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Effects of dihydropalmitinium hydroxide isolated from the roots of *Berberis chitria* on intact/spayed/oestradiol dipropionate/pregnant female gerbils (*Meriones hurrianae* Jerdon)

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Abstract. Ethanolic extract of the defatted roots of *B. chitria* was chromatographed when a compound-I ($C_{21}H_{25}O_5N$) characterised as dihydropalmitinium hydroxide was isolated. Chronic administration of DPH for 20 days reduces the weights of ovary, uterus and vagina in normal cycling gerbils. Atresia of the large follicles and vacuolization of follicular cells was conspicuous. The luteal cells and endometrial glands were shrunken. Vaginal smear showed prolonged diestrous cycle reflecting suppression of estrogenic activity. Simultaneous administration of oestradiol dipropionate and DPH to spayed gerbils failed to maintain the growth of uterus and vagina. DPH treatment to intact/spayed/pregnant gerbils inhibited protein synthesis and decreased the sialic acid and glycogen contents of the genital tract. Serum protein was low whereas serum sialic acid, cholesterol, phospholipids, triglycerides and NEFA did not change. Antioestrogenic activity of the compound is discussed. Abortifacient action of DPH is of great significance in fertility regulation using plant products.

Keywords. Dihydropalmitium hydroxide; follicular atresia; protein synthesis; sialic acid; abortifacient.

1. Introduction

Sterilization has become the single post prevalent method of family planning on an international level. It is estimated that approximately 80 million surgical sterilization procedures had been performed since the early 1950s and majority of these were in females (Green 1978).

Oliver and Boyd (1959) noted that incidence of clinical manifestation of coronary artery disease rises rapidly after surgical sterilization. Patients with bilateral ovariectomy had an excess of coronary atherosclerotia blockage and myocardial infarction (Parrish *et al* 1967). An overall assessment indicate that surgical sterilization is related with high risk of developing coronary heart diseases, that is why researchers still continue to find out safe, effective and non-surgical contraceptive methods.

Large number of medicinal plants have been reported to possess antifertility activity (Kirtikar and Basu 1935; Nadkarni and Nadkarni 1954; Chopra *et al* 1956, 1958).

Kamboj and Dhawan (1981) tested 1086 botanically identified plant materials in 50% ethanolic extract for antifertility testing. Out of this only 42 exhibited anti-implantation activity in females.

The present investigation has been undertaken to study the effect of dihydropalmitinium hydroxide (DPH) isolated and characterised from the roots of *Berberis chitria* with a view to develop a safe oral contraceptive pill from plant source. Estrogenic/antioestrogenic nature of the compound was evaluated in spayed females to pinpoint the mechanism of action.

2. Experimental

Ethanolic extracts of the defatted roots of *B. chitria* was concentrated *in vacuo* and the crude product was thoroughly extracted with dil HCl (5%). The acid-extract after neutralizing with NH_4OH (pH 8–9) was extracted with $CHCl_3$ and the extract was concentrated under reduced pressure. The concentrated extract was chromatographed over neutral alumina column (solvent phase $CHCl_3$; MeOH; 50:50) extracted with $CHCl_3$ when a compound (I, $C_{21}H_{25}O_5N$) was isolated. It was characterised as DPH (IR, H_{NMR} and MS m/e 353 ($M-H_2O$)).

Table 1. Spectral data of I

IR (cm^{-1})	1600, 1570, 1500, 1460–70, 1230, 1060, 930 and 830 cm^{-1}
H_{NMR} (in 8 ppm)	3·9 (3*H*/S), 3·95 (3*H*/S), 4.1 (3*H*, S), 4·15 (3*H*, S), 3·05–3·6 (8*H*, NCH_2S/CH_2S), 7·05–9·2 (4*ArH*), 9·52 (b.s. 1H disappeared on D_2O addition
MS	m/es—353 ($M-H_2O$), 337, 305, 278, 292, 262, 177

3. Material and methods

Sixty cycling and 10 pregnant female gerbils were divided into groups of ten each and were treated as under:

Group I: Vehicle treated females (Distilled water 0·5 ml/alt. day/animal oral).

Group II: DPH (12 mg/alt. day/animal oral for 20 days).

