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BIOCHEMICAL ALTERATIONS IN THE FEMALE GENITAL TRACT OF OVARIECTOMIZED RATS TREATED WITH AQUEOUS EXTRACT OF *MORINGA OLEIFERA* LAM

Sangeeta Shukla, R. Mathur and A.O. Prakash

School of Studies in Zoology, Jiwaji University, Gwalior-474011 (M.P.) India

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Biochemical alterations have been observed in the uterus, cervix and vagina of ovariectomized rats in relation to antifertility effect of aqueous extract of *Moringa oleifera* Lam. Its administration has increased glycogen, protein, total cholesterol, acid and alkaline phosphatases in all the organs, however, in vagina the glycogen level decreased significantly. Its combined treatment with estradiol acted synergistically, however, with progesterone it behaved antagonistically.

Key words: Moringa oleifera Lam, Biochemical constituents Reproductive organs.

INTRODUCTION

Moringa oleifera Lam., commonly known as Sahajana a member of family Moringaceae, manifests significant antifertility effect in rats [1-4]. Of the various fractions, the aqueous extract of its root evokes significant anti-implantation effect [5] at 200 mg/kg dose when given orally for 7 days after coitus (1-7 *post coitum*). Furthermore extract has also been described for its hormonal properties at different dose schedules [6]. It is known that many plants exhibiting antifertility activity also induce biochemical and physiological alterations in the reproductive organs of adult rats [7,8]. These biochemical fluctuations are usually linked with various carbohydrate, protein and lipid metabolic reactions. The present study has, therefore, been undertaken to evaluate the effect of aqueous extract of *M. oleifera* Lam. on the biochemical constituents of the reproductive organs of the ovariectomized rats.

MATERIALS AND METHODS

Fresh roots of *M. oleifera* Lam. were collected from the Campus of Jiwaji University, Gwalior and was chopped, dried and extracted with water as described earlier [3]. A dose of 200 mg/kg, prepared in gum acacia suspension was administered orally with the help of an intragastric catheter. Estradiol dipropionate (Ovocyclin, CIBA, India) and progesterone (Luteocyclin, CIBA, India) were diluted in olive oil and were administered subcutaneously.

Adult, healthy, virgin female rats of Spraque Dawley strain were selected for the present investigation. These animals were maintained under uniform husbandry conditions and were fed with "Liptom Limited" Gold mohur rat pelleted diet and water *ad libitum*. These were ovariectomized bilaterally under light ether anaesthesia and after a post operative rest of two weeks, animals were randomized into eight groups of 6 each and received different treatments in the following manner :

Group No.		Treatment
1. OVX	–	Ovariectomized control (vehicle only).
2. OVX + EDP	–	OVX + estradiol dipropionate (*s.c.*) 1 μg/rat/day for 7 days.
3. OVX + P	–	OVX + progesterone (*s.c.*) 1.25 mg/rat/day for 7 days.
4. OVX + E + P	–	OVX + EDP + P as in groups 2 and 3.
5. OVX + Ext	–	OVX + extract (oral) 200 mg/kg for 7 days.
6. OVX + Ext + EDP	–	OVX + extract as in group 6 + EDP as in group 2.
7. OVX + Ext + P	–	OVX + extract as in group 6 + P as in group 3.
8. OVX+Ext+EDP+P	–	OVX + extract as in group 5+EDP and P as in group 2 and 3.

The animals were sacrificed 48 hrs after the last treatment. The organs *viz.* uterus, cervix and vagina were excised, freed from adhering tissue, blotted and weighed to the nearest 1 mg on a single pan electric balance. Fresh tissue was processed for the estimation of glycogen [9] whereas isotonic buffered homogenate was processed for the estimation of proteins [10], acid and alkaline phosphatase activity [11], total and esterified cholesterol [12]. The results were analysed statistically using students 't' test.

RESULTS AND DISCUSSION

Table 1, 2 and 3 represent the effect of aqueous extract of *M. oleifera* Lam. on the biochemical constitu-

Table 1. Effect of aqueous extract of *M. oleifera* Lam. on various biochemical constituents in the uterus of adult ovariectomized rats. (Values are mean ± SEM and six animals were used in each set).

