EVALUATION OF RHODIOLA ROSEA IN REVERSING ACRYLAMIDE-INDUCED NEUROPATHY: A BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY

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ABSTRACT

Introduction: Acrylamide (ACR), an ACR neurotoxic substance produced in the process of cooking at high temperature, is commonly consumed via roasted and baked foods. Its exposure leads to oxidative stress, neuronal degeneration, and abnormal behavior. Growing public interest in its neurotoxicity calls for the identification of the protective agents. Rhodiola rosea (Golden Root), an adaptogenic medicinal plant, has been previously reported to possess antioxidant, adaptogenic, and neuroprotective activities.

Objective: This study evaluates the neuroprotective effects of Rhodiola rosea against ACR-induced neuropathy in mice.

Methods: Twenty-four Swiss albino mice were grouped into four sets (n=6). Group I was administered ACR (70 mg/kg, s.c., 5 days/week for 3 weeks), Groups II and III were co-administered Rhodiola rosea (100 and 200 mg/kg, p.o.) with ACR, and Group IV was the normal control. Neurobehavioral tests—grip strength, hyperalgesia, narrow beam, rope, and locomotor tests—were performed weekly. Brain tissues were analyzed for biochemical and histopathological studies following the treatment duration.

Results: ACR treatment severely deteriorated neurobehavioral responses and increased oxidative stress. Rhodiola rosea co-administration decreased these in a dose-dependent manner significantly and reversed the level of antioxidants as well as the neuronal damage. Histopathological findings confirmed this observation.

Conclusion: Rhodiola rosea demonstrates significant protective effects against ACR-induced neurotoxicity, suggesting its potential as a neurotherapeutic agent.

Keywords; Acrylamide, Neurotoxicity, Rhodiola rosea, Oxidative Stress, Neuroprotection, Behavioral Assessment.

1. INTRODUCTION

Acrylamide is a low-molecular-weight α, β-unsaturated carbonyl compound that is created through high-temperature food cooking of carbohydrate food using the Maillard reaction. Acrylamide is also widely applied in industrial applications such as the production of polyacrylamide and paper products. The electrophilic nature of acrylamide makes it highly reactive with nucleophilic sites within biological macromolecules, hence its neurotoxic, genotoxic, and potentially carcinogenic implications [1-3].

Source of Ingestion:

(a) Dietary Exposure:

Acrylamide (ACR) is mainly present in thermally treated carbohydrate foods such as potato chips, French fries, and cookies, which are heated at high temperatures (e.g., 180°C). The highest levels are detected in fried and grilled potato and grain foods, and none is detected in boiled foods. ACR is produced by pyrolysis of asparagine's and Maillard reaction products. Coffee consumption and smoking are additional sources. The EU has set the limit for acrylamide in drinking water at 0.1 μg/L due to its harmfulness.

(b) Occupational Exposure:

Acrylamide is also used in the production of polyacrylamide polymers, which are used to treat water. Occupational exposure to ACR, particularly in the monomeric state, has been reported to cause neuropathies like ataxia, muscle weakness, and numbness. Repeated exposure in animals has been shown to cause neurotoxic effects like paralysis and CNS damage [4].

1.1.Metabolism and Biotransformation

Upon systemic absorption, acrylamide is metabolized by the liver mainly through cytochrome P450 2E1 (CYP2E1) to generate glycidamide, an epoxide with high genotoxic activity. At the same time, both acrylamide and glycidamide are detoxified through conjugation with glutathione (GSH) with the help of glutathione-S-transferases (GSTs). Chronic or excessive exposures nevertheless overwhelm these detoxifying routes, increasing the toxic load **(Fig 1)** [5].

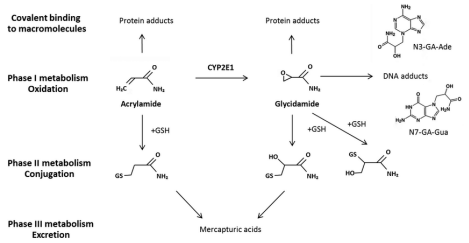

Figure 1: Biotransformation of acrylamide[20]

1.2.Mechanisms of Neurotoxicity

1.2.1 Axonal Degeneration and Impaired Axonal Transport

Acrylamide acts selectively on the distal parts of long axons, giving rise to a "dying-back" axonopathy phenotype. This action is brought about through interference with fast axonal transport processes, specifically through interference with kinesin motor proteins. This causes synaptic dysfunction and neurodegeneration progressively.