Group III: DPH (12 mg/alt. day/animal oral from 10th day of pregnancy for 10 days) (Presence of sperms in the vaginal smear was considered to be day 1 of pregnancy).

Group IV: Bilaterally ovariectomised animals treated with vehicle alone (0·5 ml DW/alt. day/animal oral on day 5 of ovariectomy for 20 days).

Group V: Ovariectomised females treated with DPH (12 mg/alt. day/animal oral after 5 days of ovariectomy for 20 days).

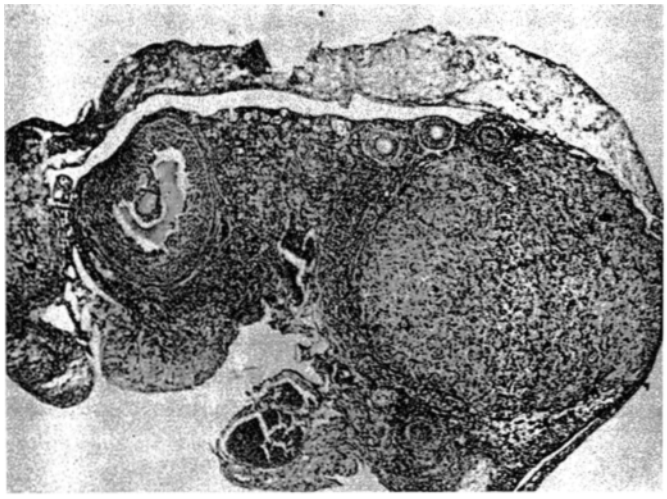
Group VI: 0·02 mg oestradiol dipropionate/alt. day s.c./animal was given after five days of ovariectomy for a period of 20 days.

Group VII: DPH: 12 mg/alt. day/animal oral + 0·02 mg oestradiol dipropionate/alt. day s.c./animal for 20 days.

On the last day of treatment the animals were killed under ether anaesthesia. Ovary, uterus, vagina and adrenal glands were removed and weighed. Right ovary, right uterine horn and a piece of vagina were fixed in Bouin's fluid. 5 μm sections were prepared and examined for histopathological changes. Left ovary, left uterine horn and the remaining part of vagina, liver and adrenal glands were frozen for biochemical estimation of protein (Lowry *et al* 1951), glycogen (Montogomery 1957), sialic acid (Warren 1959) and cholesterol (*cf* Oser 1965).

Blood was taken directly from the heart and serum analysed for lipid metabolism cholesterol (*cf* Oser 1965), triglycerides (Gottfried and Rosenberg 1973), phospholipids (Zilversmit *et al* 1950) and non-esterified fatty acids (Soloni and Sardina 1973).

Serum protein and sialic acid were analysed by routine clinical techniques. 50 corpora lutea at 32 × and 100 lutein cells at 800 × were traced with camera lucida to measure the diameter. Student's *t* test was applied in comparing means.



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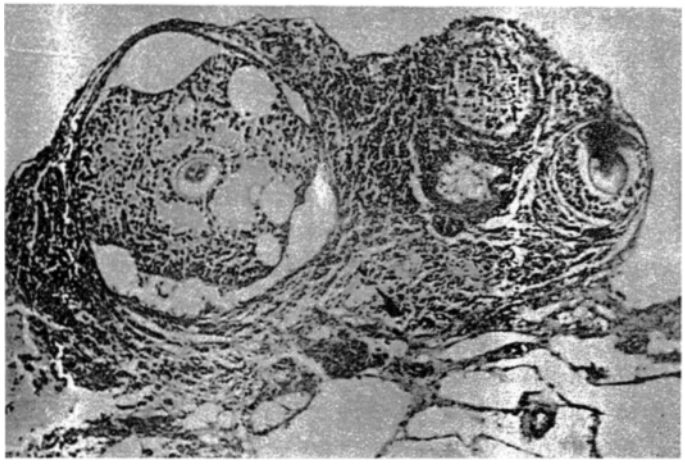

(2)

Figures 1 and 2. **1.** Control ovary showing fully developed graafian follicle with ovum (HE × 100). **2.** Section of the *Barberis* treated ovary. Note the degeneration of ovum and vacuolization of follicle cells (HE × 100).

