



Anti-Steroidogenic Effect of *Thespesia populnea* (L.) Sol. ex Correa. in Female Mice

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ABSTRACT

Thespesia populnea Corr. floral 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 peoples of Indira Gandhi Wild Life Sanctuary, Pollachi, South India. In the present study, anti-steroidogenic effect of *Thespesia populnea* (Malvacea) 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 30 days, and normal saline (0.9%) to control group. There was significant reduction in the ($P < 0.05$) wet weight of both ovaries. Cholesterol content in ovaries of METP treated mice was significantly increased ($P < 0.05$). The two ovarian enzymes 3β -, 17β -hydroxy steroid dehydrogenase and serum estrogen and progesterone also decreased ($P < 0.05$) significantly. Administration of METP in mice did not show any variation in the hematological and bio-chemical parameters. A certain number of adult female mice were ovariectomized (OVX) and tested for hormone levels with EDP and with EDP + Extract to confirm the antifertility effect of METP. It is concluded that antifertility effect of METP could be due to its anti-steroidogenic activity.

Keywords: *Thespesia populnea*; Hormonal changes; Biomarker enzymes; Anti-steroidogenesis; Hematological profiles

1. INTRODUCTION

Natural product and medicinal 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 world wide tropics. The bark has been prescribed for its traditional use as antifertility agent among tribal villagers. The oral contraceptive - pill (OCS), injectable contraceptives like combination of estrogen and progesterone, are having more side effects (Thorogood and Villard-Mackintosh, 1993; William and Stancel, 1996; Kambo et al., 1998; WHO, 2000). Hence, the search for a suitable product from medicinal plants have been tested for their anti-steroidogenic activity in mice models (Majumder et al., 1997; Gupta et al., 2004). So the present study was designed for evaluation of the dose dependent anti-steroidogenic property of methanolic extract of *Thespesia populnea* (METP) in adult female mice.

2. MATERIALS AND METHODS

2. 1. Plant material

Thespesia populnea family (Malvacea) were collected from Indira Gandhi Wild Life Sanctuary, Pollachi, South India. It was dried in shade, powdered and extracted with methanol (1:3 w/v) using soxhlet apparatus.

2. 2. Animals 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, Annamalainagar, India. They were maintained at 27 ± 2 °C with food and water ad libitum. All the experiments were carried out with the approved of institutional animal ethical committee.

2. 3. 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.

2. 4. Animal experiments

The, female mice were divided into seven 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 30 days. METP at the doses of 100, 250 and 400 mg/kg per day were given to groups 2, 3, 4 for a duration of 30 days. Body weight was noted and estrus cycle was observed every day by microscopic examination of vaginal smear. A certain number of adult female mice were ovariectomized (OVX) (Shukla *et al.*, 1989). On 16th day the OVX mice were divided into three groups as 5, 6, 7 of 8 each and tested for hormone levels. The OVX mice were administrated with estradiol dipropionate (EDP) (2 µg/100 gm body weight/day) dissolved in olive oil, subcutaneously for 7 days (Shukla et al., 1989).

2. 5. Biochemical estimation

2. 5. 1. Serum biochemical parameters

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).

2. 5. 2. Protein content

Total protein in the ovarian tissue homogenate was estimated by the method of Lowry *et al.*, (1951).

2. 5. 3. Acid and Alkaline phosphatase

The ovarian tissue was homogenized in glass homogenizer used by the method of Tennis Wood *et al.* (1976).

2. 5. 4. Cholesterol content

Ovaries were homogenized in appropriate ice cold buffer using glass homogenizer with Teflon pestle. The cholesterol was estimated by the method of Zlatkis *et al.* (1953).

2. 5. 5. Assay of 3 β -hydroxysteroid dehydrogenase

Theca cells in ovaries were homogenized in ice cold tris HCl buffer (0.1 M) (pH 7.2) and centrifuged at 16,000 g for 5 min. The supernatant was taken for the enzyme assay of the method of Bergmeyer (1974).

2. 5. 6. 17 β - hydroxysteroid dehydrogenase

Theca cells of ovaries were homogenized in ice - cold Tris HCl buffer (pH 7.2) and centrifuged at 10,000 g for 15 min at 4 °C. The supernatant was then used for enzyme assay by the method of Bergmeyer (1974).

