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EFFECT OF HEXANE EXTRACT OF *FERULA JAESCHKEANA* ON THE UTERUS OF ADULT OVARIECTOMISED RATS

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ABSTRACT: *Effect of hexane extract of Ferula jaeschkeana has been studied on the histological and biochemical constituents of the uterus of ovariectomized rats. Its administration caused remarkable increase in the height of luminal epithelium and the number of uterine glands. Its per se treatment also caused significant increase in the glycogen and protein contents and the activity of acid and alkaline phosphatase. When the extract was administered with estradiol dipropionate, a synergic effect was observed in histological and biochemical constituents of the uterus. Conjoint administration with progesterone did not antagonize the estrogenic influence of the extract.*

INTRODUCTION

Ferula jaeschkenana (Hing), a member of family umbellifare, manifest contraceptive efficacy when tested in rats (Sing *et al.*, 1985; Prakash, 1985). In hexane extract given at a dose of 25 mg/ kg / day for 7 days after coitus (1 – 7 p.c) prevents implantation in rats (Pathak and Prakash, 1989). Antifertility agents of hormonal virtues are known to induce histological and biochemical alterations in the genital tract of female rats (Prakash *et al.*,1986). Moreover, uterus being a receptacle of implantation undergoes series of physiological changes in order to meet the requirements of various reproductive events. Although hexane extract of *F.jaeschkean* imparts estrogenic activity (Pathak and Prakash, 1989) these studies have been done in immature rats and not much is known about its influence in adult cyclic and ovariectomized rats. Present investigation deals with these findings in adult ovariectomized rats so as to evaluate its extract estrogenic mode of action.

MATERIALS AND METHODS

Entire plant of *F. jaeschkeana* was collected from Nullah slopes of Srinagar (J & K). It was dried in shade, chopped, powdered and extracted with hexane as described earlier (Pathak and Prakash, 1989). Its effective dose of 25 mg/kg was prepared in gum acacia suspension and administered to rats per orally (Prakash and Mathur, 1976). Estradiol diprpionate (EDP, Ovocyclin CIBA, India) and progesterone (P, Luteocyclin, CIBA, India), dissolved in olive oil were administered subcutaneously.

Adult, virgin healthy female rats Sprague Dawley Strain (150 + 10g) were ovariectomized bilaterally under light after a post-operative rest of two weeks. These animals were divided into eight groups of 5 each and were subjected to different treatments (Table 1). After 24 h of last treatment, animals were sacrificed and uterus was excised, freed from adhering tissue and weighed. Fresh tissue was

processed for the estimation of glycogen (Seifter *et al.,* 1950) whereas isotonic buffered homogenate was processed for the estimation of proteins (Lowry *et al.,* 1951), activity of acid and alkaline phosphatase (Hawk *et al.,* 1954), total and esterified cholesterol (Zlatkis *et al.,* 1953). The results were analysed statistically using analysis of variance (ANOVA). A small piece of the uterus was also fixed in Bouin's fluid and haematoxylin-eosin stained slides were examined microscopically.

TABLE 1

Treatment for ovariectomized rats

Group No.	**Treatment**
1. Intact control	Intact rats showing estrus stage of vaginal smear
2. Ovx	Ovariectomized control (Vehicle only)
3. Ovx + EDP	Ovariectomized rats received estradiol dispropionate (s.c.) 1 g / day / rat for 7 days
4. Ovx + P	Ovariectomized rats received progesterone (s.c). 3 mg / day / rat
5. Ovx + EDP + P	Ovariectomized rats received estradiol diprovpionate as in group no.3 and Progesterone as in group no.4
6. Ovx + Ext	Ovariectomized rats received hexane extract (oral) at 25 mg / kg for 7 days.
7. Ovx + Ext + EDP	Ovariectomized rats received extract as in group 6 + estradiol dipropionate as in group 4.
8. Ovx + Ext + P	Ovariectomized rats received extract as in group 6 + progesterone as in group 4.
9. Ovx + Ext + EDP + P	Ovariectomized rats received extract as in group 6 + estradiol dipropionate as in group 3 and progesterone as in group 4.

