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Extraction Efficiency and Estrogen or Alike Activity of Ethanolic and Aqueous Extracts of different Parts of *Calotropis procera*

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ABSTRACT

In this study, estrogen or alike activities of ethanolic and aqueous extracts of shade-dried roots, branches and leaves of *Calotropis procera* Ait. (Asclepiadaceae) were evaluated. The quantity of aqueous extract for the respective parts was 8.33, 20.83 and 41.67%, and was higher than that obtained with ethanol, whilst leaves yielded greater extract than roots or branches. For the determination of the estrogen or alike activity of ethanolic and aqueous extracts of three parts of *C. procera*, 40 immature female rats were taken and divided into 4 equal groups A, B, C and D. Rats of group A served as control, while those of groups B, C and D were given ethanolic extracts of roots, branches and leaves, respectively for 14 days at the dose rate of 250 mg kg^{-1} body weight. Another 40 immature female rats were treated in the same way with aqueous extracts of three parts of the plant. After 7 days, from each group, 5 rats were killed, while the remaining 5 rats were killed 14 days after treatment. For ethanolic extracts, body weight and weights of the ovaries and uterus did not differ between treated and control groups. However, the weight of the vagina was higher ($P<0.05$) in rats of control than other three groups. Serum estradiol concentrations did not differ between rats of all groups. However, serum progesterone concentrations were higher in rats given extracts of branches and leaves than those of other two groups ($P<0.05$). Contrarily, for aqueous extracts there were no differences in body weight, weights of left ovary and uterus in treated and control rats. Weight of the right ovary was higher in rats treated with extract of leaves, while the weight of vagina in treated groups was lower than the controls. Control rats also showed higher serum estrogen concentrations than treated groups. It was concluded that ethanolic and aqueous extracts of roots, branches and leaves of *C. procera* produced no estrogen or alike activity in immature female rats.

Key Words: *Calotropis procera*; Water extract; Ethanolic extract; Reproductive organs; Serum estrogens; Rats

INTRODUCTION

Pakistan has a rich source of herbs and indigenous medicinal plants that have been used for the treatment of various ailments in man and animals since ages. These plants have been shown to possess various therapeutic activities, including antipyretic, analgesic and anti-inflammatory. Many indigenous medicinal/fodder plants have also been shown to possess oestrogen or like activities such as *Pithecellobium dulce* (Sexena & Singal, 1998), *Blepharispermum subsessile* (Agarwal *et al.*, 1999) and *Muscari racemosum* (Urbancikova *et al.*, 2002).

Calotropis procera Ait. (Asclepiadaceae), locally known as Ak, is a wild shrub, which grows up to a height of up to 2 m. The alcoholic extracts prepared from different parts of this plant have been shown to possess antimicrobial and spermicidal activity (Qureshi *et al.*, 1991). Similarly, methanolic extracts of its flowers have been evaluated for anthelmintic (Iqbal *et al.*, 2005) and analgesic activity (Dewan *et al.*, 2000). According to Hassan *et al.* (2006), aqueous and organic solvent extracts of *C. procera* also possess antifungal properties. Kamath and Rana (2002) reported that ethanolic extract of roots of *C. procera* showed oestrogenic activity, as it induced an increase in the uterine weight in immature rats when compared with controls. However, these workers did not study the oestrogenic or like activity in leaves or branches of this plant.

Before the investigation on these indigenous medicinal plants for their hormonal or like activities, their extracts in some suitable solvents are prepared. For this purpose, different solvents e.g., distilled water (Amin *et al.*, 1996), rectified spirit (Saxena & Singal, 1998), methanol (Agarwal *et al.*, 1999), ethanol (Gupta *et al.*, 2001) or acetone have been used with varying extraction efficiencies.

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In the present study, the extraction efficiencies of water and ethanol for roots, branches and leaves of *C. procera* have been evaluated. Moreover, the effects of ethanolic and aqueous extracts of three parts of *C. procera* on reproductive organs and serum estradiol concentrations in immature female rats were also investigated.

