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Effect of certain indigenous medicinal plants on follicular development and steroidogenesis in rats

S. MEHROTRA[1†], UMASHANKER[2], A.C. MAJUMDAR[3], O.P. PALIWAL[4] AND S.K. AGARWAL[5]

Division of Animal Reproduction
Indian Veterinary Research Institute, Izatnagar - 243 122 (UP)

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ABSTRACT

50% ethanolic plant extracts of *Murraya koenigii* (curry leaf plant) and *Urtica dioica* (Bichchu grass) were given P.O. in doses of 100, 300 and 1000 mg.kg^{-1} during diestrus to estrus in six groups (n=6) of 12-15 week aged female rats against a control group, based on LD_{50} studies. Rats were sacrificed during estrus, based on vaginal smear technique and dissected out to remove both the ovaries. Total number of large surface follicles were counted and ovaries were processed for histological and histochemical studies using standard procedures, to determine embedded follicular population and expression of steroidogenic enzymes i.e. Glucose-6-phosphate dehydrogenase (G_6 PD) and 3 β-hydroxy-Δ^5-steroid dehydrogenase (3βHSD), respectively. Results indicated significantly higher number of large surface and embeded follicles in Murraya 1000 mg kg^{-1} treated group of rats with relatively higher expression of G_6PD and 3βHSD as compared to control and other groups.

Key words : Medicinal plants, follicular development, steroidogenesis, rats

Use of certain plants and their preparations for fertility regulation is well documented in ancient literature. Non toxic nature, cost effectiveness and easy availability make herbal remedies as a viable alternative to hormones which are having limitations viz., high cost, variable reslts, adverse effect on reproductive performance, residual effect in food animals and their products, lack of easy availability and quick assay facilities etc. Herbal remedies having proven their efficacy by standard of both history and modern medicine are now the subject of closer scruitny by the researchers. India as a whole is the richest source of medicinal plants which are distributed in almost all parts of the country (Mohammed Ali, 1994), and holds an excellent potential for adopting phytotherapies. So far, plants, have been mainly screened for their antifertility effects in human beings and only meagre attention has been paid towards identifying plants for fertility augmentation in animals.

Hence, there is a need to explore scientifically the traditional herbal wealth in order to develop some suitable phytotherapies for fertility augmentation in livestock. The reliance on cheap, readily available, efficacious and scientifically proven plant based remedies would add substantially to our national economy. The present study was undertaken with two medicinal plants viz., *Murraya koenigii* (curry leaf plant) and *Urtica dioica* (Bichchu grass), mentioned in traditional practice in order to examine their effects on follicular development and ovarian steroidogenesis in rats.

MATERIALS AND METHODS

Preparation of plant material, acute toxicity studies and determination of LD_{50} is described elsewhere (Mehrotra *et al.*, 2003).

Effect of 50% ethanolic extract of Murraya and Urtica on follicular development in rats : A total number of 42 female rats of 12-15 week age were taken in diestrus phase as determined by vaginal smear technique (Bennet and Vickery, 1970). Animals were divided into seven groups consisting of six animals each. Group I served as control and received equivalent amount of vehicle while group I to VII received 50% ethanolic extracts of Murraya and Urtica @ 100, 300 and 1000 mg kg^{-1} P.O., respectively. During the treatment period (diestrus to estrus) all the rats were examined regularly for cycle stage and sacrificed during estrus by over dosing of chloroform. The rats were dissected out to remove both right and left ovaries and weight of trimmed and filter paper blotted ovaries were taken with the help of electronic balance (Afcoset-India) and recorded separately for individual

[1]Sr.Scientist
[2-5]Principal Scientist
[3]Principal Scientist, Physiology & Climatology Division
[4]Head, Pathology Division

[†]Corresponding author

animal. Follicles protruding from the surface of ovary were defined as large follicles whereas all others as small follicles (Hynes *et al.*, 1996). Total number of large follicles were counted and recorded for both ovaries with the help of stereo-zoom microscope (Wild-Heerburg, Germany). The ovaries were selected randomly from each group and processed for histological and histochemical studies.

