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Evaluation of *C. pareira* L stem and *T. peruviana* (pers.) K. Schum leaf for estrogenic activity

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ABSTRACT

Context: Methanolic extract of *C. pareira* stem and *T. peruviana* leaves are capable of producing anti-fertility activity in female rat by increasing estrogen level and decreasing progesterone level. **Aim:** The aim of the present study is to *C. pareira* L stem and *T. peruviana* (Pers.) K. Schum leaf for estrogenic activity. **Material and Method:** Methanolic extract were made from *C. pareira* stem and *T. peruviana* leaves (after removal of cardiac glycoside) using cold maceration process. Extracts were tested for estrogenic activity on female immature rat (50-60 gm) at dose level of 250mg/kg. Obtained data were compared with control and standard (estrogen treated) group data. **Statistical Analysis:** Statistical analysis of all data was made with statistical packages (GraphPad Instat) and ANOVA followed by Dunnett's t-test was used for statistical analysis. **Result:** Decrease in the absolute weight of the uterus and uterine weight to body weight ratio in CPS-Me and TPL-Me-G group treated were significant ($p<0.05$) as compared to control. **Conclusion:** In our earlier studies it was found that methanolic extract of *C. pareira* stem and *T. peruviana* leaves increased serum estrogen level and decreased progesterone level in pregnant rat. In the present study, these extracts decreased the absolute weight of the uterus and uterine weight to body weight ratio significantly ($p<0.05$). So, we can conclude that these two-extract possessed partial estrogenic activity.

Keywords: Body weight, *C. pareira* stem, Estrogenic activity, *T. peruviana* leaves, Uterine weight.

INTRODUCTION

Many naturally occurring substances scientifically validated for their estrogenic properties. [1, 2] Estrogenic substances exhibit biological functions just like estradiol in all mammals. Different natural estrogenic substances evoke their activities through different mechanism. Estrogenic substances exert their action by binding with estrogenic receptors (α and β) either fully or partially. [3-5] Sometimes these compounds produce their action through selective binding with receptors.

In our previous study, we have found that methanolic extract of *C. pareira* stem (CPS-Me) and methanolic extract (freed from cardiac glycosides) of *T. peruviana* leaves (TPL-Me-G) possessed a significant ($p < 0.001$) antifertility potential in female rat model. [6, 7] This conclusion was made on the basis of results obtained from cytological, implantation and hormonal screening. Both the extracts were found to extend the duration of estrous cycle especially diestrous phase and decrease mean no of implant in dose depended manner. Significant elevation in serum estradiol levels and gradual decrease in progesterone levels from day 12^{th} to 19^{th} were also observed with CPS-Me and TPL-Me-G treated female SD rats. These effects were directly related to dose.

Many anti-fertility agents obtained from plant origin were observed to produce estrogenic effect by an increase in uterine weight, uterine to body weight ratio.[8] In our previous study it was not established that whether CPS-Me and TPL-Me-G having full or partial estrogenic activity.

The aim of our present study is to evaluate CPS-Me and TPL-Me-G for estrogenic properties.

MATERIALS AND METHOD

Collection

Samples of *Cissampelos pareira* L stems and *Thevetia peruviana* (Pers.) K. Schum leaves were collected from Panchkula (30.74°N, 76.80°E) district of Haryana, India. Botanical Identification were made by NISCAIR, India with reference No. NISCAIR/ RHMD/ Consult/ 2014/ 2534/ 113-1 (*Cissampelos pareira* L. Stem) and NISCAIR/ RHMD/ Consult/ 2014/ 2534/ 113-3 (*Thevetia peruviana* (Pers.) K. Schum (Leaves).