4. Results

4.1 *Body and organ weights (table 1)*

Non-pregnant females treated with DPH showed significant ($P < 0{\cdot}01$) decrease in the weight of ovary, uterus and vagina.

In pregnant females ovarine weight was significantly ($P < 0{\cdot}05$) reduced. But the weights of uterus and vagina remain unaltered. Adrenal glands were enlarged.

Table 1. Changes in body and organ weights (ovary, uterus, vagina and adrenal glands) of the DPH/oestradiol treated intact/ovariectomised and pregnant female gerbils.

Treatment	Body weight (gms)		Organs weight (mg/100 gm body weight)			
	Initial	Final	Ovary	Uterus	Vagina	Adrenal
Control (Vehicle treatment) Gr I	68 ± 8	69 ± 9	30·3 ± 3·5	109 ± 5	58·4 ± 6·1	56 ± 2
Intact + DPH Gr. II	78 ± 5	76 ± 4	11·5 ± 0·5^{b}	80·4 ± 2·1^{b}	90·2 ± 4·3^{b}	93·8 ± 2^{c}
Pregnant + DPH Gr. III	76 ± 2·3	78·5 ± 3	14·73 ± 2·0*	109·87 ± 3·65^{a}	73·40 ± 3·08^{a}	94·84 ± 1·76^{c}
Ovariectomized Gr. IV	67 ± 7	70 ± 5	—	40·7 ± 4·7^{c}	39·2 ± 5·6^{a}	32·6 ± 4·7^{b}
Ovariectomy + DPH Gr. V	78·8 ± 7·1	80 ± 6	—	95·1 ± 7·2^{h}	93·7 ± 4·5^{i}	92·3 ± 2·4^{i}
Ovariectomy + oestradiol dipropionate Gr. VI	71 ± 7	73 ± 6	—	320 ± 17·3	72 ± 4·8	70 ± 4·8
Ovariectomy + Oestradiol dipropionate + DPH Gr. VII	51 ± 2	59 ± 5	—	224·83 ± 8·79^{n}	129·30 ± 7·19^{n}	98·67 ± 4·52^{n}

*—($P < 0·05$) compared with GI; *b*—($P < 0·01$) compared with GI; *c*—($P < 0·001$) compared with GI; *a*—Non-significant compared with GI; *h*—($P < 0·01$) compared with GIV; *i*—($P < 0·001$) compared with GIV; *n*—($P < 0·01$) compared with GVI; (all figures ± SEM).

4.2 *Histological observations*

4.2a *Ovary:* DPH (12 mg/alt. day/animal) treatment caused atresia of large follicles. Vacuolization of follicle cells was conspicuous. Corpora lutea and lutein cell nuclear diameter were reduced. Corpora lutea: control, $6{\cdot}25 \pm 0{\cdot}05$ μm; treatment, $3{\cdot}8 \pm 0{\cdot}9$ μm; lutein cell nuclear diameter: control, $5{\cdot}9 \pm 0{\cdot}04$ μm; treatment, $4{\cdot}3 \pm 0{\cdot}03$ μm.

4.2b *Uterus:* Endometrium was atrophied and the stromal glands regressed. Myometrial atrophy was severe in spayed females treated with DPH. Simultaneous treatment with estrogen could not prevent the damage.

4.2c *Vagina:* Vagina of the DPH treated females showed prolonged diestrous cycle. Estrogen induced keratinization in spayed females ceased when DPH was administered simultaneously.

4.3 *Biochemical observations*

4.3a *Protein:* Protein contents of the ovary, uterus and vagina were low in DPH treated pregnant and non-pregnant female gerbils (Groups II and III). DPH treatment suppressed the oestrogen induced protein synthesis in spayed females (Group VII, table 2).

4.3b *Sialic acid:* Pregnant and non-pregnant females treated with DPH showed reduced ($P < 0{\cdot}001$) sialic acid contents in the ovary, uterus and vagina (Groups II and III). Further reduction was seen when spayed females were treated with DPH (Group V). Simultaneous administration of oestrogen and DPH in spayed females could not restore sialic acid to normalcy (Group VII) (table 2).