Treatment	Wet weight (mg/100g b.w)	Proteins (mg/100mg)	Glycogen (mg/100g)	Acid phosphatase (mg P/100g/h)	Alkaline phosphatase (mg P/100g/h)	Total cholesterol (mg/100g)
OVX Control	45.0 ± 3.6	10.6 ± 0.9	40.5 ± 2.3	86.9 ± 5.9	296.7 ± 15.7	0.121 ± 0.006
OVX+EDP	143.6 ± 8.6[a]	11.9 ± 1.0	74.3 ± 3.4[a]	121.1 ± 6.3[a]	425.3 ± 25.4[a]	0.146 ± 0.008[a]
OVX+P	63.8 ± 3.2[a]	13.5 ± 0.7[a]	45.6 ± 2.8	201.9 ± 12.8[a]	378.7 ± 16.7[a]	0.163 ± 0.008[a]
OVX+EDP+P	122.5 ± 7.0[b]	16.2 ± 0.9[b]	60.6 ± 2.6[b]	99.7 ± 6.5	289.8 ± 15.8[b]	0.160 ± 0.008
OVX+Ext	100.0 ± 6.1[a]	11.2 ± 0.8	71.2 ± 3.4[a]	108.4 ± 6.5[a]	430.5 ± 22.8[a,b]	0.140 ± 0.007[a]
OVX+EDP+Ext	169.8 ± 8.9[b]	12.6 ± 1.0	85.7 ± 3.9[b]	130.7 ± 7.5	460.0 ± 23.0	0.156 ± 0.007[b]
OVX+P+Ext	89.8 ± 6.2[c]	15.3 ± 0.6[c]	65.3 ± 3.4[c]	160.6 ± 8.4[c]	405.3 ± 22.1[c]	0.169 ± 0.008[c]
OVX+EDP+P+Ext	134.6 ± 7.8	16.1 ± 0.8	70.6 ± 3.5	120.3 ± 6.5	325.6 ± 18.7	0.170 ± 0.009

Values are statistically significant ($P < 0.05$) when compared to OVX Control (a), EDP Control (b) and P Control (c).

Table 2. Effect of aqueous extract of *M. oleifera* Lam. on various biochemical constituents in the cervix of adult ovariectomized rats. (Values are mean ± SEM and six animals were used in each set).

Treatment	Wet weight (mg/100g b.w)	Proteins (mg/100mg)	Glycogen (mg/100g)	Acid phosphatase (mg P/100g/h)	Alkaline phosphatase (mg P/100g/h)	Total cholesterol (mg/100g)
OVX Control	16.3 ± 0.9	12.0 ± 0.8	32.5 ± 2.2	130.6 ± 6.6	190.0 ± 8.5	0.110 ± 0.006
OVX + EDP	52.0 ± 2.1[a]	16.2 ± 1.1[a]	56.3 ± 3.8[a]	219.0 ± 12.0[a]	519.9 ± 25.2[a]	0.128 ± 0.006[a]
OVX + P	34.3 ± 2.6[a]	15.3 ± 0.8[a]	38.1 ± 2.5	158.3 ± 7.2[a]	310.7 ± 15.2[a]	0.130 ± 0.007[a]
OVX+EDP+P	44.1 ± 2.5[b]	19.5 ± 1.1[b]	46.1 ± 2.2[b]	210.1 ± 10.9	440.5 ± 26.3[b]	0.133 ± 0.005
OVX+Ext	37 8 ± 2.1[a]	15.6 ± 0.9[a]	48.5 ± 2.9[a]	190.2 ± 9.7[a]	490.3 ± 24.9[a]	0.115 ± 0.005
OVX+EDP+Ext	56.8 ± 2.8	15.9 ± 0.7	59.0 ± 2.8	230.7 ± 12.2	590.0 ± 25.4[b]	0.120 ± 0.006
OVX+P+Ext	40.1 ± 2.4	18.5 ± 0.9[c]	44.3 ± 2.2[c]	167.7 ± 8.6	390.2 ± 20.7[c]	0.126 ± 0.005
OVX+EDP+P+Ext	47.3 ± 2.8	19.3 ± 1.0	50.4 ± 3.5	220.4 ± 12.1	600.7 ± 30.9	0.131 ± 0.005

Values are statistically significant ($P < 0.05$) when compared to OVX control (a), EDP control (b) and P control (c).