Histological studies : Ovaries were fixed in formal saline (10%) and embeded in paraffin wax using standard histological procedure. Serial sections of 5 μm thickness were stained with haematoxylin and eosin. Follicular size was determined by averaging two perpendicular diameters of the follicle and follicles were classified into different classes as described by Barrends *et al.* (1995).

Class I : Small preantral follicles (50-200 μm)
Class II : Large preantral follicle (201-274 μm)
Class III : Small antral follicle (275-450 μm)
Class IV : Large antral follicle (>450 μm)

Total number of follicles were counted in each group and different category follicles were expressed as percentage.

Histochemical Studies

Glucose-6-phosphate dehydrogenase (G_6PD) : Immediately after sacrificing the rats ovaries were frozen at -20°C in cryostat (IEC-Minotome). Frozen sections of 8 micron thickness were cut with the help of microtome. The sections were than taken on grease free slides and covered with G_6PD incubation media (Brandu *et al.*, 1968). The sections were kept at 37°C for 30 minutes for proper colour development which takes place due to formazan deposition. After incubation sections were washed gently with running tap water, air dried and mounted with glycerol.

3-β-hydroxy-Δ^5-steroid dehydrogenase (3BHSD) : Cryostat sections similar to G_6PD studies were made and taken on clean, dry slide followed by treatment with cold acetone (-20°C) for 20 minutes in a cuplin jar to remove lipids. After air drying the sections were poured with incubation media (Wattenburg, 1958) and kept in dark at 37°C for one hour for colour development. Rest of the procedure is similar to G_6PD studies. Enzyme localization and its inensity for both G_6PD and 3BHSD was evaluated subjectively under microscope based on deposition of purple colour formazan granules, among different experimental groups.

Data on ovarian weight and surface follicle population was analysed using one way analysis of variance (ANOVA) and the means for different experimental groups were compared by Duncan's multiple range test. Percent follicular population in histological studies were compared using normal deviate test (Fleiss, 1981).

RESULTS AND DISCUSSION

Effect on follicular development

a) **Large surface follicles** : Perusal of table 1 reveals that while mean ovarian weights didn't show any statistical difference between experimental groups, the mean values of large surface follicle were significantly higher ($P < 0.05$) in group IV animals as compared to control (12.5±0.56 vs 7.83±0.79) indicating favourable effect of Murraya 1000 mg kg^{-1} on large follicle population.

b) **Histological classes of follicles** : Data pertaining to this is presented in Table 2, which depicts that average number of follicles per ovary in group I to VII were 12.2, 10.66, 11.4, 14.4, 11.5, 10.5 and 10.5, respectively and was higher in group IV without any considerable difference between other groups. Group IV animals exhibited significantly lower ($P < 0.05$) percentage of class I and higher ($P < 0.05$) percentage of class III follicles compared to group I (46.47 vs 67.21 and 32.39 vs 13.11%). However, no significant difference was observed in class II and IV follicles between experimental groups. On pooling of class II, III and IV data the follicle

Table 1. Effect of plant extracts on ovarian weight and large surface follicles in rats (Mean±SE)

Experimental group (n=6)		Weight of ovary (mg)	No. of large surface follicle/animal
I	(Control)	30.66±0.88	7.83 ± 0.79^{bc}
II	(Mur 100)	28.93±1.05	7.16 ± 0.65^{b}
III	(Mur 300)	29.65±1.09	9.16 ± 0.70^{c}
IV	(Mur 1000)	29.53±1.32	12.50 ± 0.56^{d}
V	(Urt 100)	31.85±1.19	5.16 ± 0.47^{a}
VI	(Urt 300)	31.71±1.05	7.50 ± 0.42^{bc}
VII	(Urt 1000)	29.88±0.62	7.16 ± 0.60^{b}

Means with different superscripts differ significantly ($P < 0.05$)

