RESEARCH ARTICLE

Effect of the *Murraya koenigii* and *Moringa oleifera* Leaf Extracts on the Ovarian Follicular Population and other Organ Weights in Rats (*Rattus norvegicus*)

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Abstract

The object of the present study was to evaluate the effect of *Murraya Koenigii* and *Moringa Oleifera* plant leaf extracts on ovarian follicular population, body weight and other organ weights in the rats. A total of 42 rats with a mean body weight of 160.05 gms were divided in 7 groups with six rats in each. Group 1, which served as control rats, was administered with 0.5 ml vehicle. Group II,III and IV were administered Murraya Koenigii leaves extract at 100,300 and 1000 mg/kg/b.w. Group V, VI, and VII were administered *Moringa Oleifera* at 100,300 and 1000 mg/kg/b.w, respectively. The mean ± SE values of a number of surface ovarian follicles, small antral follicles, and overall preantral follicles were significantly higher in the Group IV, VI, and VII as compared to the control group that received *Murraya koenigii* at 1000 mg/kg/b.w and *Moringa Oleifera* at 300 mg/kg.b.w. and 1000 mg/kg.b.w. The mean ovarian weight was significantly higher in the Group IV and Group VI and VII groups compared to the control group.

Keywords: Extract, Follicle, Leaf, *Moringa Oleifera, Murraya Koenigii,* Ovary, Organ, Wistar rats.

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Introduction

Medicinal plants *Murraya Koenigii* and *Moringa Oleifera* have been documented in traditional Indian practices to promote animal fertility. *Murraya Koenigii* and *Moringa Oleifera* have been demonstrated individually to augment the reproductive function in laboratory rats, anestrous goats and buffaloes. (Kumar *et al*., 2008, Jondhale *et al*., 2009; Ogunsola *et al*., 2017, Agarwal *et al*. 2018, Zeng *et al*., 2019).

After such herbal treatment, the estrus induction response is reported to be mediated through the increase in the number of medium and large follicles in anoestrus goats (Dutt, *et al*., 2011). The bioactive compounds present in plant extracts play a significant role in modulating mammalian reproductive performance (Agarwal *et al*., 2018). The Phytoestrogens present in Murraya Koenigii modulate steroidogenesis by increasing ovulation rate and decreasing ovarian follicle atresia (Nandini *et al*., 2010) and also due to preponed FSH surge by plant's active principles and thereby leading to enhanced follicle population (Mehrotra *et al*.,2005).

Murraya Koenigii and *Moringa Oleifera* plants have many bioactive compounds (Rajbhar *et al*., 2018; Balakrishnan *et al*., 2020). *Murraya Koenigii* is a member of the Rutaceae family and is known as the "curry leaf tree" (Jain *et al*., 2017), whereas *Moringa Oleifera* is a member of the Moringaceae family and is known as the "drumstick tree" (Jain *et al*., 2017; Rajbhar *et al*., 2018). Moreover, the bioactive compounds present in plant extracts play a significant role in modulating mammalian reproductive performance, which is dose-dependent (Agarwal *et al*., 2018). The object of the present study is to determine the effect of *Murraya Koenigii* and *Moringa Oleifera* plant leaf extracts on ovarian follicular population, body weight, and other organ weights in rats.

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Materials and Methods

Animals

A total of forty-two Wistar albino (*Rattus Norvegicus)* female rats procured from a small animal house, Department of Pharmacology and Toxicology, Veterinary College, Hebbal, Bangalore, were reared and divided into seven groups (Group I, II, III, IV, V, VI, and VII) consisting of six rats in each group. Ethical approval was obtained from Institutional Animal Ethics Committee with NO. VCH/IAEC/2019/100) dated 03/12/2019 to

experiment. All the rats (8 weeks of age) were maintained in Polypropylene rat cages under standard laboratory hygienic conditions, providing balanced laboratory animal feed and water ad *libitum*.

Plant Materials

Fresh *Murraya koenigii* (Curry leaves) *and Moringa oleifera* (drumstick leaves) were obtained from the plants grown in Veterinary College Campus, Hebbal, Bangalore. Dept validated both Forestry and Environmental science plants, University of Agricultural Sciences, Bengaluru (File No. F & ES 5/163-6/2/21). The leaves were shade dried at room temperature and finely pulverized.