MATERIALS AND METHODS

Collection of plant material. *Calotropis procera* Ait. (Asclepiadaceae) plants were collected during the month of February from the fields located in and around University of Agriculture, Faisalabad, Pakistan. After leaving the whole plants under shade for one week, roots, branches and leaves were separated, cut into small pieces, further dried in shade, grinded into fine powder and stored in screw-cap bottles at room temperature for subsequent use.

Preparation of extracts. For preparation of ethanolic and aqueous extracts, suitable amount of the powdered roots, branches and leaves material of *C. procera* (Table I) was soaked in 90% reagent grade ethanol or distilled water and placed into the Soxhlet's apparatus. Golden brown material, when formed, was transferred to a Petri dish, dried in an oven into a semi-solid mass and weighed using a digital balance. The extract was stored in polythene bags in a refrigerator at 4°C for subsequent use.

Evaluation of extracts. A total of 40 immature female Sprague-Dawley rats (4-5 weeks old) were procured from the National Institute of Health, Islamabad, Pakistan and maintained under natural climatic conditions but with proper protection from severe weather conditions. They were provided with feed and water *ad libidum*. These rats were randomly divided into 4 groups A, B, C and D, with 10 rats in each group. Rats of group A served as control, while those of groups B, C and D were separately given ethanolic extracts of roots, branches and leaves of *C. procera*, respectively. Another 40 immature female rats were treated in the same way with aqueous extracts of three parts of the plant. The extracts were given orally for 14 days at the dose rate of 250 mg kg^{-1} body weight (Kamath & Rana, 2002), using a gastric tube. After 7 days, 5 rats from each group were killed, while the remaining 5 rats from each group were killed after 14 days of treatment. The weights of reproductive organs including ovaries, uterus and vagina were recorded. Blood samples were also collected from rats of each group, the serum was harvested and stored at -20°C.

Hormonal assays. Serum samples were analyzed for estradiol and progesterone concentrations, using commercially available ELISA kits (DRG Diagnostics, Germany; Reference No. EIA-2693 for estradiol & EIA-1561 for progesterone). The minimum detection limit of the assay was 9.714 pg mL^{-1} for estradiol and 0.45 ng mL^{-1} for progesterone. For estradiol assay, the cross reactivity was 0.05% for esteriol and 0.2% for estrone. For progesterone assay, the cross reactivity for other steroids was <1.10%. For both the hormones, the intra-assay and inter-assay coefficients of variation were <7.0 and <10.0%, respectively.

Statistical analysis. Mean values (± SE) of various parameters for rats of each group were computed. In order to ascertain the magnitude of the effects of treatments and days of treatment on various parameters, the data were analyzed statistically using two-way analysis of variance in completely randomized design. Duncan's Multiple Range test was applied for multiple means comparisons, where necessary.

RESULTS AND DISCUSSION

Extraction efficiency. When ethanol was used, the extract obtained from the leaves (14.17% of the dried plant material) was higher than that from the roots (5.33%) or branches (6.67%) of the same plant (Table I). Agarwal *et al.* (1999) used methanol for the extraction of 750 g dried rhizomes of *Blepharispermum subsessile* and obtained 50 g (6.67%) of extract. Gupta *et al.* (2001) obtained 5.6% crude extract from leaves of *Colebrookia oppositifolia* when 90% ethanol was used for extraction. A very low quantity of methanol extract (1.52%) was recovered from stem bark of *Albizia lebbeck* by Gupta *et al.* (2005), while relatively higher extraction efficiency (16.2%) has been reported for the methanol extract of *Petiveria alliacea* seeds (Oluwole & Bolarinwa, 1998). Kamath and Rana (2002) macerated 100 g dried roots of *C. procera* with 1000 mL of 90% ethanol and reported that the crude extract obtained in this way was 11.4% of the original plant material. However, these workers did not use leaves or branches of the plant for extraction.

When water was used, the quantity of the extract obtained from each part of the plant was higher than that obtained with ethanol (Table I). Telefo *et al.* (2002) obtained only 4% extract when they used hot water for extraction of leaves of four plants. However, the comparison in the extraction efficiency between ethanol and water is not reported thus far.

