CHAPTER TWO
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2.1. Material :

2.1.1. Selection of animals :

Healthy male albino rats (Wistar strain) procured from Hindustan Antibiotics, Pune, were selected as experimental animals to study the effects of Agnus castus. The selected rats were 2 - 3 months old, weighing 160 - 180 gms.

2.1.2. Maintanance of rat colony :

The white rat colony was maintained in animal house of the department, for record and surgery, cage cleaning and storage of food, separate rooms were reserved. The room in which experimental animals were kept was maintained at about 24° C. Exhaust fans were attached to the room to decrease odours and moisture generated by the rats. The room was well protected against the entrance of wild rats, cockroaches and any other animal which may carry infections. The rats were maintained in the animal cages. All the cages were thoroughly cleaned every alternate day with disinfectants, air dried and then rats were transfer to them. The cages were arranged on clean racks which were also cleaned every alternate day. Each rats was kept in separate cage. Rats were weighed at regular intervals of a week.

Rats of particular weight were taken for experimental observations hereafter described. The rats were supplied with water ad-libitum. Dry food pellets (Lipton India Ltd.) were used as food for rats.

The rats were divided into two groups - control and experimental. All the rats were allowed for 15 days quarantine period before the start of the experiment.

2.1.3. Plant material :

Mother tincture of berries of Agnus castus, which was used for the present investigation was procured from Dudhedia Homoeo Pharmacy, Harigaon, Maharashtra, India.

2.2. Methods :

2.2.1. Preparation of Drug :

One part of Agnus castus mother tincture was mixed with one part of double distilled water. The drug was prepared everyday just before the injection.

2.2.2. Dose and duration :

Experimental male albino rats of proren fertility were injected with 0.5 ml of the drug intraperitoneally (about 125 -

150 mg/kg. body weight/day); while control rats were injected with equal quantity of double distilled water for 5 weeks. After every week of interval 3 animals were sacrificed by cervical dislocation; 24 hours after the last dose of the treatment. All the reproductive organs were dissected out, blotted dry of blood, cleared off fat and weighed. Tissues were processed as per the requirement of the parameters.

2.2.3. Weight :

The rats of both the groups were weighed at regular intervals of a week; these body weights were recorded. Similarly weights of various reproductive organs, from each animal, after dissection were recorded.

2.2.4. Histology :

Small pieces of tissues were fixed in Bouin's fluid for 24 hours. Tissues were well washed in running water, dehydrated through ethanol grades, cleared in xylene and embeded in paraffin wax. The sections were cut at 6 - 7 μ. These paraffin sections were stained with Haematoxylene - Eosin (HE) technique for histological examinations in testes and accessory reproductive organs.

The spermatogenes is follows a seminiferous epithelial wave (Perey et al., 1961) which constitutes a series of cell associations. Two main classifications have been established for the spermatogenesis in rat for these cell associations, which are known as RG method (Roosen Runge and Giesel, 1950) and LC method (Leblond and Clermont, 1952 a; 1952 b). The RG method subdivided the process of spermatogenesis into eight stages while LC method subdivided into fourteen stages. A very high resolution optical microscopy is essential to observe clearcut differences in these stages, so instead of 8 or 14 stages used in RG method and LC method, it appeared more practical to study spermatogensis and plant extract induced alteration with reference to different types of spermatogenic cells, sertoli cells as well as in leydig cells.

2.2.5. Tubular diameter :

Morphological study of seminiferous tubules was carried out with the help of oculometer (monocular microscope) Diameters of these tubules were calculated. The measurements were recorded in mm and finally they were converted into μ m - Tubular diameter of 100 tubules was recorded and the mean value was calculated.

2.2.6. Feulgen technique :

In this investigation chromatin was demonstrated histochemically in fixed tissue sections by the Feulgen method (Feulgen, 1923; Feulgen and Rosenbeck, 1924).

a) Fixative :

Tissues were fixed on Cornoy's fixative which was prepared by adding ethanol, chloroform and acetic acid in the proportion of 160:30:10.

b) Reagents :

1)Schiff's reagent :

It was prepared and kept in a refrigerator. Before using it was allowed to reach room temperature.

2)Rinsing solution :

0.5 % aqueous potassium metabisulfite prepared fresh from a 10 % stock solution.

c) Procedure :

1) Small pieces of tissues were fixed in Cornoy's fixative for 18-21 hours. (2) Kept in double distilled alcohol for one hour. (3) Cleared in xylene and paraffin sections of 5-6

were obtained. (4) After dewaxing and hydration sections were brought to water. (5) Rinsed in cold 1 N HCl. (6) Sections were hydrolysed in preheated 1 N HCl for 7 minutes at 60° C. (7) Washed with distilled water. (8) Immersed in Schiff's reagent for 10 minutes. (9) The sides were washed in three changes of two minutes each in rinsing solution. (10) Washed thoroughly under tap water. (11) Dehydrated through different grades of alcohol. (12) Cleared in xylol and mounted in D.P.X.

d) Results :

Nucleic acid sites stained redish-pink.