

Supplementary Information

Behavioral studies

Spontaneous Motor Activity (SMA)

Animals were kept in the normal environment i.e. in a cage and their behavior was evaluated for 5 min. Neurological scoring was done as follows: **0**- rats moved around in the cage freely exploring the environment; **1**-rats showed movement but could not approach all sides of the cage and showed hesitation in moving; **2**-rats barely moved to show postural abnormalities (bent towards the paretic side); **3**-rats did not show any movement at all and their posture was bent towards the paretic side.

Flexion Test (FT)

Flexion test (FT) was done according to the method described by Kumar accordingly, scoring was done on four points: **0**-no neurological deficit was visible; **1**-animal showed contralateral forelimb flexion with wrist flexion and shoulder adduction; **2**-animal showed a reduced resistance to lateral push and **3**-animal showed movement in a circle towards the paretic side.

Grip Strength

A 50 cm long string was tied between two vertical supports and animals were evaluated based on the following scale: **0**-animal fell off; **1**-animal kept hanging onto string by two forepaws; **2**-as for **1** but attempted to climb the string; **3**- animal kept hanging onto string by using two forepaws and one or both hindlimbs; **4**-animal kept hanging onto string by using two forepaws with tail wrapped around the string; **5**-animal escaped.

Biochemical studies

Measurement of Lipid peroxidation (LPO)

TBARS are formed as a byproduct of lipid peroxidation. TBARS was estimated as follows: Initially, 0.2 ml of S1 supernatant was pipetted out into a test tube of (15×100mm) dimension and was then incubated at 37°C in a metabolic water bath shaker for 1 h at 120 strokes up and down; after that, another 0.2 ml of S1 was pipetted out in an Eppendorf tube and kept at 0°C. After incubation for 1 h, 0.4 ml of 5% TCA and 0.4 ml of 0.67% TBA was added to both the samples. The reaction mixture from the test tube was transferred to the centrifuge tube and kept for centrifugation at 3000g for 15 min. Later the supernatant was put into other test tubes which were then kept in a boiling water bath for 10 min, after that the test tubes were cooled and the absorbance measurement is done at 535 nm. The rate was expressed in terms of μmol of TBARS formed/hr/mg protein using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of reduced glutathione (GSH) level

Initially, 1.0 ml of post mitochondrial supernatant (PMS) fraction (10 %) was mixed with 1.0 ml of sulphosalicylic acid (4%). The samples were incubated at a temperature of 4°C for at least 1 hr and then subjected to centrifugation at 1200×g for 15 min at 4°C. A total of 3.0 ml of assay mixture

contained 0.4 ml filtered aliquot, 2.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.4 ml DTNB (10 mM). Immediately, the mixture became yellow colored and was read at 412 nm on a spectrophotometer (Hitachi, U-2910). The GSH content was expressed as μmol DTNB conjugate formed/ gram tissue using a molar extinction coefficient of $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of glutathione reductase (GR) activity

The assay mixture consisted of 1.65 ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml EDTA (0.5 mM), 0.1 ml NADPH (0.1 mM), 0.05 ml oxidized glutathione (1.0 mM) and 0.1 ml of 10% PMS making the total volume to 2.0 ml. The enzyme activity was determined at 25°C by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/minute/mg protein using a molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of glutathione peroxidase (GPx) activity

A total volume of 2 ml consisted of 0.1 ml EDTA (1 mM), 0.1 ml sodium azide (1 mM), 1.44 ml phosphate buffer (0.1 M, pH 7.4), 0.05 ml glutathione reductase (1 IU/ml), 0.1 ml NADPH (0.2 mM), 0.05 ml reduced glutathione (1 mM) and 0.01 ml H_2O_2 (0.25 mM) and 0.1 ml 10 % PMS. The disappearance of NADPH at 340 nm was recorded at 25°C. The enzyme activity was calculated as μmol NADPH oxidized/minute/mg protein with the molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was measured by monitoring the auto-oxidation of (-) epinephrine at pH 10.4 for 3 min at 480 nm. The enzyme activity was calculated as nmol (-) epinephrine that is protected from being oxidized/minute/mg of protein using a molar extinction coefficient of $4.02 \times 10^3 \text{ mol L}^{-1} \text{ cm}^{-1}$.

Measurement of Catalase (CAT) activity

The assay mixture consisted of 2.0 ml phosphate buffer (0.1 M, pH 7.4), 0.05 ml of PMS (10%) and 0.95 ml H_2O_2 (0.019 M) in a total volume of 3.0 ml. The absorbance was recorded at 240 nm. The catalase activity was calculated as nmol H_2O_2 consumed/minute/mg protein.

Measurement of glutathione-S-transferase (GST) activity

The reaction mixture consisted of 2.4 ml phosphate buffer (0.1 M, pH 6.5), 0.2 ml CDNB (1.0 mM), 0.2 ml reduced glutathione (1.0 mM) and 0.2 ml of cytosolic fraction in a total volume of 3.0 ml. The absorbance was recorded at 340 nm and the enzyme activity was calculated as μmol

CDNB conjugate formed/minute/mg protein using a molar extinction coefficient of $9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Measurement of protein

The protein concentration in all samples was determined as follows: Peptide bonds make a complex with alkaline CuSO_4 reagent, the complex gives a blue color with Folin's reagent. Briefly, 0.1 ml (10 % w/v) was diluted to 1 ml with water and protein is precipitated with equal volumes of TCA (10%), samples were kept overnight at 4°C and then centrifuged at $800 \times g$ for 5 min. The supernatant was decanted and discarded. The pellet was dissolved in 5 ml of NaOH (1N). Finally, 0.1 ml of diluted aliquot was taken for the development of color. 0.1 ml of aliquot was further diluted to 1 ml with water and then 2.5 ml of alkaline copper sulfate reagent containing CuSO_4 (1 %), Na_2CO_3 (2%) and sodium-potassium tartrate (2 %) was added. 10 min after the addition of alkaline CuSO_4 reagent to allow complex formation, 0.25 ml of FolinCiocalteu. Reagent (FCR) was added. After about 30 mins have lapsed a blue color developed that was read at 660 nm. Bovine serum albumin (BSA 0.1 mg/ml) was used as a standard.