

# Histomorphological Effect of Alcoholic *P.nigrum* Fruit Extract on Wistar Rat Models

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**Abstract**— Antifertility effect of alcoholic extract of *P.nigrum* fruit on male and female wistar rat model were investigated. The alcoholic fruit extracts of *P.nigrum* were administered orally to different groups of animals and further their effect on histology of ovary and testes were observed. In this research work different group of animals were administered with low (0.6ml/animal/day) and high dose (1.2ml/animal/day). After the completion of the experiment histomorphology of ovary shown decrease in follicular growth observed at low and high dose respectively compared to control ones. Testes histology revealed that the seminiferous tubular thickness at high dose decreases in diameter supporting the antifertility effect of extract.

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

## I. INTRODUCTION

The way global population rapidly increasing has turned the attention of associated reproductive health programmes worldwide towards the safe and reliable method/contraceptive that can be used to limit family size. Evaluation of herbs for antifertility effect has been in progress worldwide for several decades to identify relatively cheap widely available, widely accepted and effective contraceptive of plant origin that is non-toxic, non-hormonal, non-invasive in administration and relatively long lasting for control of population explosion. Medicinal plants in India have been screened for contraceptive potential and anti-fertility effect, since the country is always been concern about population explosion.

Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. They are erect or scandent

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 objective of this study was to investigate the antifertility potential of alcoholic extract of *P.nigrum* on male and female wistar rat model.

## II. MATERIALS AND METHODS

### [1].Plant Collection and Extract Preparation

The dry fruits of *P. nigrum* were collected from the local market of Jaipur, Rajasthan. Then they were thoroughly 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 40<sup>0</sup>c for 48 hours. Further they were powdered and used for extraction.

### [2].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 filter paper. The extracts were evaporated to dryness under reduced pressure at 90<sup>0</sup>C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition.

### [3].Animals and Treatments

In this experimental study matured male and 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 divided in different groups (A) treated female with treated males,(B)untreated females with treated males,(C)treated females with untreated males and(D) untreated females with untreated males (Control).Each Group contains four animals. They left in the same cage for a period of six weeks. They were provided with rat pellet and water ad libitum throughout the period of the experiment. All the doses used were therapeutic doses calculated for the weight of the rats. The control group (C) received the same volume of the vehicle (distilled water) alone. The extracts were administered orally using separate sterilized oral dosing needle for a period of sixty days. There were also two recovery groups for each of the test or treated groups.

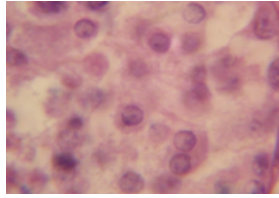
### Histomrphometric study

After the completion of the experiment the animals were sacrificed by heart puncture method. The testes and ovary from both control and treated groups were dissected out for further studies. The tissues of testes and ovary were fixed in fresh Bouin's fluid and embedded in paraffin wax. Section of 5  $\mu$ m thick were cut and stained in haematoxylin and eosin. The tissue sections were observed under a light microscope (x400 objective) for histomorphometric changes.Seminiferous tubular diameter was measured. The primary, secondary and mature follicles were also observed in microsection of ovary.

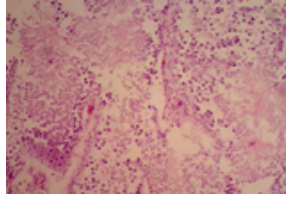
## Histomorphological Effect of Alcoholic *P.nigram* Fruit Extract on Wistar Rat Models

### Figures

#### I. Histomicrograph of rat's testes at different doses

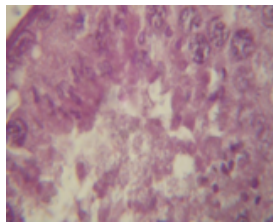


**Fig-A**



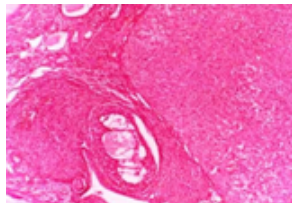
**Fig-B**

**Fig-A** The histomicrograph of rat's testes at control showing seminiferous tubules. **Fig-B** The histomicrograph of rat's testes at low dose showing seminiferous tubular structure without necrosis. **Fig-C** The histomicrograph of rat's testes at high dose showing necrosis in tubular structure

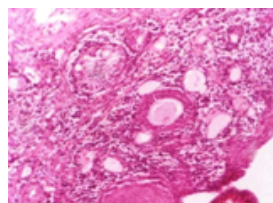


**Fig-C**

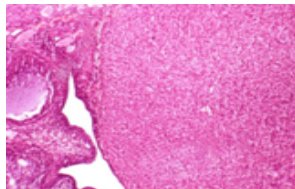
#### II. Histomicrograph of rat's ovaries at different doses



**Fig-D**



**Fig-E**



**Fig-F**

**Fig A:** The histomicrograph of ovary of control animal showing normal primary and secondary follicles. **Fig B:** the histomicrograph of ovary at low dose showing presence of primary, secondary but lacking mature follicles **Fig C:** The histomicrograph of ovary at high dose showing reduction in the number of follicles as well as atresia.

Comparison of mature follicles in the ovary of females treated groups

### III. RESULT

Histomorphologic studies of ovary in control shows normal follicular growth. At high and low dose treated female ovary there were decrease in the number of follicles observed as they step towards maturation. The histology of control male rat's testes showing the spermatozoa in seminiferous tubular lumen whereas in the case of treated males at low dose does not show any remarkable change in histomorphology. The seminiferous tubular lumen shows decrease in diameter in the

|                         |           |        |                    |
|-------------------------|-----------|--------|--------------------|
| Treated at Low dose     | 1.47±0.37 | <0.05  | significant        |
| Treated at high dose    | 0.57±0.83 | <0.001 | Highly significant |
| Untreated/Control Group | 3.8±0.357 |        |                    |

Comparison of seminiferous tubular diameter in rats testes (µm)

|                         |                |        |                    |
|-------------------------|----------------|--------|--------------------|
| Treated at Low dose     | 16.13µm±0.45µm | <0.05  | significant        |
| Treated at high dose    | 13.5µm±0.57µm  | <0.001 | Highly significant |
| Untreated/Control Group | 20.19µm±0.56µm |        |                    |

animals treated at high dose. The mean seminiferous tubular diameter in male rats treated at high dose was 13.5µm±0.57 µm where as the mean seminiferous diameter in untreated male rats at high dose was 20.19µm±0.56 µm when observed in the microscope. This difference in the seminiferous tubular diameter in treated male rats at high dose and untreated male rats is highly significant (P<0.001) statistically. The mean number of the mature follicles in the female rats treated at high dose was 0.57±0.83 while the number of mature follicles in untreated female rats was 3.8±0.357 when observed under the microscope at 4x. The difference in the number of the mature follicles is significant (P<0.05) statistically.

### DISCUSSION

This antifertility effect is very much supported by the histological section of male testes which have shown decrease in the diameter of seminiferous tubules and most of the tubules were not showing spermatozoa in the tubular lumen. In the females the failures in the follicular development at both low and high dose of extract also support the antifertility effect. Statistically difference in the number of mature follicles was significant (P<0.05) where the difference in tubular diameter statistically highly significant (P<0.001) All the above data's and micrograph suggested that alcoholic extract had some antifertility potential in both male and female wistar rat models.

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