RESULTS

(A) Effect on wet weight

Table 2 revealed that the administration of the hexane extract of *F. jaeschkeana* increased significantly the wet weight of uterus when compared to ovariectomized control groups (P<0.001). Also the administration of extract with EDP or with significantly increased the uterine wet weight when compared to EDP and P *per se* respectively.

TABLE – 2

Effect of hexane extract of *F.jaeschkeana* on the wet weight, protein and the glycogen content in the uterus of adult ovariectomized rats.

Group No.	Treatment	We weight mg/100g	Protein mg/100mg	Glycogen mg/100g
1	Intact control	135.2 ± 6.7	11.6 ± 0.56	60.1 ± 3.1
2	Ovx control	46. 4 ± 4.08 (Vs. group 2 < 0.001)	9.7 ± 1.6 (Vs. group 1>0.05)	45.6 ± 2.3 (Vs. group < 0.02)
3	Ovx + EDP	170.5 ± 6.1 (Vs. group 2 < 0.001)	11.5 ± 0.51 (Vs. group 2>0.05)	84.7 ± 4.6 (Vs. group 2>0.05)
4	Ovx + P	54.2 ± 3.1 (Vs. group 2<0.05)	12.4 ± 0.66 (Vs. group 2 >0.05)	49.4 ± 2.4 (Vs. group 2>0.05)
5	Ovx + EDP +P	138.2 ± 7.1 (Vs. group 3 < 0.001)	12.9 ± 1.67 (Vs. group 3>0.05)	83.6 ± 4.9 (Vs. group 3>0.05)
6	Ovx + Ext	98.3 ± 5.6 (Vs. group 2<0.001)	10.2 ± 1.02 (Vs. group 2>0.05)	75.6 ± 4.1 (Vs. group 2<0.001)
7	Ovx + Ext + EDP	214.8 ± 11.3 (Vs. group 3<0.001)	12.1 ± 2.23 (Vs. group 3>0.05)	89.02 ± 5.5 (Vs. group 4<0.05)
8	Ovx + Ext + P	94.3 ± 4.8 (Vs. group 4<0.001)	14.3 ± 0.72 (Vs. group 4>0.05)	60.6 ± 3.4 (Vs. group 4<0.05)
9	Ovx + Ext + EDP + P	161.7 ± 11.9 (Vs. group 5>0.05)	14.4 ± 1.02 (Vs. group 5>0.05)	82.3 ± 7.7 (Vs. group 5>0.05)

(B) Effect on biochemical constituents (Tables 2-3)

Administration of hexane extract of *F.jaeschkeana* produced significant increase in the uterine glycogen contents, activity of alkaline phosphatase, and level of total and esterifies cholesterol while no significant change was induced in the protein concentration and in the activity of acid phosphatase. When given along with EDP or progesterone, it did not induce significant change in protein contents, activity of phosphatase and in the level of esterifies cholesterol. The combined administration of progesterone and extract significantly increased the glycogen contents induced by progesterone. The combined treatment of extract, EDP and P induced significant change in the activity of alkaline phosphatase and in the level of total cholesterol, but the activity of acid phosphatase remain unchanged.

(C) Effect on histoarchitecture

The histoarchitecture of the uterus of ovariectomized control rats exhibited typical infantile condition (Fig.1). Administration of estradiol dipropionate (EDP) stimulated the features remarkably (Fig.2). Progesterone also caused similar changes but to lesser extent (Fig.3). Conjoint treatment of EDP and P induced changes but these were less marked when compared to EDP induced changes (Fig.4). The administration of hexane extract stimulated the histoarchitecture of the uterus as indicated by well organized endometrium with stroma (Fig.5). Furthermore, it acted synergistically with EDP (Fig.6). Its combined treatment with progesterone depicted stimulated luminal epithelium with loose stroma and deformed uterine glands (Fig.7), however, a conjoint treatment of EDP plus progesterone showed typically stimulated condition in the uterus as revealed by EDP treated rats (Fig. 8 c.f. Fig.6)

