of aspartate transaminase (AST), estimation of serum alanine transaminase (ALT). (Reitman and Frankel, 1957), estimation of urea (Natelson, 1957) and estimation of uric acid (Carawy, 1963).

Fertility test

The group 2,3 were used for fertility and withdrawal(withdrawal allow to free from without METP treatment of 60+30 days) as implantation number in uterine horns of female mice for a duration of 60 days METP treated female mice as 100,250,400 mg/kg body weight by followed the method of Kitchlu *et al.*,(1999).

Statistical analysis

Results are expressed as mean ±S.E.M. Statistical analysis was done by student's t-test and the difference was considered statistically significant at P≤0.05.

RESULTS

Effect of METP on the hematological parameters of female mice

METP treated 60 days duration of female mice no altered in hematological parameters of Hemoglobin, RBC, WBC when compared to control group (table 1.,fig.2).The withdrawal group of 60+30 days also no altered in hematological parameters(table 1.,fig.2).

Table 1 Effect of METP on the Haemoglobin (g/dl), RBC 10⁶/mm³ and WBC 10³ /mm³ count of control and treated female mice

| Group | Treatment | Dose/mg/kg Body wt. | HB gm/dl | RBC 10 ⁶ /mm ³ | WBC 10 ³ /mm ³ |
|-------|-----------------------|---------------------|---------------------------|--------------------------------------|--------------------------------------|
| 1 | Control (0.9% saline) | 0.5ml | 12.92±0.72 | 6.55± 0.76 | 3.46 ± 0.96 |
| 2 | METP | 400 | 12.78±0.077 ^{NS} | 6.01 ± 0.51 ^{NS} | 3.85 ± 0.54 ^{NS} |
| 3 | METP | 400 | 12.75±0.055 ^{NS} | 6.14 ± 0.57 ^{NS} | 3.71 ± 0.08 ^{NS} |
| | Withdrawal group | | | | |

Each values are represents mean ± S.E.M of six mice, Statistical significant at *P<0.05,when compared to control group

Effect of METP on biochemical parameters

METP treated 60 days duration of female mice no changes in serum biochemical profiles of serum cholesterol, serum aspartate transaminase (AST), serum alanine transaminase (ALT) serum urea, serum uric acid when compared to control group (table 2,fig.,3). The withdrawal of 60+30 days also no changes in biochemical profile.



Fig 1 Bark of *Thespesia populnea(L.)Sol.ex Correa*

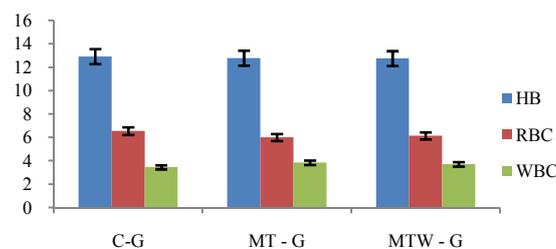


Fig.2 Effect of METP on the Haemoglobin (g/dl), RBC 10⁶/mm³ and WBC 10³ /mm³ count of control and treated female mice

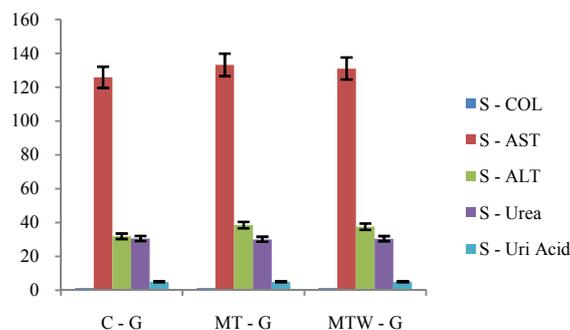


Fig 3 Effect of METP on the serum cholesterol(mg/100ml), serum AST,ALT(IU/L) serum urea, uric acid(mg/dl) in female mice

