Influence of *Calotropis procera* Roots on Biochemistry of Reproductive Organs of Ovariectomized Rats

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Biochemical alterations have been observed in the uterus, cervix and vagina of ovariectomized rats treated with alcoholic extract of *Calotropis procera* roots administered in the presence and absence of ethynilestradiol. Ovariectomy resulted in the decrease of glycogen and protein content as well as activity of acid and alkaline phosphatase in the uterus, cervix and vagina. Administration of the alcoholic extract of *Calotropis procera* increases glycogen and protein content and activity of acid and alkaline phosphatase in uterus and cervix with increasing weights, whereas, the glycogen contents decreased significantly in vagina. Combined treatments of the alcoholic extract with ethynilestradiol act synergistically confirm the estrogenic action of alcoholic extract of *Calotropis procera* roots in adult ovariectomized rats.

Key words: *Calotropis procera*, ovariectomy, ethynilestradiol, estrogen

Calotropis procera (asclepiadaceae) is a shrub, reaching 15 feet height, with thick twisted branches, the young ones blunty quadrangular, bark ash colored, covered with a minute white woolly down. The Principle constituents of this plant are starch, mucilage, a bitter principle (mudar) and a small quantity of acrid resin. Mudar is an alternative, tonic and diaphoretic, and in large doses emetic. It is said to have been employed with benefit in numerous obstinate cutaneous diseases, syphilitic affections, dysentery, diarrhea and chronic rheumatism[1]. Its roots and latex is used as purgative, toothache, emetic and specific for guinea warm[2]. Alcoholic extract of *Calotropis procera* has been found to possess antimicrobial and spermicidal activity and its administration at the dose of 250 mg/kg body weight to female albino rats from day 1 to day 7 of pregnancy has been reported to prevent 100% implantation along with significant uterotrophic activity[3]. In the view of its remarkable antiimplantation and uterotrophic activity the present investigation deals with the effect of *Calotropis procera* on biochemical constituents of reproductive organs of ovariectomized female rats.

Calotropis procera roots were collected in summer season from campus of Dr. H. S. Gour University, Sagar, India and authenticated in the Department of Botany, Dr. H. S. Gour University, Sagar, where a Voucher Specimen has been deposited for further reference. Extract and dose was prepared as described earlier[3].

One week acclimatized adult healthy virgin female Wistar rats (110±10 g) obtained from Departmental animal house of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar. They were housed in polypropylene shoebox type cages with stainless still grill top and bedded with rice husk. The animals were provided commercial diet and water *ad libitum*. The study was conducted after obtaining the approval from the Institutional Animal Ethics Committee (Registration No. 379/01/ab/CPCSEA). Only those animals showing 3 regular estrus cycles were selected and bilaterally ovariectomized and 2 weeks later, these were divided into five groups containing six rats in each group. Group I received vehicle only (CMC suspension 0.2 ml) and served as intact control (without ovariectomy). Group II received vehicle only (CMC suspension 0.2 ml) and served as ovariectomized control. Group III received alcoholic extract at 250 mg/kg body weight dose orally and group IV administered ethynilestradiol 1 µg/rat/day, im. Group V received alcoholic extract and ethynilestradiol at above dose.

Treatment was continuing for 7 d. After 48 h the last dose, animals were killed and uterus, cervix and vagina were excised, freed from adhering tissues, weighed and processed for glycogen[4], protein[5] and activity of acid and alkaline phosphatase[6] estimation.

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The results were analyzed statistically using student "t" test.

Ovarian hormones are known to alter biochemical constituents of the female reproductive tract. Administration of estrogen or progesterone to adult or ovariectomized rats is known to elevate significantly wet weight of uterus, cervix and vagina albeit the estrogen is found to exert more pronounced action[7-9]. On the basis of this inherent virtue, the estrogenic and antiestrogenic nature of many contraceptive agents has been assessed. Table 1 reflects the response of alcoholic extract of *Calotropis procera* on wet weight of uterus, cervix and vagina. Alcoholic extract significantly increases the wet weight of uterus, cervix and vagina ($p<0.05$) when compared to control group and when it administered with ethynilestradiol also significantly enhances ($p<0.05$) the wet weight of same organs when compared to ethynilestradiol group. These findings are in agreement with the estrogenic nature of the extract as it increased the wet weight of reproductive organs of the ovariectomized rats. Synergistic actions with ethynilestradiol further corroborate its estrogenic activity.

Effect of alcoholic extract on glycogen and protein contents is summarized in Table 2. Ovarian hormones apparently bring about mobilization of glycogen in the female reproductive organs. Estrogen is known to increase glycogen content in uterus and cervix of adult rats on the contrary it is reported to be decreased in vagina of ovariectomized rats[10-12]. Present study indicate that alcoholic extract therapy to spayed rats elevated ($p<0.05$) the glycogen contents of the uterus and cervix, but has an opposite effect on the vagina. Further its conjoint administration with ethynilestradiol act in a synergistic way and these findings corroborate that glycogen content in the reproductive organs is estrogen motivated. Further the decrease in vaginal glycogen contents says that in this tissue the estrogen increases the glycogen consumption rate than its storage[13].

Protein contents in the female reproductive tract are also regulated by the ovarian hormones. Estrogen and progesterone separately or in combined form significantly increased the protein contents of reproductive tract[14]. Our present findings also support the aforementioned information that alcoholic extract behaved in estrogenic way as it is increased protein contents ($p<0.05$) in all the parts of reproductive organs of ovariectomized rats (Table 2). Increase in the protein content of reproductive organs generally led to increase the uterine weight[15] as it has been observed in the present findings.

