CHAPTER - II

MATERIALS AND METHODS

II. MATERIALS AND METHODS

The present work is designed to study the effects of industrial stresses in powerloom sector on different organs of the rat (*Rattus norvegicus*). The materials required to carry out this work and methods used to study the same, are included under this chapter.

A. MATERIALS

1. Experimental Animal :

For present study the adult male albino rats (*R. norvegicus*) were used to carry out experimental work scheduled under this study. Male rats were preferred since this study is the beginning of the large systematic study of effects of industrial stresses on rat organs. There are many metabolisms which are essentially influenced by male and female hormones. In female cyclic alterations in hormones (oestrous cycle) have to be considered when prolonged exposure to stress decided. The adult male albino rats weighing about 220-250 gms were used. These rats were kept in well ventilated, disinfected cages of size (14 x 9 x 6 inches) with adequate space for their movements. The temperature of the room was maintained to $25^0C \pm 2^0C$. The animals were fed with standard pellet feed (Lipton India Ltd.) and water *ad libitum*.

These rats were obtained from the animal house of Department of Zoology, Shivaji University, Kolhapur. Experimentations were conducted according to INSA. Ethical guidelines for use of animals in scientific research.

2. CHEMICALS

Chemicals used for histological study :

For histological study following chemicals of AR grade were used :

i. 10% buffered formalin
ii. Hematoxylene
iii. Eosin
iv. Alcohol
v. Paraffin wax
vi. Xylene
vii. DPX

Chemicals used for total tissue protein estimation :

Following chemicals of AR grade were used to estimate total tissue proteins :

i. Lowry's 'A' solution
ii. Lowry's 'B' solution
iii. Lowry's 'C' solution
iv. Folin Ciocalten- Phenol reagent
v. Standard protein solution

The chemicals required for preparation of some of the above solutions were obtained from B.D.H. Chemicals Pvt Ltd., Poole England.

3. SITE OF EXPOSURE OF EXPERIMENTAL ANIMALS

To study the effects of industrial stresses in powerloom sector on different organs of male albino rats (*R. norvegicus*), the experimental animals were exposed to the powerloom sector in Ichalkaranji. Ichalkaranji is a town place, situated 30 Kms. away from Kolhapur city. In Ichalkaranji there were about 4500 powerloom units, weaving cloth in private as well as co-operative sector. For present study one powerloom unit from the private sector was selected to expose the experimental rats, to observe and study the effects of stresses in powerloom sector on different organs of male albino rats. The cages of experimental rats were kept at the place where the workers were working in the powerloom.

B. METHODS

I. Experimental Protocol :

The following experimental schedule gives the details of experimental work. The experimental rats were grouped into three sets labelled as S_1, S_2 and S_3. Each set includes three rats. Depending upon exposure period they were labelled as P_1, P_2 and P_3. A set of control was also maintained. The experimental rats were exposed to adverse working environment in the powerloom sector. The sets were as below (Table No.1).

Table No. 1
EXPERIMENTAL PROTOCOL

Sr. No.	Name of the experim-ental group	Number of rats in each group	Groups of the exposed rats	Period of exposure in hrs/ day	Exposure Schedules	Recovery period	Day of sacrifice
1.	S_1	3	Rat 1 – P_1 Rat 2 – P_2 Rat 3 - P_3	6 hrs/ day 6 hrs/ day 6 hrs/ day	Exposed once for one day Exposed once for two successive days Exposed once for three successive days	Exposure followed by 15 days recovery	On 15th day
2.	S_2	3	Rat 1 - P_1 Rat 2 - P_2 Rat 3 - P_3	6 hrs/ day 6 hrs/ day 6 hrs/ day	Exposed twice for one day Exposed twice for two successive days Exposed twice for three successive days	Each exposure followed by 15 days recovery.	On 30th day
3.	S_3	3	Rat 1 - P_1 Rat 2 - P_2 Rat 3 - P_3	6 hrs/ day 6 hrs/ day 6 hrs/ day	Exposed thrice for one day Exposed thrice for two successive days Exposed thrice for three successive days	Each exposure followed by 15 days recovery.	On 45th day
4.	Control	3	Rat - 1 Rat - 2 Rat - 3	These rats were maintained in laboratory under standard laboratory conditions at room temperature, without being exposed to any kind of stress			

II. Sacrifice Method :

The rats of all three sets and also of control were killed by cervical dislocation. The adrenals, kidneys, heart, stomach and duodenum from all, experimental and rat (control) were removed, weighed and used for further study.