Extraction

a. The air-dried, powdered plant material of *C. pareira* (stem 100g) was freed from fatty substances by cold maceration with petroleum ether for 24 hours at room temperature with occasional shaking. The solid to solvent ratio was 1: 10 i.e. 10 ml petroleum ether was used per 1gm of plant materials. The mark was air dried for overnight. Defatted and dried plant materials of *C. pareira* were extracted with methanol through the cold maceration process (Plant materials were soaked in methanol for 48 hrs at room temperature, with occasional shaking). The proportion of plant materials and methanol was 1gm (plant material) in 10ml (methanol). Extracts were filtered off and separated from solid materials using Whatman No. 1 filter paper. Extracts were dried in rotary evaporator at temperature 50°C as like as our previous study.[6]

b. *T. peruviana* leaves are rich source of toxic cardiac glycosides [9, 10] and hence, it was an essential requirement to remove glycosides from these leaves. Leaves were defatted by boiling with petroleum ether for 2hrs using soxhlet apparatus. These defatted leaves were made free from cardiac glycoside by boiling leaves with 80% methanol: ethanol (8:2) for 3 hours at 45°C by the modified method of Oluwaniyi et al, 2007. [11] These cardiac glycoside free leaves were then extracted by cold maceration method at room temperature using methanol for 48 hrs. The extract was concentrated to dark brown semi-solid mass. It was further dried using rotary evaporator.[7]

Phytochemical screenings

Methanolic extracts *C.pareira* stem and Glycoside-freed *T. peruviana* leaves (TPL-Me-G) were subjected to qualitative investigation for presence or absence of alkaloids, glycosides, flavonoids, terpenoids and phytosterols using chemical methods and thin layer chromatography (TLC).[12, 13]

Animals

Immature female SD rats (55-60g) were divided into 4 group (n=6) namely A, B, C, D. All the animals used for this experiment were bred in the animal house of the National Institute of Pharmaceutical Education and Research, Mohali, Punjab, India. The animals were housed in polypropylene cages and maintained in an environmentally controlled room provided with a 12: 12 h light and dark cycle for each 24 h period at a temperature of approximately 25° C. Animals were provided with sufficient quantity of food pellets and potable water. Animal studies were performed in the animal house of Rayat Institute of Pharmacy, Ropar, Punjab, India with due permission from the Institutional Animal Ethics Committee (approval no. is RIP/IAEC/2010–11/25 dated 26/ 7/ 11).

Administration of drug for *in-vivo* experimentation

Carboxyl methyl cellulose suspension (CMC; 1% w/v) was prepared in distilled water. CPS-Me, CPR-Me and TPL-Me-G was reconstituted in CMC suspension to make the desired concentration for all pharmacological evaluations. The suspension was administered to experimental animal by intragastric tube.

Standard Drug

17-β-estradiol benzoate oil base injection (5mg/ml) was used as standard drug and procured from Macmillon Pharmaceutical Ltd., Amritsar, India as gift sample.

Estrogenic/non estrogenic activity

To find out the estrogenic /non estrogenic activity of CPS-Me and TPL-Me-G wet uterine weight (mg) to body weight (gm) ratio was measured. Immature female SD rats (55-60g) were divided into 4 group (n=6) namely A, B, C, D. Group A received vehicle only; Group B received estradiol 0.05µg/animal subcutaneously. Group C, D received CPS-Me and TPL-Me-G 250 mg/kg respectively through oral route for seven days. On 8th day all animals were sacrificed by decapitation and uteri were removed promptly. Prior to scarification body weight of all animals has been taken. After scarification uterine weight of each animal was examined and the uterine weight to body weight ratio was calculated. Assessment of estrogenic activity was performed according to the method of Kanno *et al,* 2001. [14]

Statistical analysis

Data were expressed as mean ± S.E.M. The data were statistically analyzed using statistical packages (GraphPad Instat) and statistical analysis was done by ANOVA followed by Dunnett's t-test. The significance level considered was $p<0{\cdot}05$ compared with control.

RESULT

Phytochemical screenings

a. Phytochemical investigation of *C. pareira* stem methanolic extracts revealed that were rich in most active constituents like alkaloids, flavonoids, terpenoids, carbohydrates, proteins, and tannins which matched with our previous studies (Samanta *et al,* 2014). Results are tabulated in [Table No 1].

Table 1: Phytochemical investigation of *Cissampelos pareira* stem methanolic extracts (CPS- Me)

Test for active constituents	CPS- Me
Carbohydrate	+
Amino acids	+
Alkaloids	+
Triterpene	+
Saponine	–
Flavonoids	+
Glycosides	–
Tannins	+

(+) Identification tests gave positive results. (-) Identification tests gave negative results.

b. Phytochemical investigation of Glycoside-freed *T. peruviana* leaves methanolic extracts found to possess alkaloids, terpenoids, amino acids and tannins which also resembled with our previous studies (Samanta *et al,* 2016) [Table No 2].