Table 2 Effect of METP on the serum cholesterol (mg/100ml), serum AST,ALT(IU/L) serum urea, uric acid(mg/dl) in female mice

| Group | Treatment | Dose/mg/kg Body wt. | Serum cholesterol | Serum AST | Serum ALT | Serum urea | Serum uric acid |
|-------|-----------------------|------------------------|--------------------------|---------------|--------------------------|--------------------------|-------------------------|
| I | Control (0.9% saline) | 0.5ml | 1.25±0.003 | 125.80 ±52.03 | 31.87±8.11 | 30.54±1.89 | 5.0±0.002 |
| II | METP | 400 | 1.25±0.003 ^{NS} | 133.17 ±59.02 | 38.45±9.12 ^{NS} | 30.09±2.01 ^{NS} | 5.0±0.002 ^{NS} |
| III | METP withdrawal | 400 | 1.25±0.003 ^{NS} | 131.0± 59.11 | 37.48±6.11 ^{NS} | 30.44±1.55 ^{NS} | 5.0±0.002 ^{NS} |

Each values are represents mean ± S.E.M of six rat, Statistical significant at *P<0.05,when compared to control group

Effect of METP on anti-fertility test in female mice

METP treated female mice after 60 days mated with male (2:1ratio,two female:one male) for a doses of 100,250, 400 mg/kg body weight showed significantly reduced in implantation site in uterine horns of (P≤0.05) when compared to control group of implantation site of female mice.

Effect of METP on Withdrawal in female mice

The mice METP treated with 400 mg/kg body weight were showed turn of normal in withdrawal of METP in implantation site was noted in 60+30 days. Resulted in withdrawal period showed 20% possibility of fertility noted in METP treated mice uterine horns (table,3).

In earlier study, hamster treated with pure compound of *Dipsacus mitis* root extract showed significant reduction in number of implantation (Kitchlu *et al.*,1999). The high dose of METP treated female mice for a withdrawal study showed 20% implantation turn normal position as related eliminated the METP effect. It has been suggested that previous study of antifertility activity of *Croton roxburghii*, *Zizyphus jujube* bark extract to female mice showed changes in ovary, ovarian steroidogenic enzymes, blood and biochemical profiles are reversible on withdrawal (Gupta *et al.*,2004).

Table 3 Effect of METP on the ant-fertility and withdrawal of female mice

| Group | Treatment | Dose/mg/kg Body wt. | Number of mice | | % Antifertility activity | Number of implantation |
|-------|----------------------|----------------------------|----------------|---------------------------------|-----------------------------|---------------------------|
| | | | Mated | Pregnant on day10 th | | |
| 1 | Control(0.9% saline) | 0.5ml | 6 | 6 | 0 | 9.84±0.77 |
| | | 100 | 6 | 5 | 10 | 9.44±0.24 ^{NS} |
| | | 250 | 6 | 3 | 50 | 4.13±0.12* |
| 2 | METP(60 days) | 400 | 6 | 0 | 100 | Nil |
| | | METP withdrawal(60+30days) | 400 | 6 | 1 | 80 |

Each values are represents mean ± S.E.M of six rat, Statistical significant at *P<0.05,when compared to control group

DISCUSSION AND CONCLUSION

The METP reduced at estrus and diestrus phase where minimum activity of steroidogenesis as a result anti-implantation effect found in METP treated female mice. In this regard Gupta *et al.* (2004) reported the estrus cycle in mice treated with *Croton roxburghii* and *Zizyphus jujuba* barks investigated for its antisteroidogenic activity.

In the present study, there was no significant changes in the haematological and biochemical parameters where no altered in METP treated female mice groups. In this regard earlier study reported the METP treated female mice showed anti-steroidogenic effect and that no altered in haematological and serum biochemical profile (Chandru and Jayakumar, 2016). The METP 250mg/kg body weight treated female mice for a duration of 60 days showed significant implantation where found in uterine horns on 6th and 8th day pregnancy of female mice. This is earlier record abortifacient type of antifertility effect can be produced by compounds in mice models (Farnsworth *et al.*, 1976; Desta,1994). Administered METP 400mg/kg body weight for a duration of 60 days showed 100% anti-implantation recorded in the present study. In this connection earlier study Singh (1990) reported 100% antifertility effect of *Sesbania sesban* seed treatment in rat. In the present study medium and high dose of METP treatment showed significant inhibition in the implantation when compared with saline control.

The present study resulted METP is more potent as well as non-toxic in the general body metabolism and also reversible in action. It is concluded that the anti-fertility effect of METP in mice model could be safe Antifertility herbal agent.

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