Effect of *Andrographis paniculata* Wall. ex Nees root extract fractions on estrogen, FSH, LH, progesterone and ovary of female albino rats, *Rattus norvegicus*

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The fractions (F) 3 and 4 derived from the root extract of *Andrographis paniculata* Wall. ex Nees were used in the present investigation to study the contraceptive action of the plant on albino rats. Rats were treated with 500 mg/kg body weight/day of fraction 3 and 4 of the extract for a period of 48 days. The total body and ovary weight, levels of hormones, such as estrogen, LH, FSH and progesterone, histological changes in the ovary and uterus were observed. There was no remarkable change in total body weight of *A. paniculata* treated rats, whereas the size of the ovary was reduced in treated rats as compared to control. The levels of LH, FSH, estrogen and progesterone were found to be raised in treated groups when compared with control and there were remarkable changes noted in the histology of ovary and uterus of experimental rats. The obtained results suggest that the presence of active principle in the fractions 3 and 4 of *A. paniculata* might be responsible for the contraceptive efficacy of the plant extract. These alterations are possibly due to the interference of secondary metabolites of the plant on the physiology of the sexual cycle of female rats.

**Keywords:** *Andrographis paniculata*, Creat, Follicle Stimulating Hormone, Luteinizing hormone, Progesterone, Estrogen, Ovary, Uterus.

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**Introduction**

Medicinal plants have been utilized for many ailments since the earliest days of mankind. The traditional medical practitioners have expertise in using herbal medicine for curing the diseases and for controlling various physiological activities like birth control. The whole medicinal plant or their byproducts have always provided an exciting opportunity to develop new therapeutic agents for different ailments. Some plants have also been reported to exhibit a contraceptive property and inhibition of the implantation activity due to a disturbance in oestrogen-progesterone balance\(^1\),\(^2\). There are numerous ways in which herbs are used to disrupt fertility, some herbal preparations impress upon the uterus or sometimes block and even disturb the production of hormones\(^3\). The cold water infusion of the roots of *Lithospermum ruderale* Douglas ex Leh.\, taken as a drink for a period of six months, induced sterility thereafter. Experiments with rats showed that *L. ruderale* prolonged diestrus period, thus reduce or block estrous\(^4\). Several plant products inhibit male and female fertility and may be developed into contraceptive\(^5\). Females fed a diet with *Lithospermum* developed atrophy or atresia of their sex organs without a decrease in body weight or change in the growth of the pituitary or thyroid\(^6\). The administration of *Curcuma longa* L. extracts showed significant increase in the estrogen level and induce inhibitory effect on FSH and LH resulting in failure to ovulate. But there are few investigations on the effect of major active principles andrographolide and neoandrographolide of *Andrographis paniculata* Wall. ex Nees on hormone and reproductive status of the female rat. Therefore, in the present study an attempt has been made to record the effect of isolated fractions 3 and 4 from the root of this plant on reproductive hormones and ovary of female rats.

**Materials and Methods**

The plant *A. paniculata* (Plate 1a & b), commonly known as Creat specimens were collected from Kolli Hills (Namakkal District, Tamil Nadu) during February-June 2011. After the taxonomical identification, the roots were cut into small pieces and well rinsed with distilled water for 3 to 4 times. The cleaned roots were shade dried under a roof then

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root pieces were powdered using mechanized grinder. The root powder (500 g) was subjected into Soxhlet extractor using methanol and then the extract was concentrated in a rotator evaporator. The extract (30 g) was subjected to column chromatography on silica gel cleaned with mixture of chloroform: methanol of increasing polarity. The maximum concentration of fractions 3 (F3) and 4 (F4) were collected in separate tubes and concentrated in a vacuum evaporator.

Animals

Female albino rats (Rattus norvegicus), 3-4 months old and weighing better (140 to 160 g) with proved fertility were properly marked and six number of animals were housed in a polypropylene cages under controlled environmental conditions of humidity and under 12 h light and 12 h dark conditions. The temperature was maintained at 28±1°C. They were fed with standard pellet feeds and water ad libitum. Vaginal smear of each rats were obtained daily and rats with normal estrus were selected for the treatment. The animals were used as per the IAEC guidelines.