4.3c *Glycogen:* Glycogen contents were low in the uterus and vagina of DPH treated pregnant/non-pregnant and spayed females (table 2).

4.3d *Cholesterol:* No significant change was seen in adrenal gland cholesterol (table 2).

4.3e *Serum analysis:* DPH (12 mg/alt. day/animal) administration to pregnant gerbils bring about a significant lowering in serum protein, whereas no such change was seen in the DPH treated non pregnant females (table 3). Serum sialic acid, cholesterol, phospholipids, triglycerides and NEFA did not show a significant change in all the treated animal groups (table 3).

5. Discussion

DPH (12 mg/alt. day/animal) given to female gerbils on the 10th day of pregnancy showed abortifacient property. DPH caused widespread degenerative changes in the ovary, uterus and vagina. The atretic follicles and shrunken luteal cells indicate the suppression of pituitary gonadotropins. (Dufour *et al* 1979; Arya *et al* 1979).

Clark *et al* (1973) suggested that the antiestrogenic activity of U-11, 100 A was

Table 2. Biochemical changes in protein, sialic acid, glycogen and cholesterol in ovary, uterus and vagina of DPH/oestradiol dipropionate treated intact/ovariectomised/or pregnant female gerbils.

Treatment	Protein (mg/gm)			Glycogen (mg/gm)			Sialic acid (mg/gm)			Cholesterol (mg/gm)
	Ovary	Uterus	Vagina	Uterus	Vagina	Liver	Uterus	Vagina	Vagina	Adrenal
Control Gr. I (Vehicle treatment)	215 ± 8	190 ± 10	219 ± 5·7	3·4 ± 0·01	2·8 ± 0·03	4·10 ± 0·01	7·1 ± 0·3	6·5 ± 0·4	7·7 ± 0·2	31·3 ± 2·3
Intact + DPH Gr. II	135·6 ± 14·3^{b}	138·4 ± 5·31^{b}	152·3 ± 2·7^{c}	1·31 ± 0·02^{c}	1·45 ± 0·08^{c}	9·30 ± 0·05^{c}	2·85 ± 0·2^{c}	2·1 ± 0·05^{c}	2·64 ± 0·05^{c}	32·3 ± 0·3^{M}
Pregnant + DPH Gr. III	—	63·7 ± 1·6^{c}	94·2 ± 1·4^{c}	1·06 ± 0·16^{c}	1·60 ± 0·23^{b}	3·13 ± 0·05^{c}	—	3·10 ± 0·32^{b}	3·25 ± 0·18^{c}	37·2 ± 4·7^{M}
Ovariectomized Gr. IV	—	117 ± 11^{b}	130 ± 11^{c}	1·42 ± 0·01^{c}	1·56 ± 0·03^{c}	3·13 ± 0·02^{c}	—	3·9 ± 0·1^{b}	4·4 ± 0·1^{c}	—
Ovariectomy + DPH Gr. V	—	152·3 ± 2·7^{c}	120·9 ± 2·6^{N}	0·99 ± 0·05^{i}	1·12 ± 0·09^{h}	5·48 ± 0·06^{i}	—	1·99 ± 0·10^{i}	1·74 ± 0·03^{i}	42·2 ± 5·9
Ovariectomy + Oestradiol propionate Gr. VI	—	337 ± 30	138 ± 13	2·3 ± 0·05	2·7 ± 0·01	5·0 ± 0·02		6·3 ± 0·5	4·2 ± 0·2	—
Ovariectomy + DPH + Oestradiol dipropionate Gr. VII	—	179·9 ± 2·6^{l}	217·8 ± 2·1^{l}	4·52 ± 0·28^{l}	4·31 ± 0·66^{p}	9·31 ± 0·27^{r}		2·31 ± 0·16^{l}	1·93 ± 0·003^{r}	31·4 ± 0·5

b—($P < 0·01$) compared with GI, *c*—($P < 0·001$) compared with GI, *M*—Non-significant compared with GI, *g*—($P < 0·05$) compared with GIV, *h*—($P < 0·01$) compared with GIV, *i*—($P < 0·001$) compared with GIV, *N*—(Non-significant) compared with GIV, *j*—($P < 0·05$) compared with GVI, *l*—($P < 0·01$) compared with GVI, *r*—($P < 0·001$) compared with GVI, *p*—Non-significant compared with GVI, Biochemical estimations: means of six determinations.