2. 6. Hormone assay

2. 6. 1. Estradiol

Serum estradiol was estimated using RIA kit obtained from diagnostic products corporation (DPC), USA.

2. 6. 2. Progesterone

Progesterone concentration in serum was estimated by solid - phase RIA' procedure using kits obtained from diagnostic system laboratory (DSL) USA.

2. 7. Hematological parameters

Estimation of haemoglobin (Hb) Drabkin and Austin (1932), RBC and WBC count (Arthur, 1980) and estimation of differential count (Arthur, 1980).

2. 8. Statistical analysis

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

3. RESULTS

3. 1. Effect of METP on estrus cycle, wet weight of ovaries and body weight

METP arrested the normal estrous cycle at diestrus phase at the doses of 100, 250, 400 mg/kg. The wet weight of ovaries were reduced significantly ($P < 0.05$) (Table 1). OVX+ EDP (group 6), OVX+ EDP + extract (group 7) treated group showed significant increase in the wet weight of uterus when compared to OVX control group (5) (Table 3). There was no significant change in their body weight in any of the groups.

3. 2. Effect of METP on cholesterol content

Mice from METP treated group 2, 3, 4 at the doses of 100, 250, 400 mg/kg showed significant ($P < 0.05$) increase in the cholesterol content of ovary (Table 2). OVX + EDP (or) EDP + Extract treated group 6, 7 showed significant increase ($P < 0.05$) in cholesterol content of uterus as compared to OVX + control group (Table 3).

3. 3. Effect of METP on 3β - HSD and 17β - HSD

The activities of two key steroidogenic enzymes 3β -HSD and 17β -HSD were inhibited significantly ($P < 0.05$) at the doses of 250, 400 mg/kg body weight (Fig. 1,2, TG-3, TG-4).

3. 4. Effect on serum estrogen level

Administration of METP extract to the group (TG = treated group) TG-2, TG-3, TG-4 in the doses 100, 250, 400 mg/kg showed significant ($P < 0.05$) reduction in the serum estradiol level as compared to control group (CG = control group) (Fig. 3, CG-1). Mice from OVX control (0.9% saline) group showed low level of serum estradiol when compared to OVX + EDP either EDP + extract treated OVX group (Table 3).

3. 5. Effect on serum progesterone level

The mice treated with 400 mg/kg of body weight extract showed significant ($P < 0.05$) decrease in the level of serum progesterone when compared with the control (0.9% saline) group I (Fig. 4, CG-1, TG-4).

3. 6. Effect of METP on biochemical parameters

High dose of METP treatment (400 mg/kg b wt.) group 4 showed significant ($P < 0.05$) in the activity of acid phosphatase in ovary when compared to the control. However there was a significant ($P < 0.05$) decrease in the activity of alkaline phosphatase in ovaries of the same group (Table 2). But in mice treated with METP there was no significant change in the hemoglobin, RBC, WBC, polymorphs, lymphocytes, eosinophils and serum cholesterol, AST, ALT, urea, uric acid (Table 4, 5). Administration of METP to the mice (2, 3, 4 group) at the

doses of 100, 250, 400 mg/kg body weight did not show any observable changes in the protein content of both ovary (Table 2).