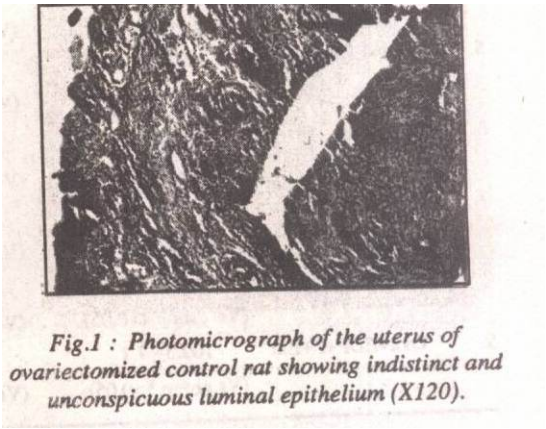

Fig.1 : Photomicrograph of the uterus of ovariectomized control rat showing indistinct and unconspicuous luminal epithelium (X120).

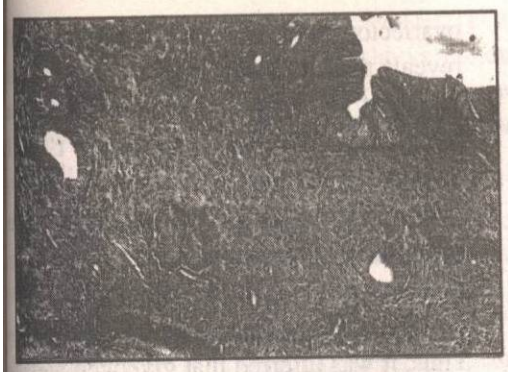

Fig.2 : Rats treated with estradiol dipropionate. Note increase in the height of endometrial epithelium with basal nuclei (X120).

Fig.3 : Rats treated with progesterone showing secretory utering glands (X120).

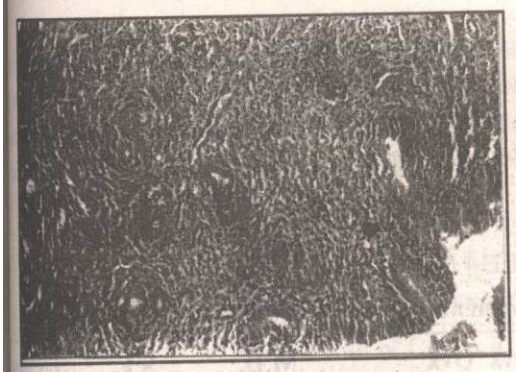

Fig.4 : Rats treated with estradiol dipropionate conjointly with progesterone showing ill defined uterine glands (X120).

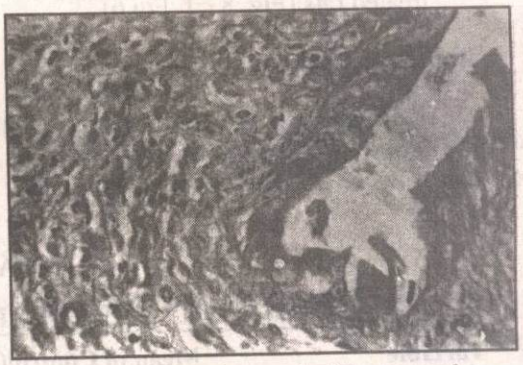

Fig.5 : Photomicrograph of the uterus of ovariectomized rats treated with hexane extract showing well organized endometrium with loose stroma (X400).

Fig.6 : Rats treated with estradiol dipropionate. and hexane extract showing stimulation in the luminal epithelium (X400).

Fig.7 : Rats treated with hexane extract and progesterone depicting the increase in the height of lumnal epthelum (X400).

Fig.8 : Rats treated with hexane extract plus estradiol dipropionate plus progesterone indicating stimulated features with an increase in the number of uterine glands (X120).

TABLE 3

Effect of hexane extract of *F. jaescheana* on the activity of acid and alkaline phosphatase in the uterus of adult ovariectomized rats.

