
Methodology

3. METHODOLOGY

This chapter explains the methodology involved in the estimation of various parameters in this study.

COLLECTION OF THE *Drosophila* STOCK

The wild type *Drosophila melanogaster* stock was collected from the *Drosophila* Stock Centre, Manasagangotri, Mysore University.

PREPARATION OF MEDIUM

One litre of water was boiled, 100g of jaggery was added to it and cooked well. Then 100g of sooji was added, mixed and boiled well. Following this, 10g of agar and 7.5ml of propionic acid were added and cooked well. The mixture was cooled and transferred to bottles. Yeast granules were layered on the top of the medium and the bottles were plugged with cotton.

CULTURING OF *Drosophila*

Drosophila was cultured in the above media at 25°C under 12 hours light and 12 hours darkness. They were maintained in the *Drosophila* colony laboratory in the University campus.

PREPARATION OF THE HOMOGENATE

The male and female *Drosophila* were identified and separated as soon as they hatched. The virgin animals were maintained in the above mentioned diet and then divided randomly for the various treatments.

PREPARATION OF THE PLANT EXTRACT

Wheat seeds were collected from the local market at Coimbatore. They were soaked for 24 hours in tap water and sowed. The fresh leaves of the 4th day plants were collected. They were washed in running tap water to remove the dirt particles and blotted gently between the folds of tissue paper to remove any water droplets. The leaves were then homogenized in water (1g/ml) using a micropestle. The homogenate was centrifuged at low speed to remove large particles and used for the treatment.

TREATMENT GROUPS

The following treatment groups were set up for each parameter for male and female animals separately.

1. Untreated group
2. *Triticum aestivum* leaf extract treated group
3. H₂O₂ low dose (20 mM)
4. H₂O₂ low dose (20 mM) + *Triticum aestivum* leaf extract treated
5. H₂O₂ high dose (30 mM)
6. H₂O₂ high dose (30 mM) + *Triticum aestivum* leaf extract treated
7. CCl₄ low dose (125 mM)
8. CCl₄ low dose (125 mM) + *Triticum aestivum* leaf extract treated
9. CCl₄ high dose (195 mM)
10. CCl₄ high dose (195 mM) + *Triticum aestivum* leaf extract treated

The test agents (H₂O₂ and leaf extract) were mixed with the diet. The exposure was given for seven days. At the end of the treatment period, the flies were anaesthetised using diethyl ether and their wings were clipped off.

They were then weighed, crushed and homogenized in ice-cold phosphate buffer (30mg of flies / 300µl buffer) using a sonicator (Sonics, USA).

The homogenate was quickly aliquoted after clarification by centrifugation and frozen at -85°C (Ilshin Ultra Deep Freezer, Korea) till analysis. All the analyses were performed in as short a duration as possible.

PARAMETERS ANALYSED

As the volume of homogenate obtained was very less compared to the volume obtained in other traditional animal models, all the assays were performed using a nanospectrophotometer Optizen 3220 UV Bio (Korea), wherein the assay volume required ranges from 0.7 to 4 µl.

ENZYMIC ANTIOXIDANTS

a) SUPEROXIDE DISMUTASE

Superoxide dismutase, one of the most important cellular antioxidant, was assayed by the method of Kakkar *et al.* (1984). The detailed procedure is given in Appendix I.

b) CATALASE

Catalase activity in the homogenates was assayed by the method proposed by Luck (1974). The procedure is given in Appendix II.

c) PEROXIDASE

Peroxidase activity was estimated by the method of Reddy *et al.* (1995). Appendix III presents the detailed procedure adopted.

NON-ENZYMIC ANTIOXIDANTS

a) ASCORBIC ACID

Ascorbic acid was estimated by the method reported by Roe and Keuther (1943). Appendix IV explains the method.

b) TOCOPHEROL

Vitamin E, a scavenger of free radical, was estimated by the method of Varley *et al.* (1981). The steps involved in the assay are given in Appendix V.

c) REDUCED GLUTATHIONE

The method proposed by Moron *et al.* (1979) was used for the estimation of reduced glutathione.