Experimental design

Rats were divided into 3 groups containing 6 animals in each group. A group was maintained as control and the remaining groups II and III were administered orally 500 mg/Kg body weight of the fractions 3 and 4 by orally using intragastric tube for 48 days daily. The 500 mg/Kg body weight/day of fractions 3 and 4 were selected as an effective concentration based on the tentative experiments. The direction of the treatment was based on the estrus cycles. Ovaries were dissected out and freed of extra deposition by washing with saline solution for further morphological observation and analysis.

Hormone assay

Blood samples were collected by cardiac puncture. The serum was separated out and was used to estimate the levels of estrogen, LH, FSH and progesterone concentrations by Chemi Luminescent Immuno Assay (CLIA) and Enzyme Linked Immunosorbent Assay (ELISA).

Histology

Small pieces of ovary were fixed in 10% neutral buffered formalin and processed for embedding in paraffin. Sections of 5 to 6 µm were cut and stained with hematoxylin and eosin for histological analysis as per standard approved procedure.

Statistical analysis

The obtained results were treated with statistics and calculated the mean ± SD values.

Results and Discussion

Effect on size of the ovary

No change was observed in the development of reproductive organs such as ovary and uterus in the control. But in treated rats with F3 and F4 fractions of root extract exhibited a drastic reduction in the growth of the ovary and uterus (Plate 2). The size of the ovary comparatively was not developed as in that of control rats. The administration of root extract
fractions (F3 and F4) of *A. paniculata* remarkably inhibited the growth of the ovary and uterus in experimental rats. The significant alterations in the ovary and uterus might be due to the inhibitory effect on metabolites of the fractions on the hormonal pathway of experimental animals as earlier reported that the leaf extract of this plant possesses reproductive inhibitory activity and it is attributable to poor development of ovarian tissues\(^{12}\).

**Hormonal changes**

A significant alteration was accounted in the concentration of steroid hormones such as serum estrogen, LH, FSH and Progesterone. In control rat, the concentration of estrogen (6.0 IU/mL), LH (3.50 m IU/mL), FSH (2.10 m IU/mL) and the progesterone (0.20 ng/mL) were estimated. But in F3 and F4 fractions administered rats for 48 days showed a significant increase in the level of estrogen, LH, FSH and progesterone at 266.6, 108.5, 123.8, 600.0 and 333.3, 114.2, 147.6, 775.0%, respectively in F3 and F4 than control (Figs. 1 & 2). The estrogen and progesterone were drastically raised than the control in F3 and F4 treated rats (Table 1). Plant estrogens are known to inhibit enzymes involved in steroidogenesis\(^ {13}\) and cause significant changes in the reproductive physiology to lead infertility. The reproductive and general metabolic effects in mature and immature rats are manipulated with the ingestion of phytoestrogenic substance\(^ {14}\). Similarly, *Lithospermum officinale* L. and *Lycopus virginicus* L. proved to be more potent than the conventionally used potassium iodide (KI) in blocking hormonal secretion\(^ {15}\). All the hormones were remarkably elevated in rats administered with fractions 3 and 4, particularly the levels of estrogen and progesterone were found to be highly increased than in the case of the control rats, which could be due to the modulation in the synthesis of steroid hormones as a result of presence of the active principle in the F3 and F4. The changes in FSH level in the proestrus and estrus stages in the extract administered group

[Image of graphs and tables]
indicate the disturbance of estrous cycle and ovulation process through the suppression of FSH. A high dose of ethanolic and aqueous extract of Carum carvi L. has been shown to cause a reduction in the concentration of LH, FSH and Progesterone hormones. Further, it is reported that the administration of the extracts showed a significant increase in the estrogen level and induce inhibitory effect on FSH and LH resulting in failure to ovulate. Phytoestrogens have noxious effect leading to impaired fertility in domestic animals as well as disturbance of the normal gestation process. Weak relative binding capacity of the phytoestrogens can have significant hormonal effects. The root extract of Mimosa pudica L. has antifertility effect as it prolongs the estrous cycle and disturbs the secretion of gonadotropin hormones in albino mice. Phytoestrogens may have effects on the reproductive system and in particular that they possess estrogenic activity.