Group No.	Treatment	Acid phosphatase	Alkaline phosphates	Total cholesterol	Esterifies cholesterol
1	Intact control	102.0 ± 5.1	370.2 ± 18.0	0.160 ± 0.008	0.042 ± 0.001
2	Ovx + Control	90.8 ± 6.0 (Vs. group 1>0.05)	314.8 ± 16.17 (Vs. group 1<0.05)	0.134 ± 0.004 (Vs. group 1<0.02)	0.037 ± 0.002 (Vs. group 1<0.05)
3	Ovx + EDP	108.5 (Vs. group 2>0.05)	456.5 ± 22.7 (Vs. group 2<0.001)	0.0150 ± 0.003 (Vs. group 2<0.02)	0.046 ± 0.003 (Vs. group 2>0.05)
4	Ovx + P	186.3 ± 12.7 (Vs. group 2<0.001)	381.7 ± 18.7 (Vs. group 2<0.001)	0.166 ± 0.006 (Vs. group 2<0.02)	0.041 ± 0.002 (Vs. group 2>0.05)
5	Ovx + EDP +P	119.9 ± 8.6 (Vs. group 3<0.05)	336.8 ± 17.3 (Vs. group 3<0.001)	0.184 ± 0.005 (Vs. group 3<0.001)	0.045 ± 0.003 (Vs. group 3>0.05)
6	Ovx + Ext	100.6 ± 6.6 (Vs. group 2>0.05)	415.8 ± 18.3 (Vs. group 2<0.001)	0.163 ± 0.004 (Vs. group 2<0.001)	0.051 ± 0.003 (Vs. group 2<0.02)
7	Ovx + EDP + Ext	124.1 ± 6.2 (Vs. group 3>0.05)	507.3 ± 22.2 (Vs. group 3>0.05)	0.170 ± 0.005 (Vs. group 3<0.02)	0.052 ± 0.004 (Vs. group > 0.05)
8	Ovx + P + Ext	161.6 ± 8.5 (Vs. group 4>0.05)	400.0 ± 17.6 (Vs. group 4>0.05)	0.199 ± 0.006 (Vs. group 4<0.02)	0.048 ± 0.001 (Vs. group 4<0.02)
9	Ovx + EDP+ P + Ext	109.3 ± 7.1 (Vs. group 5>0.05)	457.7 ± 23.4 (Vs. group 5<0.001)	0.222 ± 0.004 (Vs. group 5<0.001)	0.051 ± 0.002 (Vs. group 5>0.05)

Statistical Analysis

Analysis of variance for the date on all the biochemical contents in the uterus of ovariectomized is shown in Table 4(A). It reveals that the calculated 't' values for the variables glycogen and alkaline phosphatase are greater than that of tabulated 't' at 0.05 level of significance with 8 degree of freedom (for one tail test). Hence we may reject the null hypothesis of no difference between the experimental (Ovx + Ext) and control (Ovx) groups in glycogen and alkaline phosphatase. Thus, it was inferred that glycogen and alkaline phosphates were significantly more in experimental (Ovx + Ext) groups in comparison to control (Ovx) group.

In Table 4(B) calculated't' ration for all the variables were less than tabulated value of t at 0.05 level at 8 degree of freedom (for one tail test). Hence the null hypothesis of no difference between experimental (Ovx + E + Ext) and control (Ovx + E) groups for all the variables were retained. Thus it was concluded that there was no improvement in any of the variables due to the experiment.

In Table 5 (AP the table 't' ration for the variables glycogen and protein were greater than that of tabulated 't' at 0.05 level of significance with 8 degree of freedom (for one tail test). Hence we may reject the null hypothesis of no difference between the experimental (Ovx + P + Ext) group and control (Ovx + P) groups in glycogen and protein. It was also inferred that glycogen and protein contents were significantly more in experimental (Ovx + P + Ext) group in comparison to control (Ovx + P) group while there was no improvement in acid and alkaline phosphatase of control and experimental groups.