Estrogenic/non estrogenic activity

The absolute weight of the uterus and uterine weight to body weight ratio was decreased in CPS-Me treated group and TPL-Me-G treated group ($p<0.05$) as compared to control. However, there was no significant change found in CPR-Me treated group [Table No 3].

Table 2: Phytochemical investigation of Glycoside-freed *T. peruviana* leaves methanolic extract

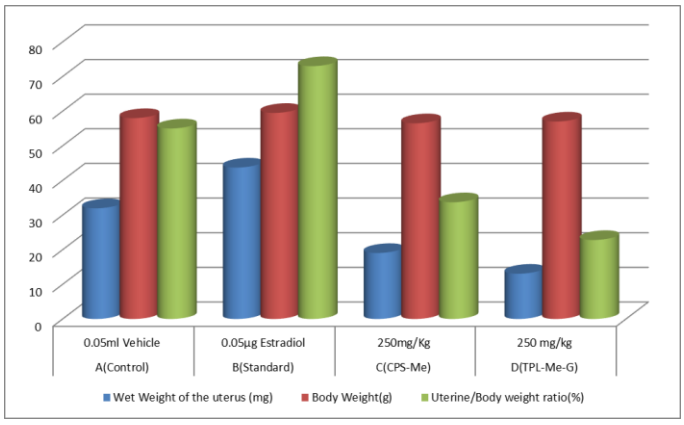
Test for active constituents	TPL-Me-G
Carbohydrate	+
Amino acids	□
Alkaloids	□
Triterpenes	□
Phytosterol	□
Saponine	□
Flavonoids	□
Glycosides	□
Tannins	□

(+) Identification tests gave positive results. (-) Identification tests gave negative results

Table 3: Effect of CPS-Me and TPL-Me-G on uterus and uterine /body weight ratio

Groups	Dose	Wet Weight of the uterus (mg)	Body Weight(g)	Uterine/Body weight ratio (%)
A (Control)	0.05 ml vehicle	31.95± 0.7	58 ± 0.74	55
B (Standard)	0.05 µg Estradiol	43.72± 0.65	59.5± 0.96	73
C (CPS-Me)	250 mg/Kg	19.05± 0.32*	56.5± 0.77*	33.7
D (TPL-Me-G)	250 mg/kg	13.07± 0.87*	57± 1.2	22.8

*p<0·05 (Significant) compared with control



Graph 1: Evaluation of Estrogenic Activity of CPS-Me and TPL-Me-G

DISCUSSION

Decrease in the absolute weight of the uterus and uterine weight to body weight ratio in CPS-Me and TPL-Me-G group treated were significant ($p<0.05$) as compared to control. Anti-fertility properties of many plant extracts were reported to be exhibited by estrogenic activity such as an increase in uterine weight, protein synthesis, the uterine content of glucose; cholesterol, water intake and fluid retentivity thereby alter the surroundings of the uteri which may result in retarded implantation of fetuses in the uterus.[8,15] In contrast, CPS-Me and TPL-Me-G produced antifertility activity by increasing the estrogen and decreasing the progesterone level but did not increase the uterine weight. These results may suggest that flavonoids (major) and some other constituents present in these extracts may produce selective estrogenic activity. Not total but selective estrogenic activity of alkaloids of *Senna alata* were reported by Yakubu and Musa (2012) in their study effects of post-coital administration of alkaloids from *Senna alata* (linn). Roxb) leaves on some foetal and maternal outcomes of pregnant rats. [16] Partial estrogenic activity matched with our results but in our study the prime constituents were flavonoids.

CONCLUSION

Both CPS-Me and TPL-Me-G did not increase the uterine weight but increase serum estrogen level on female rat. So, it can be concluded that both CPS-Me and TPL-Me-G have selective estrogenic activity. Studies on human are essential to establish (CPS-Me and TPL-Me-G) their usefulness as antifertility agents. Being selective estrogenic drug both CPS-Me and TPL-Me-G are promising for treatment of breast cancer and menopause and further research could be carried out in these field.

Conflict of interest

The authors declare no conflict of interests.

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