Preparation of Extract

The powder prepared from shade dried leaves was extracted directly with 80% methanol using the soxhlet apparatus. Ten gm of leaves powder was mixed with the 200 ml of 80% methanol and ran in the soxhlet apparatus for 72 hours. at 64°C and the extracts obtained in powder form were stored in desicator until further use. The extracts were tested qualitatively for the presence of bioactive compounds, i.e., Tannins and Phenols (black color), flavonoids (Yellow color precipitate), and Phenols (Red color) by applying Ferric chloride test, Lead acetate test, and Dilute iodine test, respectively.

Animal Treatment

Group I (served as a control) rats were administered 0.5 mL of Dimethyl sulphoxide (DMSO, a vehicle) by oral gavaging technique once daily. The methanolic extract of *Murraya Koenigii* was administered at 100 mg, 300 mg, and 1000 mg /kg.b.w. (Group II, II, and IV) and methanolic extract of *Moringa Oleifera* was administered at 100 mg, 300 mg, and 1000 mg /kg.b.w. (Group V, VI, and VII) respectively. The required dose of the plant extract was prepared by dissolving the dried extracted material taken from desiccators in a known quantity of 10% DMSO such that 0.5 mL of the sample containing 100, 300, and 1000 mg of extract respectively was orally administered by gavaging once a day for three consecutive estrous cycles starting from the stage of estrous. The vaginal cytology method described by Nandini et al. (2010) identified different stages of the estrous cycle.

On the day of estrous (10 weeks old), rats were sacrificed under anesthesia induced by injections of ketamine (40 mg/kg b.wt) and xylazine (10mg/kg b.wt). At the end of the experiment, they were dissected. The left and right ovaries, uterus, liver, pancreas, and kidney were collected in the normal buffer formalin (10% Neutral buffered formalin: NBF). Their weights were measured. The ovaries were trimmed and blotted with filter paper, and the weight of the ovaries was recorded with the help of an electronic digital balance. Similarly, weights of other organs were also recorded.

Follicular Population Studies

The total number of surface ovarian follicles of both the ovaries was counted under a stereo zoom microscope. The average of two perpendicular follicular diameters was used to calculate follicular diameter (Barrends *et al*., 1995). The ovarian follicles were categorized as follows: Small preantral follicles: 50-200 µm; Large preantral follicles: >200-270 µm; Small antral follicles: >270-450 µm; Large antral follicles: >450 µm.

Bodyweight and Other Organs Weights

The body weight of the rats was measured before starting of experiments and at the end of experiments before sacrifice. The ovary, uterus, liver, pancreas, and kidney were taken immediately after sacrifice, and mean weights were calculated according to the procedure described by Nandi *et al*., (2006).

Statistical Analysis

Follicular population and other organ weights data between experimental and control groups were analyzed by a computer-assisted statistical software package (Graph pad Prism, San Deigo, USA). Data was analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Significance and non-significance of difference between mean values were determined at 5% level of significance ($p < 0.05$)

Results and Discussion

Phytochemical Tests

The qualitative phytochemical tests (Ferric chloride test, Lead acetate test, and Dilute iodine test) indicated that the plant extracts contain phytochemicals Tannins, Phenols, flavonoids.

Follicular Population

The mean ± SE values of a number of surface ovarian follicles, small antral follicles, and overall preantral follicles were significantly higher in Group IV, VI, and VII that received *Murraya koenigii* at 1000 mg/kg/b.w and *Moringa oleifera at 300* mg/kg.b.w. and 1000 mg/kg.b.w as compared to control group. Among the treatment groups, *Murraya koenigii* at 100 and 300 mg/kg b.w. and *Moringa oleifera* at 100 mg/kg/b.w. was not enough to produce a significant effect with respect to folliculogenesis. The results are presented in Table 1.

The stereo zoom microscopic examination of ovaries revealed that the population of surface follicles on both ovaries increased significantly in Group IV, VI, and VII. This enhanced follicle population was attributed to phytoestrogens present in *Murraya koenigii* and *Moringa oleifera* that potentially increased the growth and development of follicles and decreased ovarian follicular atresia. This reduction of atresia might be due to the FSH surge caused by phytoestrogens. The present study results were in conformity with the reports of Nandini *et al*. (2010).