Table 3. Changes in the protein, sialic acid, cholesterol, phospholipids, triglycerides, NEFA in the serum of DPH/oestradiol dipropionate treated intact/ovariectomised/or pregnant female gerbils.

Treatment	Protein	Sialic acid	Cholesterol mg/100 ml	Phospholipids	Triglycerides	NEFA meq/l
Control (Vehicle treatment) Gr. I	11723 ± 279	34·5 ± 5·8	144 ± 13·5	160 ± 15·0	70·1 ± 6·5	0·259 ± 0·01
Intact + DPH Gr. II	10890 ± 190[c]	28·3 ± 6·6[c]	103 ± 9·5[c]	128 ± 8·0[c]	87·9 ± 8·9[c]	0·229 ± 0·015[c]
Pregnant + DPH Gr. III	8615·4 ± 283[b]	33·1 ± 0·9[c]	115 ± 4·8[c]	—	85·7 ± 2·4[c]	—
Ovariectomised Gr. IV	9529 ± 312[a]	25·0 ± 1·5[c]	127 ± 5·0[c]	115 ± 8·3[c]	86·4 ± 9·0[c]	0·241 ± 0·01[c]
Ovariectomy + DPH Gr. V	9853 ± 93[m]	30·2 ± 2·0[m]	113 ± 1·2[m]	116 ± 12·1[m]	90·7 ± 1·2[m]	0·223 ± 0·012[m]
Ovariectomy + Oestradiol dipropionate Gr. VI	11584 ± 268	36·4 ± 2·7	111 ± 4·8	100 ± 2·4	—	0·206 ± 0·01
Ovariectomy + DPH + Oestradiol propionate Gr. VII	10915 ± 233[n]	30·5 ± 1·4[n]	119 ± 17·8[n]	—	92·9 ± 2·1	0·321 ± 0·01[n]

a — ($P < 0·01$) compared with GI, *b* — ($P < 0·001$) compared with GI, *c* — Non-significant compared with GI, *m* — Non-significant compared with GIV, *n* — Non-significant compared with GVI, Biochemical estimations: means of six determinations.

associated with its ability to inhibit uterine cytoplasmic receptor replenishment. It is possible that DPH could act by a similar mechanism.

Raj *et al* (1981) noted the drastic reduction in the levels of steroids and LH with concomitant reduction in ovarian weight. The reduction in hormonal levels correlates well with the onset of persistent diestrous smear in the DPH treated gerbils. DPH may suppress the luteal function as evidenced by the shrunken luteal cells. Since these are the sites of progesterone biosynthesis, the present finding raises the possibility that DPH may have some potential as an interceptive agent even after implantation.

Increase in uterine weight is commonly accepted as a measure of the estrogenicity of the compound (Jones and Edgren 1973). In spayed gerbils DPH inhibited increase in weight of uterus and vagina induced by oestrogen, reflects the antioestrogenic nature of the compound.

Simultaneous administration of oestrogen to DPH treated spayed gerbils failed to maintain the growth of uterus and vagina (Drasher 1952; Punnonen and Rauramo 1974).

DPH treatment in intact/pregnant gerbils inhibited protein synthesis in the genital tract further reflects the anti-oestrogenicity of the compound (Mohla and Prasad 1969).

Genital tract glycogen is controlled by ovarian steroids (Gregoire *et al* 1973). The decrease in glycogen contents of uterus and vagina after DPH administration to intact/spayed/pregnant gerbils possibly reflects reduced synthesis of ovarian steroids (Bitman *et al* 1965). Coppola and Ball (1965) and Galleti and Gardi (1973) reported that the uterine/vaginal sialic acid concentrations are dependent on ovarian hormone. In the present study sialic acid contents were low in all the treatment groups confirms the antioestrogenic nature of DPH.

In conclusion, DPH has all the potential to be developed as a fertility regulating agent due to its anti-estrogenic/abortifacient action.

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