In Table 5(B) the calculated 't' ration for the variable alkaline phosphatase contents have greater than that of tabulated t at 0.05 level of significance with 8 degree of freedom (for one tail test). Hence we may rejected the null hypothesis of no difference between the experimental (Ovx + E + P + Ext) group and control (Ovx + E + P) groups in alkaline phosphates. Thus, it may be concluded that the alkaline phosphatase contents were significantly more in experimental (Ovx + E + P+ Ext) group in comparison to control (Ovx + E + P) group while there was no improvement in glycogen, protein and acid phosphates of control and experimental groups.

TABLE 4 (A & B)
't' ratio for the control and experimental groups in ovariectomized rats

Variable (A)	Mean of Control (Ovx Ovx) and Experimental (Ovx + Ext) groups		M.D.	S.E.	t-value
	Experimental	Control			
1. Glycogen	60.6	49.0	11.6	4.13	2.80
2. Protein	14.46	12.4	2.06	.93	2.20
3. Acid phosphatase	161.66	186.39	24.69	13.70	1.80
4. Alkaline phosphatase	400.00	381.74	18.25	22.99	0.79

Variable (B)	Mean of Control (Ovx + E) and Experimental (Ovx + Ext + E) groups		M.D.	S.E.	t-value
	Experimental	Control			
1. Glycogen	82.32	83.64	1.32	8.19	0.16
2. Protein	14.48	12.92	1.56	1.75	0.89
3. Acid phosphatase	109.32	119.94	10.62	10.03	1.05
4. Alkaline phosphatase	457.74	336.8	120.94	26.06	4.64

For one tail test tab t.05 (8) = 1.86

TABLE 5 (A & B)
't' ratio for the control and experimental groups in ovariectomized rats

Variable (A)	Mean of Control (Ovx Ovx) and Experimental (Ovx + Ext) groups		M.D.	S.E.	t-value
	Experimental	Control			
1. Glycogen	75.02	45.64	29.38	4.66	6.30
2. Protein	10.28	9.84	0.37	1.76	0.21
3. Acid phosphatase	100.60	90.80	9.79	8.09	1.21
4. Alkaline phosphatase	415.88	314.82	101.06	21.90	4.61

Variable (B)	Mean of Control (Ovx + E+P) and Experimental (Ovx + Ext + E+P) groups		M.D.	S.E.	t-value
	Experimental	Control			
1. Glycogen	89.02	84.74	4.27	6.43	0.66
2. Protein	17.18	11.52	0.65	2.04	0.32
3. Acid phosphatase	124.14	108.8	15.34	8.27	1.85
4. Alkaline phosphatase	507.36	456.56	50.80	28.45	1.78

For one tail test tab t.05 (8) = 1.86

DISCUSSION

Bio chemical and histological alterations in the female genital tract are dependent on the female sex steroids estrogen and progesterone. Exogenous administration of these hormones disturb the intrinsic hormonal equilibrium and thus affect the normal genital physiology through morphological, histological and biochemical modifications (Evans and Simson, 1950). It has been observed that ovariectomy which ceases the secretion of estrogen and progesterone reduced the level of biochemical constituents and when the hormones are administered exogenously, the loss is restored (Woessner, 1973). Uterus being the receptacle of implantation is more sensitive towards these changes. Uterine histological structures which are stimulated under estrogenic influence include and increase in the height of the liminal epithelium, vascularity with loose stroma and enlargement of uterine glands (Datta *et al.,* 1968, Karkun and Mehrotra, 1973). In the present study the administration of the hexane extract to spayed rats stimulated the histoarchitecture of the uterus as revealed by an increase in the height of liminal epithelium depicting thereby a response parallel to that of estrodiol dipropionate. These absorptions demonstrate the estrogenic nature of the extract as reported earlier in immature ovariectomized rats (Pathak and Prakash, 1989).

The administration of estrogen or progesterone to adult ovariectomized rats significantly increased the wight of uterus (Lerner, 1969; Karkun and Mehrotra, 1973). The estrogenic and antiestrogenic nature of many contraceptive agents has been assessed in bilaterally ovariectomized immature rats (Edgren and Calhaun, 1957; Kholkute and Udupa, 1976; Pakrashi and Saha, 1977). The present finding also reveal that the administration of hexane extract of *F. jaeschkena* increased the wet weight of uterus, which may be accounted for its potent estrogenic nature (Pathak and Prakash, 1989). Concurrent administration of EDP acted synergistically which curter corroborate its estrogenic action. Similar type of findings have also been reported earlier wherein plant extracts of estrogenic

nature increased the uterine weight in immature rats (Saxena *et al.,* 1985).