There seems to be a wide variation in the extraction efficiency reported for different plants, even when the same solvent was used for extraction. Besides variations in the purity of the solvent, differences in the plants used and extraction protocols could be responsible for these variations in extraction efficiencies among different studies. In this study, the extraction efficiency for the same plant was higher with water compared to ethanol (Table 1). It is well known that the steroid sex hormones (estrogen, testosterone & progesterone) are lipid soluble (Carruthers, 1986). Under such cases, only ethanolic extracts would be suitable for the investigations of steroid sex hormone or like activities of the plant. However, if steroid hormone or alike activities are based on some specific metabolites, aqueous extracts would also be

Table I. The extraction efficiency for different parts of *Calotropis procera* in ethanol and water

Part of the plant	Dried plant material (g)	Extract obtained (g)	Extraction efficiency (%)
Ethanol			
Roots	15.00	0.80	5.33
Branches	15.00	1.00	6.67
Leaves	12.00	1.70	14.17
Water			
Roots	15.00	1.25	8.33
Branches	12.00	2.50	20.83
Leaves	12.00	5.00	41.67

useful. These results also revealed that leaves of *C. procera* yielded higher amount of ethanol extract than roots or branches of the same plant. A similar trend was observed when water was used for the extraction. The exact cause of this variation is not yet known.

Evaluation of plant extracts. With the administration of ethanolic extract, the body weight of rats did not differ between treated and control groups (Table II). Similarly, the effects of treatments on the weight of the ovaries and uterus were non-significant. However, the weight of the vagina was higher ($P<0.05$) in rats of group A (control group) than those of other three groups. The values of body weight and weights of ovaries and vagina were higher at 14 days than at 7 days of treatment ($P<0.05$). Serum estradiol values did not differ between rats of the four groups. However, serum progesterone concentrations were higher in rats of group C and D (given extracts of branches & leaves) than those of other two groups ($P<0.05$). Duration of treatment had no effect on serum concentrations of estradiol as well as progesterone.

In rats given aqueous extracts of roots, branches and leaves of *C. procera*, the body weight was highest in group D given leaves extract and lowest in control group, the difference was non-significant (Table III). Similarly, the treatments had no effect on the weights of the uterus and the left ovary. For the right ovary, the weight was higher ($P<0.05$) in rats of group D (given leaves extract) than groups A (control) and B (given roots extract). The weight of the vagina was higher in control compared to all other groups ($P<0.05$). The values of body weight and weights of reproductive organs were higher at 14 than at 7 days of treatment ($P<0.05$).

Serum estradiol concentration differed among all the groups, the value was highest in control group and lowest in rats of group D given leaves extract (Table III). Serum progesterone concentration was higher in rats of groups A (control) and B (given roots extract) than those of groups C (given branches extract) and D (given leaves extract). The serum concentrations of both these hormones were higher at day 7 than at day 14 of the experiment ($P<0.05$).

An earlier study in India (Kamath & Rana, 2002) has shown that administration of ethanolic extract of roots of *C. procera* to immature female rats (25-30 days old) at the rate of 250 mg kg^{-1} body weight for three days increased the weights of the uterus, cervix and vagina compared to controls; the extract also potentiated the estrogenic activity of ethynyl estradiol; these effects were attributed to estrogenic activity of the extract. However, in the present study, ethanolic and aqueous extracts of roots, branches and leaves of *C. procera* showed no effect on the body weight of immature female rats. Similarly, no differences could be seen in the weights of internal reproductive organs of the treated and control rats (Tables II & III). Moreover, there were no differences in the serum estradiol concentrations in the rats of ethanolic extract treated and control groups. In aqueous extract treated rats, serum estradiol concentrations were lower compared to control rats. This indicates that the ethanolic and aqueous extracts of roots, branches and leaves of *C. procera* do not have any significant estrogen or like activity (Tables II & III). Circosta *et al.* (2001) observed that the ethanolic and aqueous extracts of roots of *C. procera* did not show any oestrogenic activity when tested in immature female bilaterally ovariectomized rats. However, these extracts interrupted the normal estrus cycle in 60 and 80% of adult rats, respectively. In that study, the treated rats exhibited prolonged dioestrus stage of the oestrus cycle, with consequent temporary inhibition of ovulation.