Our data further demonstrated that alcoholic extract act as an estrogenic agent (Table 3). An increase in the uterine acid and alkaline phosphatase activity has been reported after estrogen treatment. When alcoholic extract administered increased ($p<0.05$) the activity of both acid and alkaline phosphatase in all the reproductive organs of ovariectomized rats. Its combined treatment with ethynilestradiol act synergistically. These findings clearly reveal that alcoholic extract increased the activity of acid

TABLE 1: EFFECT OF ALCOHOLIC EXTRACT OF *CALOTROPIS PROCERA* ON UTERINE, CERVICAL AND VAGINAL WET WEIGHT OF OVARIETOMIZED RATS

Group	Treatment (P.O.) (7 d)	Uterus	Cervix	Vagina
1.	Intact control (Vehicle only 0.2 ml)	55.10±2.03	24.01±1.64	45.89±1.34
2.	OVM. control (Vehicle only)	46.19±1.93	16.01±0.62	37.82±1.73
3.	OVM+Extract (500 mg/kg)	54.39±4.33*	23.21±2.89*	46.16±4.91*
4.	OVM+EED (1 μg/rat/IM)	70.1±6.89	52.01±2.89	74.16±2.71
5.	OVM+Ext+EED (500 mg/kg+ μg/rat/IM)	81.01±9.05*	61.82±2.73*	82.82±2.73*

Values are mean±SE and expressed as mg/100 g. *P <0.05, OVM= ovariectomized, EED= ethynilestradiol, Ext.= extract, IM= intramuscular, PO= per oral

TABLE 2: EFFECT OF ALCOHOLIC EXTRACT OF *CALOTROPIS PROCERA* ON UTERINE, CERVICAL AND VAGINAL GLYCOGEN AND PROTEIN CONTENTS OF OVARIECTOMIZED RATS

Group	Treatment (PO) (7 d)	Glycogen			Protein		
		Uterus	Cervix	Vagina	Uterus	Cervix	Vagina
1.	Intact control (Vehicle only 0.2 ml)	46.12±1.14	34.21±1.42	36.19±1.04	48.15±1.17	23.24±1.11	16.01±1.04
2.	OVM control (Vehicle only)	38.29±3.43	28.36±2.89	25.29±2.01	31.76±0.68	11.14±1.04	9.76±0.72
3.	OVM+ Extract (500 mg/kg)	48.59±2.36*	35.31±2.63*	17.40±1.27*	47.12±0.69*	22.84±0.70*	15.2±0.42*
4.	OVM+EED (1μg/rat/IM)	72.96±2.56	59.25±1.80	13.46±2.17	63.79±0.66	35.39±0.65	32.89±0.68
5.	OVM+Ext+EED (500 mg/kg+ 1μg/rat/IM)	86.31±4.32*	77.59±2.36*	09.71±1.48*	71.84±0.70*	40.41±0.76*	52.87±0.85*

Values are mean±SE and expressed as units/mg tissue weight. *p<0.05, OVM= Ovariectomized; EED= ethynilestradiol; IM= intramuscular, PO= per oral

TABLE 3: EFFECT OF ALCOHOLIC EXTRACT OF *CALOTROPIS PROCERA* ON UTERINE, CERVICAL AND VAGINAL ACID AND ALKALINE PHOSPHATASE ACTIVITIES OF OVARIECTOMIZED RATS

Group	Treatment (PO) (7 d)	Acid phosphatase			Alkaline phosphatase		
		Uterus	Cervix	Vagina	Uterus	Cervix	Vagina
1.	Intact control (Vehicle 0.2ml)	121.13±1.61	135.17±2.16	112.14±1.07	312.14±3.41	201.18±4.13	151.17±1.08
2.	OVM control (Vehicle only)	83.11±4.06	119.63±5.45	89.36±3.54	291.8±12.91	183.60±7.56	134.87±6.58
3.	OVM+ Extract (500 mg/kg)	101.99±6.86*	141.24±9.38*	103.63±8.63*	310.7±22.58*	198.53±20.54*	142.22±9.34*
4.	OVM+EED (1μg/rat/IM)	149.46±5.78	178.10±9.61	159.54±6.52	369.85±19.50	239.92±29.59	182.20±9.73
5.	OVM+Ext+EED (500 mg/kg+ 1μg/rat/IM)	163.15±8.67*	191.38±10.85*	170.83±8.63*	383.37±22.5*	251.45±24.55*	193.72±12.64*

Values are mean±SE and expressed as units/mg tissue weight. *p<0.05, OVM= Ovariectomized; EED= ethynilestradiol; IM= intramuscular, PO= per oral

and alkaline phosphatase which may be due to its estrogenic influence. The role of these two enzymes in the reproductive organs has been studied by a number of workers but no clear picture is yet available in relation to steroidal applications. As the acid phosphatase is located mainly in the lysosomes, which increased enzymatic activity signifies the disintegration of the complex organelle and the liberation of hydrolytic enzymes. Histochemical studies revealed that acid phosphatase showed positive reaction with the permeable membrane; therefore it may alter the cell permeability resulting in increased absorption of nutrient material by the cell. Alkaline phosphatase is believed to be involved in the growth and secretory function of the tissue cells, metabolism of carbohydrate and lipids, nucleic acid and increase in the cell permeability. Therefore increase in activity of acid and alkaline phosphatase in reproductive organs may result in the alterations of secretory functions by influencing the cell permeability, thereby changing the reproductive milieu especially uterine environment which is directly involved in the implantation of eggs[16-18]. On the basis of these observations it may be concluded that alcoholic extract of *C. procera* owing to its potent estrogenic nature alters the biochemical milieu of the reproductive tract which lead to change the normal status of the reproduction in female reproductive tract of rat and thus produce significant antifertility effect.

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