CHAPTER - TWO

MATERIAL AND METHODS

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2.0 Introduction :

The laboratory materials and different methods used in the present study are described in brief in this chapter.

2.1 Materials. :

2.1.1 Selection of animals: -

Male albino rats of Wistar strain (Institute of Virology, Pune.) were selected as experimental animals. The selected rats were about 90 – 100 days old weighing about 200 – 250 gms. each.

2.1.2 Maintenance of colony: -

Albino rats were maintained in separate cages in the animal house of Department of Zoology, Willlingdon College, Sangli. under standard laboratory conditions (27° ± 2 (12:12 dark : light intervals)). An exhaust fan was attached to the room to decrease odour and moisture generated by rats. The room was protected against the entrance of wild rats, cats, cockroaches etc. which may carry infections.

The rats were equally divided in to two groups, control group C and experimental group T (Treated). Each group was of 15 rats.

All the cages were cleaned every alternate days with disinfectants, air dried and were kept in separate cages. The cages were arranged on clean racks which were cleaned every day. Rats were weighed at regular intervals of 24 days and rats with particular weight were selected for observations. The rats were fed with dry pelleted diet (Hindustan Lever Ltd., Bombay) and water ad libtum.

2.1.3 Plant Material

2.1.3.1 Fresh leaves and soft stems of oscimum sanctum – Tulsi were selected from nearby areas of vishrambag, Sangli and Jaysingpur town.

2.1.4. Preparation and administration of dose: -

The fresh leaves were washed, and ground and mixed with wheat flour to the extent of 10% and fed to the experimental animals along with pelleted diet. Record was kept on the total food consumption and it was calculated that each rat consumed on an average 400 mg of Tulsi leaves/100gm body weight per day . Rats were first given Tulsi pellets everyday in the morning (around 10 A.M.) and then the standard diet. Rats could conveniently eat the whole Tulsi pellets and there was no left over. Control rats were given equal quantity of only wheat flour pellets.

2.2. Methods:

2.2.1. Body weight: -

The rats of both control and Treated (experimental) groups were weighted at regular intervals of 24 days on electronic balance and the body weights were recorded.

2.2.2. Organ Weight: -

Three animals from each group were sacrificed at intervals of 24 days, 48 days and 72 days of treatment and finally after termination of treatment till 120 days. Testes, epididymes (caput and Cauda) , seminal vesicles, prostate gland and Cowper's gland were removed at regular

intervals from sacrificed animals and separately weighed , and their weights were recorded.

2.2.3. Histoarchitecture :

Small pieces of tissues were fixed in aqueous Bouin's fluid for 24 hours, tissues were well washed under running tap water, dehydrated through ethanol grades, cleared in xylene and embedded in parafin wax. Thin sections of the tissues were cut on microtome (at 7 μ).

These paraffin sections were dewaxed in xylene and stained with routine Hacmatoxylene – Eosin (H-E) technique for histoarchitectural studies . Ten random sections per organ were examined for qualitative assessment of spermatogenesis. Two main methods of study of spermatogenesis have been established in rats which are known as LC method (Leblond and Clermount, 1952a , 1952 b) and R G method (Roosen-Runge and Gisel, 1950). The LC method involves 14 different stages, while R G Method involves only 8 stages in the spermatogenetic cycle . A very high resolution optical microscopy is essential to observe clearcut differences in these stages. So, instead of these two methods , it appeared more practicable to describe spermatogenesis with reference to tunica propria , basement membrane, spermatogonia, primary spermatocyte , secondary spermatocyte , spermatid, and spermatozoa in the lumen.

2.2.4. Tubular diameter: -

Morphological variations in seminiferous tubules in testes and epididymal tubules were studied with the help of occulometer (monocular microscope) Tubular diameter in testes and epididymis were calculated . All the measurements were recorded in millimeter and finally they were

converted in μ m. Tubular diameters about 50 tubules were recorded and mean values were calculated.

2.2.5. Statistical Methods (Fisher 1950, Gupta 1991): -

$\overline{X}$ => Arithmetic Mean of X – independent variable

a) $\overline{X} = \frac{\sum X}{N}$

X- is an independent variable

N- is the number of variations

b) S.D. ➔ Standard Deviation.

$$S.D. = \sqrt{\frac{\Sigma (x - \bar{x})^2}{N}}$$