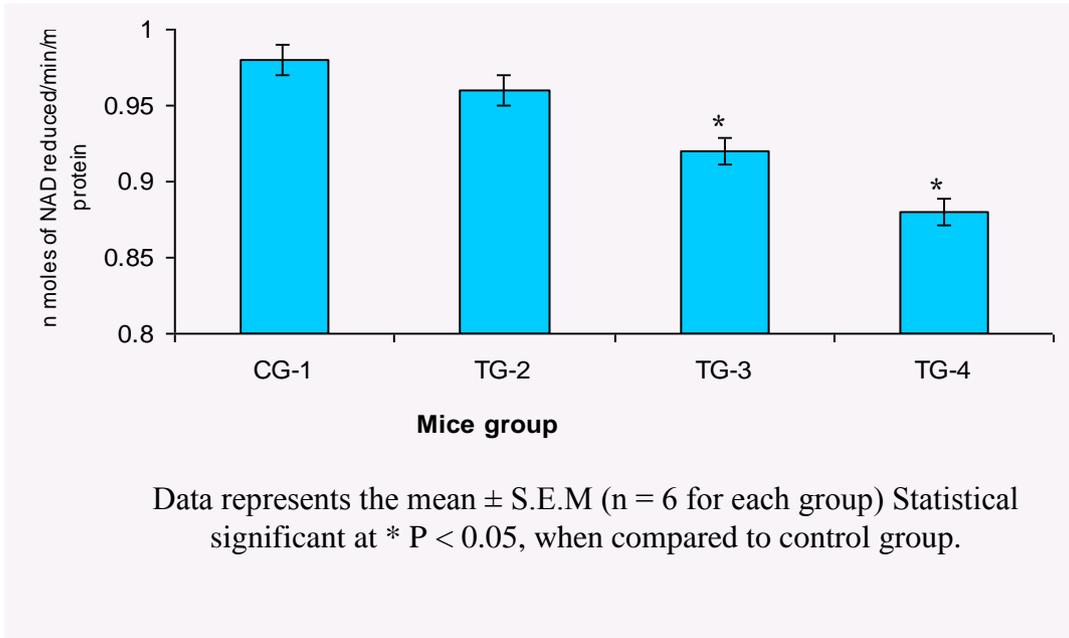


Fig. 1. Effect of METP on ovarian 3 β -HSD of control and treated female mice.

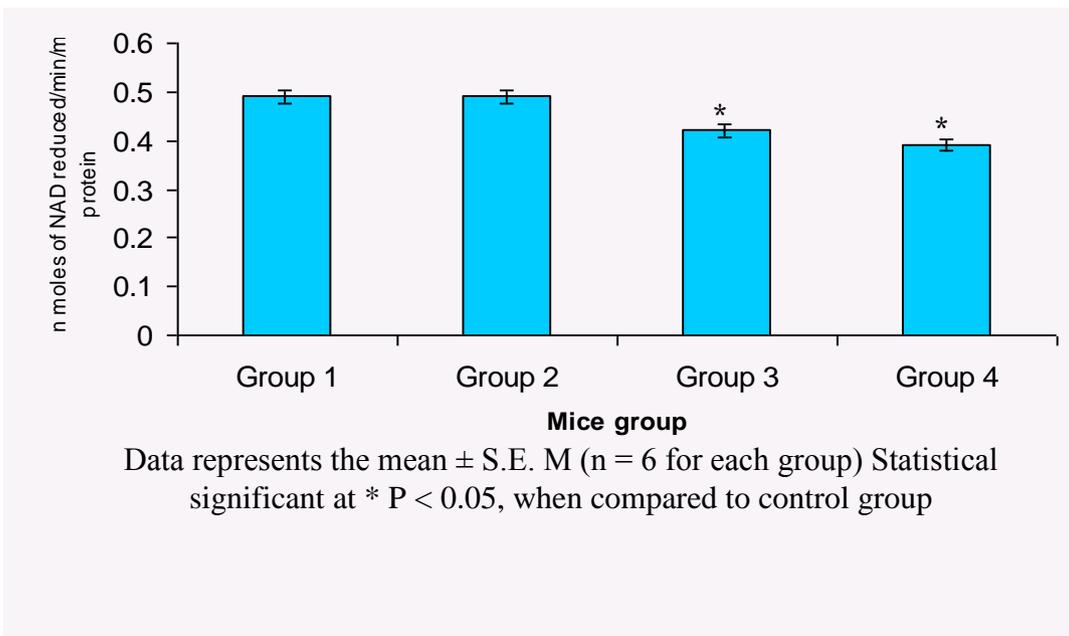


Fig. 2. Effect of METP on the ovarian 17 β -HSD of control and treated mice.