Table 1: Effect of *Murraya Koenigii* and *Moringa Oleifera* extract on the ovarian follicular population of 10 wk old rats (Values are Mean ± SE of 6 rats in each group)

Follicular population	*Control Group I*	*Group II*	*Group III*	*Group IV*	*Group V*	*Group VI*	*Group VII.*
		Murraya Koenigii extract			*Moringa Oleifera extract*		
Surface follicles	11.3 ± 0.49	12.5 ± 0.34	13.0 ± 0.73	18.3 ± 0.42 **	12.5 ± 0.76	16.5 ± 0.76*	20.6 ± 0.66***
Small PF	46.6 ± 0.88	50.1 ± 1.4	49.3 ± 1.4	73.2 ± 2.8**	52.2 ± 1.7	80.0 ± 1.7**	95.2 ± 2.7***
Large PF	30 ± 1.88	35.17 ± 1.32	36.3 ± 2.17	50.8 ± 1.77**	33.3 ± 2.91	45.0 ± 2.0*	60.17 ± 1.81***
Small AF	25.5 ± 1.47	23.3 ± 2.02	27.3 ± 2.21	48.3 ± 3.14***	32.3 ± 1.62	43.3 ± 1.25***	50.6 ± 3.71***
Large AF	13.3 ± 0.84	14.1 ± 0.60	14.0 ± 0.85	15.3 ± 0.84	13.6 ± 0.95	17.3 ± 0.84*	20 ± 0.81**
Atretic follicle	48.3 ± 0.95	43.0 ± 1.71	46.0 ± 3.38	28.3 ± 1.20***	43.1. ± 1.66	25.5 ± 1.05*	26.3 ± 01.40**

PF: Preantral follicle, AF: Antral follicle

P values (as compared to control): *<0.05, **<0.01, ***<0.001

Table 2: Effect of *Murraya Koenigii* and *Moringa Oleifera* extract on ovarian weight and other organ weight of 10 wk old rats (Values are Mean ± SE of 6 rats in each group)

Organ weight	*Control Group I*	*Group II*	*Group III*	*Group IV.*	*Group V*	*Group VI*	*Group VII*
		Murraya Koenigii extract			*Moringa Oleifera extract*		
Ovarian weight (mg)	75.6 ± 1.47	75.5 ± 2.52	79.5 ± 1.33	99.8 ± 3.11 **	78.3 ± 2.17	95.8 ± 3.91 *	99.3 ± 3.24 **
Uterine weight (mg)	406.2 ± 6.08	395.0 ± 34.03	382.0 ± 7.74	420.0 ± 23.09	383.8 ± 17.28	396.7 ± 21.55	397.2 ± 39.2
Liver weight (g)	6.2 ± 0.79	6.6 ± 0.66	6.5 ± 0.67	7.0 ± 0.68	5.8 ± 0.54	6.8 ± 0.79	7.3 ± 0.76
Pancreas weight (mg)	550.0 ± 18.44	570.8 ± 10.52	575.3 ± 11.75	577 ± 22.8	579.2 ± 13.69	555.7± 17.86	562.3 ± 21.13
Kidney weight (mg)	670.0 ± 13.39	690.3± 8.56	686.2 ± 14.24	687.5 ± 10.94	676.7 ± 14.59	695.8± 4.97	693.8 ± 8.42

P values (as compared to control): *<0.05, **<0.01, ***<0.001

Body and Other Organs Weights

The mean initial and final body weights of the rats were found to be non-significant amongst groups. The mean ovarian weight was significantly higher in Group IV and Group VI and VII compared to the control group (Table 2). The improvement in the mean ovarian weight might be due to the cumulative effect of bioactive compounds present in the plant extracts Kaempferol and Quercetin (Santos *et al*., 2019 and Santini *et al*., 2009). No significant difference was observed between the mean uterine, liver, pancreas, and kidney weights compared to control groups.

Conclusion

The *Murraya koenigii* and *Moringa oleifera* are the potential plants that can cause the significant positive role in follicular development, folliculogenesis, and desired effects on the development of ovaries.

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