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Antifertility Effect of Alcoholic P.nigram Fruit Extract on Adult Female Wistar Rat Models

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***Abstract*— In this research work effect of P.nigram fruit extract on the fertility, microscopic structure of ovary and the associated changes in the serum levels of female reproductive hormones in mature female albino rats had been observed. The animals were divided in different group's control and treated. The treated group further subdivided into animals treated with low and high dose respectively.The animals were administered with low (0.6ml/animal/day) and high dose (1.2ml/animal/day) of alcoholic fruit extracts of P.nigram orally to sixty days .At the end of the experiment the animals were sacrificed and ovary collected for histological and serological studies. Microscopic sections of the ovary revealed decrease in the number of follicle. Significant changes observed in the level of reproductive hormone.**

***Index Terms*—Antifertility, Fertility, Ovary, Follicles**

I. Introduction

Medicinal Plants have increasingly become an integral part of the human society in combating various diseases since the dawn of civilization. Piper nigrum which is commonly known as black pepper Black pepper is a flowering vine in the family Piperaceae.It is mostly cultivated for its fruit which is usually used as a spice and seasoning.Friuts of this plant were used in asthama,bronchitis,fever,arthritis,cough etc in ayurveda. The genus piper belongs to piperaceae has over 700 species distributed in both hemisphere. The piper species have high commercial, economical and medicinal importance. Plants belonging to genus piper are reputed in the Indian ayurvedic system of medicine for their medicinal properties. They are erect or scan dent herbs, shrubs or infrequent tree. The fruit, known as a peppercorn when dried, is approximately 5 millimeters (0.20 in) in diameter, dark red when fully mature, and, like all drupes, contains a single seed. The growing population always drew attention for a reliable and safe contraceptive of plant origin. The objective of this study was to investigate the antifertility potential of alcoholic extract of P.nigram on female wistar rat model.

II. Materials and Methods

A. Plant Collection and Extract Preparation

The dry fruits of P. nigram were collected from the local market of Jaipur, Rajasthan.Then they were throughlly washed in distilled water and the surface water was removed by air drying under shade. The fruits were subsequently dried in a hot air oven at 400c for 48 hours. Further they were powdered and used for extraction.

B. Preparation of Crude Extract

100gms of dry powdered fruits of Piper nigrum were extracted successively with double distilled water, Methanol (400ml.) for 10-12 hrs through double Soxhlet apparatus method. Then collected solutions were filtered through Whatman No-1 filer paper. The extracts were evaporated to dryness under reduced pressure at 900C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition further use.

C. Animals and Treatments

In this experimental study matured female wistar rats (130-150gms) were used.The animals of different groups administered with low (0.6ml/animal/day) and high dose (1.2ml/animal/day) respectively for 60 days. The animals were grouped into two parts and each part into two subgroups having four animals each. The animals were provided with rat pellet and water ad libitum throughout the period of the experiment. The control group received the same volume of distilled water. The extract was administered orally using sterilized oral needle during the experiment.

D. Histological studies

The tissues of the ovary were collected after the sacrifice of the animal by heart puncture method and fixed in fresh Bouin's fluid and embedded in the paraffin wax. They blocked in paraffin and cut horizontally at 5µ thickness and stained with eosin and hemotoxyline and further observed in light microscope for any histological changes. Ovary of untreated control (Fig-A) showed normal histological features. The ovary of treated at low dose (Fig-B) showing mature follicle, secondary follicle and degenerative follicle. The histological section of ovary treated at high dose (Fig-C) showing secondary follicle, degenerative follicle but no mature follicle.

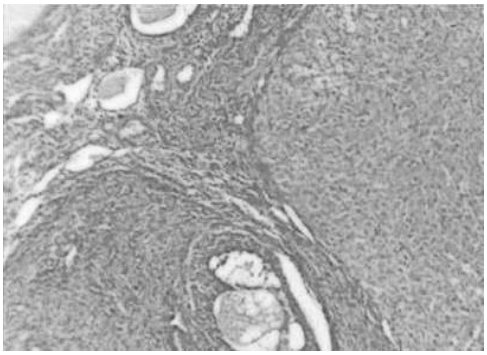

Fig-A

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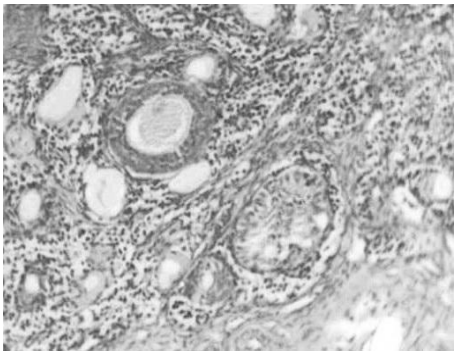