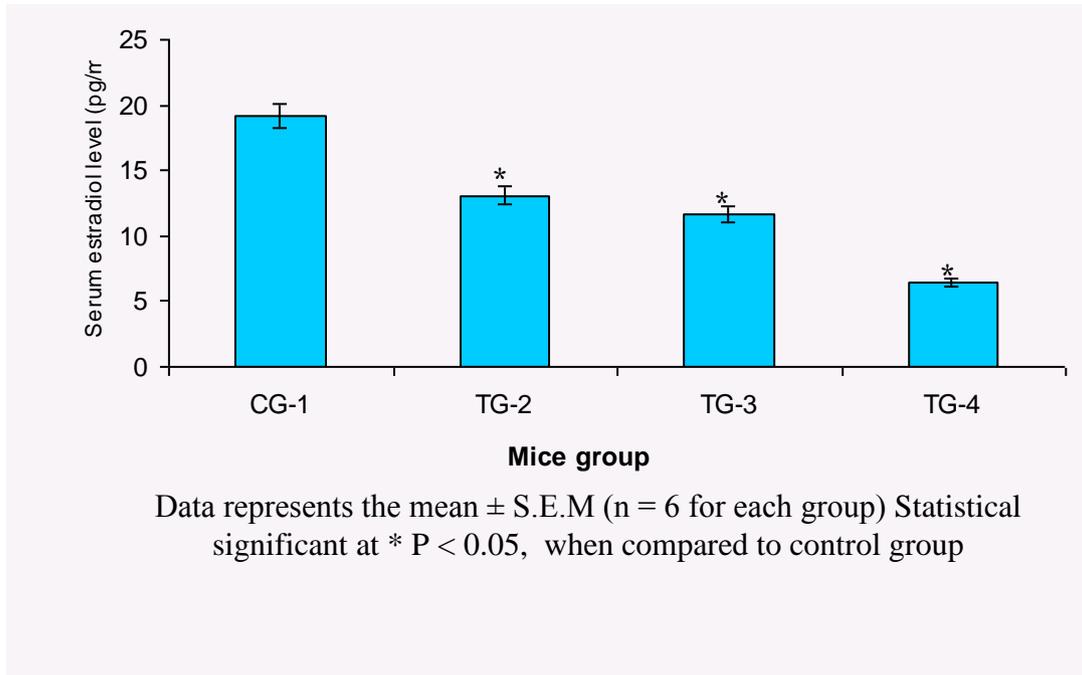


Fig. 3. Effect of METP on the serum estradiol level of control and treated female mice.