Table 2. Effect on follicular

Table 2. Effect on folliculogenesis in rats (histological studies)

Experimental group (n = 6)	No. of ovaries examined	Total no. of follicles counted	No. of follicles/ ovary	Size of follicle (μm)			
				Class I (50-200)	Class II (201-274)	Class III (275-450)	Class IV (>450)
I (Control)	5	61	12.2	41^{a} (67.21)	9 (14.75)	8^{a} (13.11)	3 (4.91)
II (Mur 100)	6	64	10.66	35^{ab} (54.68)	15 (23.43)	12^{ab} (20.31)	1 (1.56)
III (Mur 300)	5	57	11.4	31^{ab} (54.38)	13 (22.80)	11^{ab} (19.29)	2 (3.50)
IV (Mur 1000)	5	72	14.4	33^{b} (46.47)	10 (14.08)	23^{b} (32.39)	6 (8.33)
V (Urt 100)	6	69	11.5	31^{b} (52.54)	11 (18.64)	16^{ab} (27.11)	1 (1.69)
VI (Urt 300)	4	42	10.5	27^{ab} (64.28)	4 (9.52)	10^{ab} (23.80)	1 (2.38)
VII (Urt 1000)	6	63	10.5	35^{ab} (55.55)	12 (19.04)	13^{ab} (20.63)	3 (4.76)

Figures in parenthesis indicate percentage
Percentage with different superscript differ significantly ($P < 0.05$) in a column

population differed significantly ($P < 0.05$) in group IV from that of control.

In rats, follicles that are destind for ovulation in one cycle begin to grow during the previous cycle (Bennet and Vickery, 1970). Butcher and Kirkpatrick-Keller (1984) confirmed the occurrence of recruitment of large follicles for next ovulation between proestrus and estrus in response to increase in plasma FSH. They also suggested the possibility of recruitment of follicles bearing greater number of FSH receptors.

We speculate that initiation of treatment in present study during diestrus might have preponed the FSH surge thereby causing early recruitment leading to enhanced large follicle population during same estrus. In support of this, the data of histological study clearly shows the depletion of class I follicle in Murraya 1000 mg kg^{-1} group with concomitant rise of class III ($P < 0.05$) and IV ($P > 0.05$) follicles compared to control animals indicating rapid turnover in higher class.

Fortune (1994) opined that in rats recruitment of follicles and most atresia occur around the antrum formation i.e. 0.4 to 0.5 mm follicle diameter. Reduction of atresia by FSH surge might be further potentiated by plant's active principles directly also, thereby enhancing recruitment as another possible mechanism of action.

Steroidogenesis : Histochemical localization revealed marked expression of G_6PD and 3BHSD in the form of purple formazan deposit in granulosa cells of preovulatory follicles. In preantral and antral follicles granulosa cells G_6PH was moderately less while 3BHSD could not be demonstrated. Activity of G_6PD and 3BHSD enzymes were present in theca cells, corpus luteum of any stage and interstitial cells of ovary.

Our findings with pattern of G_6PD and 3BHSD in rat ovary are in resemblence with Pupkin *et al.* (1966) and Teerds and Dorrington (1993). Both the enzymes displayed similar pattern of distribution with relatively higher activities in group III and IV animals followed by VII and least in I, respectively. Although the histochemical methods employed do not permit exact quantitation, the relative intensity of staining indicated the differences in endocrine function of ovary, among experimental groups. The higher steroidgenic activity under present study in medium and higher dose of Murraya (300 and 1000 mg kg^{-1}) followed by Urtica 1000 mg kg^{-1} group compared to control indicates primarily estradiol 17-β which could be in response of theca and granulosa

cells to gonadotrophins. Estrogens are potent mitogens in granulosa cells *in vivo* (Richards, 1980), and might have stimulated granulosa cells mitosis in the developing follicles, thereby potentiating the follicular development.

It may be concluded that plant *Murraya koenigii* has potential to augment the ovarian function in terms of follicular development and steroidogenesis in rats.

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