III. Histologic Study :

The histological techniques used to study the adrenals, kidneys, heart, stomach and duodenum of experimental and rat (control) were routinely used and were reviewed in detail by Thompson (1966).

i. Procedure for Fixation :

The pieces of adrenals, kidneys, heart, stomach and duodenum were fixed in neutral buffered formalin for about 12 to 18 hours at room temperature, preventing autolysis.

ii. Washing :

After fixation, washing is essential. Fixative was washed by running tap water. Washing of tissue was done for 18-24 hours.

iii. Dehydration of tissue :

It is for removing the water from tissues were passed through various grades of alcohol such as 30%, 50%, 70%, 90% and absolute alcohol. In each grade tissues were kept for about

1.5 to 2 hours. The time of dehydration depends on type and size of tissue.

iv. Clearing of tissues :

For clearing xylene was used as a clearing agent.

v. Infiltration or Incubation :

After clearing, tissues subjected to cold and hot embeding. The embeding medium used was paraffin wax (M.P. 60^0-62^0C). Incubation was done for about 1 hour to 2 hours, depending upon size and nature of the tissues. After infiltration tissues were placed in molten paraffin wax in moulds. The blocks then trimmed.

vi. Sectioning :

Trimmed blocks were attached to the block holder and sectioned with rotary microtome by selecting required thickness setting (5μ to 7μ). Wax ribbon strips of sections were spread on slides (3 x 1 inch), on which egg albumen was applied as an adhesive (Bolles Lee, 1921), allowing space for a label.

vii. Staining :

During staining slides were firstly deparaffined by keeping them in xylene. The deparaffined slides hydrated by passing them through series of downgrades of ethanol (10 minutes in each

grade). Then stained with Harri's Haematoxylene (for 7-8 minutes). Excess stain removed by washing them and with distilled water. Then slides were dehydrated by passing them through alcoholic grades. At absolute alcohol grade slides were stained with eosin for 1 minute. Then washed with absolute alcohol, cleared in xylene and mount in DPX (Kirk Patric and Lendrum, 1939, 1941).

IV. Total Tissue Protein Estimation :

Animals from all 3 sets and also from control, killed by cervical dislocation. Immediately after killing, adrenal, kidney, stomach, duodenum, heart removed, weighed and used for protein estimation.

Preparation of Homogenate :

10 mg of each tissue was taken and crushed separately by using mortal and pistle by adding 10ml of water in it. These homogenate mixtures filtered into separate beakers. These filtrates were used as homogenates for protein estimation.

Protein Estimation :

Proteins were estimated by the Lowry's method (Lowry's *et al.* 1951). This method requires following reagents, which were prepared as follows :

1. Lowry's 'A' Solution : 2 gms of Na_2CO_3 + 400 mg NaOH were dissolved in 100ml distilled water.

2. Lowry's 'B' Solution : 500 mg $CuSO_4$ + 1 gm Na/K – Tartarate were dissolved in 100ml distilled water.

3. Lowry's 'C' Solution : 50ml Lowry's 'A' solution + 1ml Lowry's 'B' solution. Mixed at the time of use only.

4. Folin Ciocalten – Phenol Reagent : 100gm sodium tungstate (Na_2WO_4. $2H_2o$) and 25 gm Na-molybdate ($NaMOO_4.2H_2o$) were dissolved in 700 ml distilled water with 100ml concentrated HCl and 50ml 85 per cent phosphoric acid. Mixture was refluxed for 10 hours in an all glass apparatus, 150gm of lithium sulfate was added to 50ml distilled water with few drops of Br_2. Mixture was boiled for 15 minute to remove excess bromine. The mixture was cooled and diluted to 1 litre and then filtered. The reagent thus prepared was diluted with 2 volumes of water before use.

5. Standard Protein Solution : 13 mg Bovine albumin + 250ml of distilled water + NaOH.

Preparation of Unknown :

Into 10ml test tube 0.5 ml homogenate + 1 ml distilled water + 3ml Lowry's 'C' reagent were added, shake well and kept for 15 minutes to form Cu-protein complex.

After 15 minutes added 0.5ml Folin's reagent and kept for 1 hour and readings were taken at 660nm on Bosch and Lomb spectrophotometre.

Preparation of Standard Solution :

In other test-tube 0.5ml standard Bovine albumin solution + 1 ml distilled water + 3ml Lowry's C reagent, taken and kept for 15 minutes. After 15 minutes, 0.5 Folin's reagent was added and was kept for 1 hours to develop maximum colour.

Total tissue proteins were expressed as mg/gm of tissue.