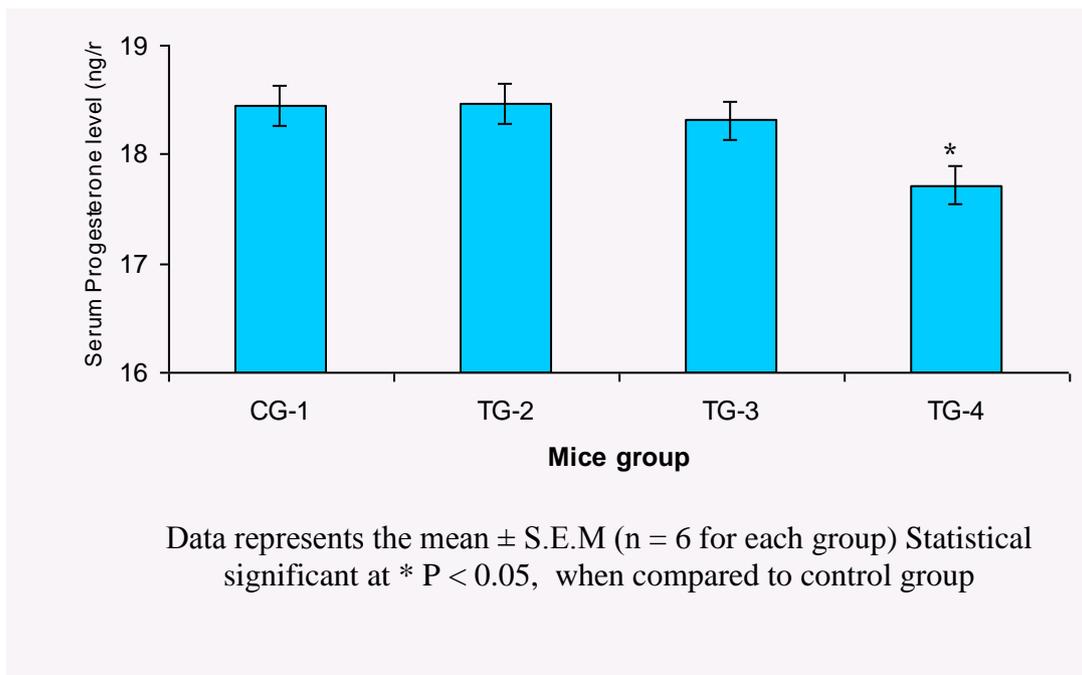


Fig. 4. Effect of METP on the serum progesterone level of control and treated female mice.

Table 1. Effect of METP treatment on the body and ovarian weight of Mice.

Group	Treatment	Dose / mg / kg body wt.	Body wt. (g)	Wt. of ovary (mg)
1	Control (saline 0.9%)	0.5 ml	27.9 ± 0.8	10.3 ± 0.7
2	METP	100	27.6 ± 0.7	10.1 ± 0.2
3	METP	250	27.4 ± 0.5	6.7 ± 0.2*
4	METP	400	27.1 ± 1.7	5.2 ± 0.4*

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

Table 2. Effect of METP treatment on the content of protein, cholesterol, and enzymes of acid and alkaline phosphatase in mice ovary.

Group	Treatment	Dose / mg / kg body wt.	Protein (µg/100 mg fresh tissue)	Cholesterol (mg/100 mg fresh tissue)	Acid phosphatase (µ moles of phenol liberated / min / 100 mg protein)	Alkaline phosphatase (µ moles of phenol liberated / min / 100 mg protein)
1	Control (saline 0.9%)	0.5 ml	12.08 ± 0.09	1.82 ± 0.004	0.052 ± 0.004	0.152 ± 0.02
2	METP	100	12.09 ± 0.13	1.82 ± 0.005	0.054 ± 0.005	0.151 ± 0.04
3	METP	250	12.11 ± 0.20	2.08 ± 0.002*	0.055 ± 0.004	0.150 ± 0.04
4	METP	400	12.08 ± 0.16	2.19 ± 0.004*	0.059 ± 0.003*	0.126 ± 0.03*

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

Table 3. Effect of METP treatment on the body weight, wet weight of uterus, content of cholesterol, and serum estradiol, progesterone level in ovariectomized (OVX) mice.

Group	Treatment	Dose / mg / kg body wt.	Body wt. (g)	Wt. of uterus (mg)	Cholesterol (mg/100 mg fresh tissue)	Estradiol (Pg / ml)	Progesterone (ng / ml)
5	Control (saline 0.9%)	0.5 ml	25.5 ± 0.2	24.3 ± 1.6	0.73 ± 0.009	3.12 ± 0.18	0.85 ± 0.12
6	OVX + EDP	(2 µg/100 gm of body wt. / day)	25.0 ± 1.2	33.4 ± 1.8*	0.76 ± 0.009*	9.18 ± 0.12*	0.86 ± 0.14
7	OVX + EDP + Extract	(2 µg/100 gm of body wt. / day) + extract 400 mg/kg	25.5 ± 0.1	32.1 ± 1.0*	0.77 ± 0.011*	9.12 ± 0.11*	0.86 ± 0.11

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

Table 4. Effect of METP treatment on the biochemical parameters of serum in adult female mice.

Group	Treatment	Dose / mg / kg body wt.	Serum cholestrol (mg/100 ml)	Serum aspartate transaminase (IU/L)	Serum alanine transaminase (IU/L)	Serum urea (mg/dl)	Serum uric acid (mg/dl)
1	Control (saline 0.9%)	0.5 ml	1.25 ± 0.03	125.80 ± 52.03	31.87 ± 8.11	30.54 ± 1.89	5.0 ± 0.02
2	METP	100	1.25 ± 0.003	125.76 ± 45.02	32.45 ± 7.12	30.58 ± 2.07	5.0 ± 0.01
3	METP	250	1.25 ± 0.02	128.11 ± 32.04	33.11 ± 4.08	30.61 ± 1.49	5.1 ± 0.01
4	METP	400	1.25 ± 0.002	129.15 ± 57.06	34.97 ± 10.01	30.57 ± 2.14	5.0 ± 0.01

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

Table 5. Effect of METP treatment on the hematological profiles in adult female mice.