Protein synthesis in the uterus is considered to be regularly by estrogen and progesterone. Datta *et al.,* (1968) and Mehrotra (1976) have reported that estrogen stimulated the protein contents in the uterus. Furthermore, the protein concentration in the uterus is known to be increased significantly after the administration of estrogen and/or progesterone. Our present findings reveal that the hexane extract alone or in combination of EDP of P increased total proteins in uterus. The increase in the protein concentration generally led to increase in the uterine weight (Hider *et al,* 1969) as reported earlier.

It is known that normal functioning of the reproductive organs is due to proper mobilization of glycogen in different parts of the female genital tract. It is also known that the ovarian hormones viz. estrogen and progesterone are responsible for this movement (Walaas, 1952; Nalbandov, 1970). Estrogen administration is known to cause significant elevation in uterine glycogen contents of adult rats. (Wood *et al.,* 1968; Mohla and Prasad, 1969). Role of progesterone is controversial. It may cause an increase (Datta *et al.,*1968) or decrease (Gregoire *et al.,* 1967) in the uterine glycogen level. Datta *et al.,* 1968) have reported a significant reduction in the glycogen level in the uterus after the administration of progesterone to ED; primed animals when compared to EDP *per se* treated rats. Present findings have clearly revealed that the administration of hexane extract caused a significant elevation in the uterine glycogen contents of Ovx rats. Administration of the extract to EDP primed rats acted in a synergistic way. Similarly its combined administration with progesterone acted antagonistically. Therefore, it is quite clear on the basis of present findings that the administration of extract enhanced the contents in the reproductive organs strictly under its estrogenic influence.

Ovarian hormones, estrogen and progesterone have also been known to alter the activity of acid and alkaline phosphatase in the genital tract. Karkun and Mehrotra (1973) and Prakash (1979) have reported that an increase in the activity of alkaline phosphatase in the uterus of Ovx rats is also estrogen motivated. Hayashi and Fishman (1961) have reported that the administration of progesterone increased the activity of alkaline phosphatase, creased the activity of alkaline phosphatase, however, the activity is inhabited (Gracia Bunnel and Brandes, 1966) in the uterus of Ovx rats. On the contrary, Leathem (1959) have reported that progesterone does not have any effect although estradiol increases the activity of alkaline phosphatase in the uterus of Ovx rats. The combed treatment of estrogen and progesterone could not change the activity of acid phosphatase but suppressed significantly the estrogen induced alkaline phosphatase activity. The administration of hexane extract of *Ferula jaeschkeana per se* increased the activity of these enzymes in the uterus of Ovx rats. The combined treatment with estrogen or progesterone acted synergistically, however, with progesterone acted synergistically, however, with progesterone more pronounced effect has been observed. These findings clearly reveal that the treatment of hexane extract of *F. jaeschkeana* may be due its estrogenic effect. This view is supported by the fact that when the extract was administered to EDP primed rats, the activity of alkaline phosphatase increased significantly when compared to EDP *per se* treated animals. It is assumed that hexane extract is involved in the metabolism of protein and carbohydrates

and simultaneously it may also be involved to increase the permeability of a cell.

Various workers have reported the presence of cholesterol in different parts of genital tract. Further, the effect of ovarian hormones has been studied on the level (Rosenman *et al.*, 1952). Later, Moskowitz *et al*. (1956) have reported that 17 estradiol increased cholesterol level only which may due to the increased mass of tissue due to increased protein synthesis under their estrogenic influence as suggested earlier.

Thus, it can be concluded that the hexane extract *F. jaeschkeana* induced significant alternations in the histoarchitecture and biochemical constituents in the female genital tract under the estrogenic influence as a result of which implanting receptacle may become refractive to predispose the blastocysts away from the attachment site. Studies are in progress to confirm this mechanism of action in pregnant rats.

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