Fig-B

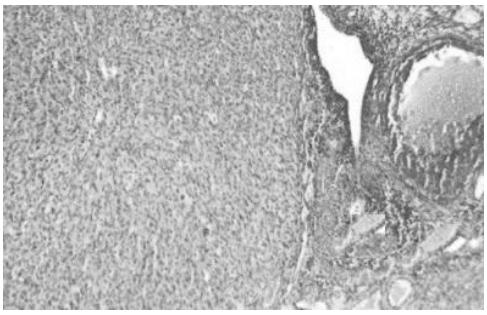

Fig-C

III. RESULT

A. Hormonal Analysis

Blood removed from the animal by cardiac puncture method was collected and centrifuged at 2000rpm to separate the serum for the measurement of FSH, LH, Progesterone and estradiol.The quantitative determination of hormones was done by Enzyme- linked- immunosorbent -assay (ELISA).

Female reproductive hormones

At low dose

B. Follicle stimulating hormone (FSH)

Mean serum concentration of FSH of female rats in group which is orally treated at low dose was 62.11±1.33 (Iu/l) while the FSH concentration in untreated /control rats 36.36±4.41(lu/l).The FSH level in treated rats was significantly high(P < .001)compared to untreated female rats.

C. Estradiol

Mean serum concentration of estradiol of female rats in group which is orally treated at low dose was 22.87±3.25 (Iu/l) while the estradiol concentration in untreated /control rats 37.03±1.16 (lu/l).The estradiol level in treated rats at low dose was statistically significant (P < 0.05) compared to untreated female rats as it is about 55% lower in concentration.

D. Leutinizing hormone

Mean serum concentration of LH of female rats in group which is orally treated at low dose was 38.87±1.52 (Iu/l) while the LH concentration in untreated /control rats 39.89±0.92(lu/l).The LH level in treated rats at low dose was statistically non significant(P < 0.05)compared to untreated female rats.

E. Progesterone

Mean serum concentration of progesterone of female rats in group which is orally treated at low dose was 27.82±3.73 (ng/ml) while the progesterone concentration in untreated /control rats were 37.93±1.03(ng/ml).The progesterone level in treated rats at low dose was 53 % lower and statistically significant(P < 0.05)compared to untreated female rats of this group.

At high dose

F. Follicle stimulating hormone (FSH)

Mean serum concentration of FSH of female rats in group which is orally treated at high dose was 50.79±1.03 (Iu/l) while the FSH concentration in untreated /control rats were 34.18±1.43(lu/l).The FSH level in female rats treated dose is observed 42% higher than the untreated ones and considered significantly high (P < .001) statistically.

G. Estradiol

Mean serum concentration of estradiol of female rats in group which is orally treated at high dose was 35.87±1.24 (pg/ml) while the estradiol concentration in untreated /control rats 53.52±1.77 (pg/ml).The estradiol level in treated rats at high dose was statistically highly significant (P < 0.001) compared to untreated female rats as it is about 31% lower in concentration.

H. Leutinizing hormone

Mean serum concentration of LH of female rats in group which is orally treated at high dose was 25.45±1.37 (Iu/l) while the LH concentration in untreated /control rats was 14.33±1.45 (lu/l). The LH level in female rats treated at high dose was observed 60% higher than the untreated ones and considered significantly high (P < .001) statistically.

I. Progesterone

Mean serum concentration of progesterone of female rats in group which is orally treated at high dose was 18.75±2.37 (ng/ml) while the progesterone concentration in untreated /control rats were 31.43±1.57 (ng/ml). The progesterone level in female rats treated at high dose was observed 35.50% lower than the untreated ones and considered significantly high (P < .001) statistically.

J. Statistical Analysis

Student's t test was used to analyse the data. Values were considered significant at P<0.05.The mean number of mature follicles in female treated at low dose were 1.47±0.37 and statistically significant (P<0.05).The mean number of mature follicles at high dose were 0.57±0.83 and statistically highly significant (P<0.001).

Comparison of Mature Follicles in The Ovary of Females Treated Groups

Treated at Low dose	1.47±0.37	P<0.05	significant
Treated at high dose	0.57±0.83	P<0.001	Highly significant
Control Group	3.8±0.357		

IV. Discussion

The histological sections of the ovaries treated at low dose were not as much affected as by the high dose treatment. Ovaries sections at low dose have shown all types of follicle including primary, secondary and mature follicles. The germinal epithelium, stromal organization and vascularity do not shown any change at this dose. Progesterone, Estradiol and LH are lower in the treated animals compared to the control ones.FSH concentration was higher in both the animals treated at low and high dose. It reflects the fact that alcoholic extract of P.nigram affect the maturation and growth of follicles in the ovary. The excess release of FSH might be due to the result of decreasing estrogen level. Statistically difference in the number of mature follicles was significant (P<0.05). The above data's micrograph and serological studies suggested that alcoholic extract had some antifertility potential in female wistar rat models.

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