Qureshi *et al.* (1991) observed that the crude alcoholic extracts prepared from roots and flowers of *C. procera* made most of the spermatozoa immotile *in vitro*, whereas non-saponifiable extracts from stem, leaves and roots did not affect the sperm motility. It may be possible that the adverse effects of the extract of this plant on sperm viability were cytotoxic, as this plant is known to contain cytotoxic and anti-tumor cardinolides (Hartwell, 1976).

The findings of Kamath and Rana (2002) could not be confirmed from the present study. Differences in the extraction procedure and protocol of the study may be attributed for this discrepancy. In our study, the extract was prepared by using a Soxhlet's apparatus and rats were medicated for 7 or 14 days. However, in the study of Kamath and Rana (2002), the extract was prepared by macerating 100 g of powdered roots with 1000 mL of 90% ethanol for 72 h with occasional shaking and the extract obtained was concentrated to dryness. Moreover, the immature female rats were given the extract orally for only three days. Differences in agro-climatic conditions between the two countries can also be a reason for these differences. The plants used in the present study were collected during the month of February when they were not at flowering stage. They could have shown some estrogenic or alike activity if they were collected during summer (at full bloom stage). Previously, oral administration of crude ethanolic extract from flowers of this plant was found to markedly affect the testicular tissue in male gerbils, causing degenerative changes in different

Table II. Weights of reproductive organs and serum hormone concentrations in rats given ethanolic extracts of different parts of *Calotropis procera* (mean±SE)

Parameters	Days of treatment	Group A (control)	Group B (roots)	Group C (branches)	Group D (leaves)	Mean
Body weight (g)	7	83.00±10.11	70.75±5.68	89.75±5.74	85.00±5.05	82.13 ± 3.59b
	14	107.75±11.14	100.50±12.74	120.00±7.47	120.00±2.68	112.06 ±4.70a
	Mean	95.38±8.39	85.63±8.56	104.88±7.19	102.50±7.12	97.09 ± 3.96
Weight of the right ovary (mg)	7	21.08±8.32	15.98±3.47	26.60±3.59	23.42±0.67	21.77 ± 2.39b
	14	36.35±5.20	30.45±5.35	29.70±3.68	38.42±4.32	33.73 ± 2.31a
	Mean	28.71±5.38	23.21±4.02	28.15±2.45	30.92±3.48	27.75 ± 1.96
Weight of the left ovary (mg)	7	21.58±9.90	14.10±3.28	28.95±5.34	21.52±2.50	21.54 ± 3.00b
	14	34.32±5.41	27.78±3.68	27.53±1.93	35.75±4.41	31.34 ± 2.06a
	Mean	27.95±5.75	20.94±3.45	28.24±2.64	28.64±3.57	26.44 ± 1.99
Weight of the uterus (mg)	7	110.73±60.02	53.75±11.75	136.18±43.21	92.55±10.71	98.30± 18.61
	14	147.97±31.84	120.83±31.51	135.43±22.19	140.97±28.86	136.30 ± 13.16
	Mean	129.35±32.23	87.29±20.07	135.80±22.49	116.76±16.93	117.30 ±11.72
Weight of the vagina (mg)	7	61.12±13.74	46.30±12.90	40.73±0.90	33.10±2.61	45.31 ± 5.02
	14	119.63±9.24	89.32±14.21	83.40±5.78	86.35±15.21	94.67 ± 6.46a
	Mean	90.37±13.45A	67.81±12.04B	62.06±8.51B	59.72±12.34B	69.99 ± 5.99
Serum E2 conc. (pg mL^{-1})	7	70.50±5.00	77.00±1.73	83.00±4.66	92.50±14.52	80.75 ± 4.17
	14	81.50±7.14	79.75±2.06	78.75±8.07	94.75±3.57	83.69 ± 3.07
	Mean	76.00±4.54	78.38±1.35	80.88±4.39	93.63±6.93	82.22 ± 2.56
Serum P4 conc. (ng mL^{-1})	7	5.60±2.61	7.68±2.86	15.25±8.38	22.73±8.63	12.81 ± 3.32
	14	14.05±4.79	11.75±3.40	19.95±3.48	25.25±5.65	17.75 ± 2.40
	Mean	9.83±2.99B	9.71±2.20B	17.60±4.29A	23.99±4.80A	15.28 ± 2.06