Table 3. Effect of aqueous extract of *M. oleifera* Lam. on various biochemical constituents in the vagina of adult ovariectomized rats. (Values are mean ± SEM and six animals were used in each set).

Treatment	Wet weight (mg/100g b w)	Proteins (mg/100mg)	Glycogen (mg/100g)	Acid phosphatase (mg P/100g/h)	Alkaline phosphatase (mg P/100g/h)	Total cholesterol (mg/100g)
OVX Control	40.1 ± 2.4	11.6 ± 0.8	52.1 ± 3.4	81.3 ± 5.5	140.5 ± 8.4	0.102 ± 0.006
OVX+EDP	77 1 ± 3.2[a]	13 3 ± 0 8[a]	31 4 ± 3.7[a]	131.4 ± 6.1[a]	230.3 ± 14.6[a]	0.126 ± 0.005[a]
OVX+P	60.3 ± 3.1[a]	13.8 ± 0.6[a]	48.5 ± 2.6	108.6 ± 5.4[a]	168.4 ± 8.4[a]	0.120 ± 0.006[a]
OVX+EDP+P	67.5 ± 3.7[b]	17.5 ± 0.9[b]	41.7 ± 2.6[b]	114.0 ± 5.4[b]	180.3 ± 10.4[b]	0.118 ± 0.005
OVX+Ext	71.3 ± 4.5[a]	12.4 ± 0.6	27.4 ± 1.4[a]	110.0 ± 6.8[a]	210.3 ± 12.5[a]	0.104 ± 0.006
OVX+EDP+Ext	90.3 ± 4.2[b]	15 4 ± 0.8[b]	30.1 ± 2.0	148.0 ± 5.8[b]	280.5 ± 13.2[b]	0.130 ± 0.006
OVX+P+Ext	64.5 ± 3.5	18.1 ± 0.9[c]	42.7 ± 2.3	110.0 ± 5.8	178.1 ± 9.2	0.132 ± 0.006
OVX+EDP+P+Ext	79.8 ± 4.4	18.0 ± 0.9	40.3 ± 2.5	119.0 ± 6.7	199.7 ± 8.9	0.121 ± 0.005

Values are statistically significant ($P < 0.05$) when compared to OVX Control (a), EDP Control (b) and P Control (c).

ents in the genital tract of ovariectomized rats. Administration of estradiol dipropionate and progesterone *per se* increased the wet weight glycogen contents, protein concentration activity of acid and alkaline phosphatase and the level of total cholesterol in the uterus (Table 1), cervix (Table 2) and vagina (Table 3); however, estrogen exerted a more pronounced effect. Further, vaginal glycogen (Table 3) showed significant depletion after the administration of estradiol dipropionate. In their conjoint treatment progesterone tends to antagonise the estrogen induced gain, however, protein content was significantly increased after their treatment.

Administration of the aqueous extract of *M. oleifera* Lam. *per se* elevated significantly the wet weight and other biochemical constituents in the uterus (Table 1), cervix (Table 2) and vagina (Table 3) of spayed rats, however, the vaginal glycogen contents has been decreased when compared to OVX control. Administration of the extract conjointly with estradiol dipropionate further augmented the effect when compared with EDP *per se*. Concurrent administration of progesterone and extract provoked a marginal increase in the wet weight, glycogen contents, activity of acid and alkaline phosphatases and the level of total cholesterol, however, considerable rise is observed in the protein contents of uterus, cervix and vagina.