Effect on ovary architecture

In control rats the ovary revealed a normal architecture exhibiting various stages of follicles along with the Graffian follicle. The primary and secondary follicles were appeared with a distinct nucleus. All the follicles showed the normal features such as intact oocytes, antral follicle and zonal pelucidal (Plate 3). However, the entire ovary was found to be shrunken and the primary and secondary follicles did not appeared distinct with nucleus in rats administered with the fraction 3 of root extract (Plate 4). The antral follicle was highly disintegrated without Oocyte. On the other hand the different stages of the follicles were not found in the animals treated with the fractions 4 of the plant extract and few follicles without Oocyte were also observed. No primary and secondary follicles were seen (Plate 5) as in the case of control rat. These changes might be due to inhibitory effect of the extract. Similarly, phytoestrogens have been shown to interfere in estrogen negative feedback by binding to estrogen receptors in anterior pituitary or hypothalamus and indirectly alter ovarian steroidogenesis. The Aspilia africana (Pers.) C. D. Adams. leaf extract was found to cause inflammation of the fallopian tube, degeneration in the ovarian cortex, in stroma cells of the ovary and disruption of endometrium of the uterus.

Plate 3—Ovary of control animal with normal architecture

Plate 4—Histopathological changes in the ovary of rat treated with A. paniculata leaf extract fraction F3

Plate 5—Histopathological changes in the ovary of rat treated with A. paniculata leaf extract fraction F4
The uterus of the control rats exhibited normal features (Plate 6) having a single layered columnar epithelial cell with elongated nuclei at the base of the cells, highly folded epithelial lining and endometrial glands. The uterine endometrium of fraction 3 of extract treated animals revealed some degree of inhibition of endometrial development as evidenced by poor development of endometrial epithelium and endometrial glands. These uterine changes were effective in causing disintegration of endometrial tissues in the uterus and the rat’s uterine wall showed highly disintegrated cuboidal cells without nuclei in all the cells especially at apical and middle aspects of the cells (Plate 7). Similar changes were observed in the endometrial epithelium of uterine wall of animals treated with fraction 4. The endometrial epithelium disorganized and contains many degenerating epithelial cells with many vacuoles. The columnar epithelial cells exhibit the presence of the degenerating materials (Plate 8). Single layered columnar epithelial cell with elongated nuclei at the base of the cells, highly folded epithelial lining, and endometrial glands were observed in the F3 treated rats. This might be due to antiestrogenic activity of F3 of the plant *A. paniculata* leaf extract. The uterine endometrium of the extract treated animals revealed some degree of inhibition of endometrial development as evidenced by poor development of endometrial epithelium and endometrial glands. The extract of *Plumbago rosea* L. leaves treated rat’s endometrial epithelium consisted of spindle-shaped cells with basal nuclei and dilated the endometrial glands. The stroma consisted of loose and edematous fibroblast-type cells with edema. In F4 of the plant extract treated rats uterine showed highly disintegrated cuboidal cells without nuclei in all the cells especially at apical and middle aspects of the cells. The methanolic extract of *Abrus precatorius* L. seeds have been shown to cause reversible alterations in the estrous cycle pattern and completely blocked ovulation in Sprague-Dawley rats. Administration of alcoholic extract of Neem flower disrupted the estrous cycle in Sprague-Dawley rats and caused a partial block in ovulation.
Conclusion

The present results clearly indicate that the oral administration of 500 mg/kg body weight/day of fraction 3 and 4 of root extract of *A. paniculata* for a period of 48 days significantly changed the total body and size of the ovary, levels of hormones, such as estrogen, LH, FSH and progesterone, histological changes in the ovary and uterus. These changes suggest that the presence of active principles in the fractions 3 and 4 of this plant possess more contraceptive efficacy.

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