Values with different small letters in a column for each parameter or capital letters within a row differ significantly (P<0.05)

Table III. Weights of reproductive organs and serum hormone concentrations in rats given aqueous extracts of different parts of *Calotropis procera* (mean ± SE)

Parameters	Days of treatment	Group A (control)	Group B (roots)	Group C (branches)	Group D (leaves)	Mean
Body weight (g)	7	159.47±4.88	154.38±3.45	149.45±6.25	168.60±3.83	157.98±2.65b
	14	186.83±4.33	194.39±7.20	201.50±7.01	193.65±6.97	194.09±3.21a
	Mean	173.15±5.17	174.39±7.13	175.48±9.04	181.13±5.35	176.03±3.35
Weight of the right ovary (mg)	7	37.45±4.84	33.02±2.88	39.42±1.95	43.68±6.75	38.39±2.25b
	14	48.53±2.13	46.83±6.20	53.52±2.46	60.23±3.04	52.28±2.08a
	Mean	42.99±3.03B	39.92±3.87B	46.47±2.60AB	51.96±4.32A	45.34±1.82
Weight of the left ovary (mg)	7	33.35±1.80	32.92±1.11	41.62±2.54	35.55±2.90	35.86±1.25b
	14	49.80±3.33	50.00±5.00	55.67±2.13	56.53±3.42	53.00±1.81a
	Mean	41.57±3.07	41.46±3.55	48.64±2.64	46.04±3.81	44.43±1.66
Weight of the uterus (mg)	7	176.02±20.77	176.87±7.59	218.65±24.91	211.12±20.87	195.66±10.02b
	14	318.32±32.62	247.82±28.16	315.68±23.35	252.67±17.70	283.62±14.01a
	Mean	247.17±28.29	212.34±17.54	267.17±21.88	231.89±14.47	239.64±10.66
Weight of the vagina (mg)	7	49.52±3.23	40.90±1.36	42.62±2.62	36.68±1.98	42.43±1.48b
	14	128.66±1.78	95.40±11.37	102.64±9.46	98.32±8.62	106.25±4.86a
	Mean	89.09±12.06A	68.15±9.86B	72.63±10.19B	67.50±10.20B	74.34±5.29
Serum E2 conc. (pg mL^{-1})	7	160.02±6.96	145.96±3.97	148.31±5.13	118.64±1.84	143.23±3.89a
	14	161.33±5.67	143.00±7.45	115.83±4.73	113.00±2.86	133.29±4.88b
	Mean	160.67±4.28A	144.48±4.05B	132.07±5.92C	115.82±1.83D	138.26±3.17
Serum P4 conc. (ng mL^{-1})	7	25.42±3.74	22.75±2.57	13.12±2.67	13.22±0.99	18.63±1.70a
	14	12.67±3.05	12.81±1.58	13.96±1.06	13.57±0.65	13.25±0.56b
	Mean	19.04±2.69A	17.78±2.08A	13.54±1.37B	13.39±0.57B	15.94±0.97

Values with different small letters in a column for each parameter or capital letters within a row differ significantly (P<0.05)

stages of spermatogenesis and sertoli cells (Garg, 1979). In the present study, serum progesterone concentrations in rats given ethanolic extracts of branches and leaves were higher than in rats of control group and those given extract of roots. Although branches and leaves seemed to possess some progesterone like activity; however, this could not be confirmed when rats were treated with aqueous extract of *C. procera.*

CONCLUSION

A higher quantity of aqueous extract was obtained compared to ethanol extract of the same plant and leaves yielded more extract compared to roots and branches. Moreover, ethanolic and aqueous extracts of roots, branches and leaves of *C. procera* did not show any estrogen or like activity in immature female rats.

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