Group	Treatment	Dose / mg / kg body wt.	Hemoglobin in (g/dl)	Red blood cell ($10^6/\text{mm}^3$)	White blood cell ($10^3/\text{mm}^3$)	Polymorphs (%)	Lymphocytes (%)	Eosinophits (%)
1	Control (saline 0.9%)	0.5 ml	12.92 ± 0.72	6.55 ± 0.76	3.46 ± 0.96	40.12 ± 2.16	84.25 ± 3.82	1.69 ± 0.17
2	METP	100	12.90 ± 0.65	6.52 ± 0.66	3.51 ± 0.85	40.14 ± 1.42	84.25 ± 2.94	1.67 ± 0.11
3	METP	250	12.84 ± 0.43	6.48 ± 0.59	3.65 ± 0.45	40.28 ± 1.68	84.25 ± 2.98	1.66 ± 0.24
4	METP	400	12.81 ± 0.86	6.43 ± 0.54	3.75 ± 0.55	41.45 ± 2.10	84.26 ± 2.90	1.65 ± 0.23

Each value represents mean ± S.E.M of six mice

Statistical significant at *P < 0.05, when compared to control group.

4. DISCUSSION AND CONCLUSION

The METP reduced the wet weight of ovaries and arrested the normal estrus cycle at diestrus phase where minimum activity of steroidogenesis. In this regard Gupta *et al.* (2004) reported a reduced ovarian weight, arrested the estrus cycle in mice treated with *Croton roxburghii* and *Zizyphus jujuba* barks investigated for its antisteroidogenic activity.

In the present study, there was a significant increase in the ovarian cholesterol content recorded in extract treated group when compared with control group. The possibility for increase in ovarian cholesterol content could be due to increase in the local synthesis of cholesterol in the ovary or diminished utilization of the cholesterol towards estrogen. The possibilities of marked decrease in serum estradiol level recorded following METP treatment could be due to inhibition of steroidogenesis. Since there was a significant decrease in the 3β - and 17β -HSD, enzymes activity which are the key enzymes of steroidogenesis, it is suggested that the decrease in serum estradiol level could be attributed to the inhibition of steroidogenesis in METP treated mice. In this regard an earlier study designed to find the antisteroidogenic activity of *Cuscuta reflexa* stem, *Corchorus olitorius* seed showed the decrease of 3β - HSD activities leading to the inhibition of steroidogenesis (Gupta *et al.*, 2003). The decrease in serum progesterone level could be due to inhibition of 3β -, 17β -hydroxy steroid dehydrogenase. In the METP (400 mg/kg) treated mice there was a significant changes in ovarian ACP and ALP, as reported earlier with the study of Malvacea family (*Hibiscusrosa sinensis*) by Prakash (1979).

The OVX mice extract experiments were designed to study the other effects of the extract. It has been observed that there was a significant increase in wet weight of uterus in OVX + EDP group 6; OVX + EDP + Extract mice group 7, as reported earlier with the study of *Hibiscus rosasinensis* by Prakash *et al.* (1990). There was a significant increase in the uterine cholesterol content and serum estrogen level in OVX + EDP and OVX + EDP +

Extract treated mice which could be due to exogenous, but could not be due to the extract as there was no significant difference between OVX+EDP and OVX+EDP + Extract.

METP did not have any toxic effects which was evident from the unaltered hematologic profile and biochemical parameters of serum AST, ALT, serum cholesterol, urea and uric acid. There was no significant change in serum cholesterol level which suggested that the extract might not have hyperlipidemic effect. The antifertility effect of methanolic extract of the bark of *Thespesia populnea* might be due to its anti-steroidogenic effect mainly with dose of 250 and 400 mg/kg body weight. Since there is no toxic effect, it is concluded that the bark could be used as a safe anti-steroidogenic agent.

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