Ovarian hormones, estrogen and progesterone are known to alter biochemical constituents of the female reproductive tract through their generalized mechanism [13,14]. Administration of estrogen or progesterone to adult ovariectomized rats is known to elevate significantly the wet weight of the uterus [15-17], cervix [18] and vagina [19] albeit, the estrogen is found to exert more pronounced action. On the basis of this inherent virtue, the estrogenic and antiestrogenic nature of many contraceptive agents has been assessed [20-23]. The present findings are in agreement with the above observations. Further, the aqueous extract of *M. oleifera* Lam. increased the wet weight of the uterus, cervix and vagina of the ovariectomized rats, thereby depicting the extrogenicity of the extracts. Synergistic action with estradiol further corroborate its estrogenic activity [6].

A typical estrogen is known to increase the glycogen contents in the uterus [24-27] and cervix [18] of adult rats and also in immature animals [28,29], however, in vagina the glycogen contents are decreased significantly [3]. Progesterone, an active antiestrogen, plays a controversial role. It has been reported to reduce significantly the glycogen contents in the uterus of adult rats [18,25] but simultaneously other authors [24,31,32] have reported no change.

An increase in the uterine acid phosphatase activity has been reported in the immature rats and adult spayed rats after estrogen [16,18,33] and progesterone therapy [34,35]. However, no significant change has also been reported in acid phosphatase activity in the uterus of ovariectomized rats treated with progesterone [36]. Similarly, an increase in uterine alkaline phosphatase activity in both intact and ovariectomized mice and rats after estrogen therapy have been reported by many workers [16,26,29,33]. Progesterone is known to increase [36] or inhibit [34,37,38] or even provoke no change [29,39] in the activity of alkaline phosphatase. It has been reported that the level of uterine cholesterol is not much changed during different phases of the oestrous cycle, ovariectomy and also by steroidal treatment [40]. Rosenman *et. al.* [41] have reported a decreased cholesterol synthesis as a result of estrogen treatment. Further Moskowitz *et. al.* [42] reported that after 6 weeks of estradiol 17 β treatment, rat showed increased cholesterol.

Our present findings reveal that the administration of aqueous extract of *M. oleifera* Lam. to ovariectomized rats elevated the wet weight, glycogen content, protein concentration, activity of acid and alkaline phosphatase in the uterus, cervix and vagina although the glycogen contents in vagina were decreased and level of total cholesterol showed marginal increase. These findings clearly reveal estrogenic mechanism of aqueous extract of *M. oleifera*. Additionally its synergistic action with estradiol further strengthenes the estrogenic property which has already been observed in immature ovariectomized rats [6].

The increase in the uterine and cervical glycogen contents after the treatment with the extract may be due to the increase in the input of more estrogen which consequently increase the glycogenetic process of the carbohydrate metabolism as it is known that estrogenic substances increase the rats of hexokinase reaction [43,44]. The decrease in the vaginal glycogen contents after the treatment with extracts say that in this tissue the estrogen increases the glycogen consumption rate than its storage. The increased uterine glycogen due to estrogenic nature of the aqueous extract of *M. oleifera* Lam. may also be involved in the rhythmic uterine contractions [45] which may further propel the blastocysts from the uterus and thus provoke their anti-implantation action. Therefore, the increased uterine glycogen level as observed in the present study may be involved in providing the ready made energy for the uterine contractions and thus helps in the expulsion of the fertilized eggs from the uterus. This explanation is further strengthened by the fact that the administration of exogenous glycogen in the uterus of pregnant rats terminates

the pregnancy [46]. Increased activity of the acid and alkaline phosphatase in the genital tract under the influence of aqueous extract of *M. oleifera* may be involved in removing the debris or lysed cells [47,48] or it may alter the permeability of the cell membrane resulting in the increased absorption of nutrient material by the cell [49].

Therefore, on the basis of our present study it may be concluded that the aqueous extract of *M. oleifera* Lam. provoked significant alterations in the genital tract probably due to its estrogenic nature. It is expected that due to the disturbances in these biochemical transformations, the extract may create unfavourable conditions in the uterus which may become the cause of infertility.

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