1.2.2 Neurotransmitter Disruption

Experimental data show that acrylamide disrupts synaptic transmission by modifying dopaminergic and cholinergic neurotransmission pathways. This interference on neural communication is responsible for the behavioral and cognitive impairments reported in exposed subjects [6].

1.3.Oxidative Stress and Mitochondrial Dysfunction

A characteristic feature of acrylamide neurotoxicity is an excessive production of reactive oxygen species (ROS) due to direct mitochondrial damage as well as CYP2E1-mediated metabolism.The elevated ROS levels initiate lipid peroxidation (as indicated by increased malondialdehyde, MDA), protein carbonylation, and DNA strand breaks. Mitochondrial membrane potential ($\Delta\Psi m$) is considerably reduced, leading to faulty synthesis of ATP and the activation of apoptotic cascades. At the same time, the antioxidant defense system—comprised of GSH, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—is severely compromised [7].

1.4.Neuroinflammation

Acrylamide activates microglia and astrocytes, which result in pro-inflammatory mediator upregulation such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and inducible nitric oxide synthase (iNOS). The nuclear factor NF-κB pathway is pivotal in mediating such inflammation. Chronic neuroinflammation also accelerates neuronal damage and synaptic degeneration [7].

1.5.Protective Role of Polyphenols

Polyphenols, a sophisticated naturally occurring phytochemicals including flavonoids, phenolic acids, stilbenes, and lignans, have attracted widespread attention owing to their neuroprotective activity. These compounds like quercetin, curcumin, epigallocatechin

gallate (EGCG), and resveratrol exert multifaceted protective effects against acrylamide-induced neurotoxicity.

1.5.1 Antioxidant Mechanisms

Polyphenols are potent scavengers of free radicals and augment endogenous antioxidant defense. Polyphenols replenish the exhausted stores of GSH, SOD, CAT, and GPx and inhibit lipid peroxidation and protein oxidation. Most polyphenols also activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway that regulates the gene transcription of the antioxidant response element (ARE)-target genes such as heme oxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1).

1.5.2. Mitochondrial Protection

Polyphenolic compounds contribute to mitochondrial integrity via stabilization of ΔΨm, mitigation of mitochondrial ROS, and conservation of ATP synthesis. They also adjust mitochondrial biogenesis via PGC-1α signaling.

1.5.3. Anti-Inflammatory Activity

The stimulation of NF-κB is inhibited by polyphenols, and their release is subdued for TNF-α, IL-1β, and other cytokines. Polyphenols also dampen the transcription of COX-2 and iNOS, thus suppressing neuroinflammatory cascades [8].

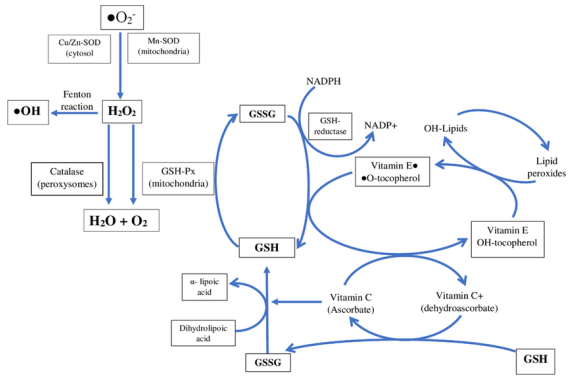

Figure 2: Mechanism of Antioxidant defense system[21]

1.6.Current Methods for Assessing Neurotoxicity in Laboratory Models

1.6.1 In Vivo Models

Multidisciplinary determination of neurotoxicity is necessary owing to the inherent complexities of the nervous system. Effects of neurotoxicity might be assessed in terms of neurophysiological tests (e.g., EEG, evoked potentials), neuropathological techniques (e.g., histochemistry, immunohistochemistry), or behavioral examination. Evaluation schemes can be specifically prepared on a case-by-case basis by utilizing an approach on successive tiers: Tier 1 being simple repeated dose toxicity testing, Tiers 2 and 3 specific determination of distinct effects of neurotoxicity. Guidelines presented by the U.S.

Environmental Protection Agency (EPA) and the OECD, specifically OECD Test Guideline 424, suggest tests to identify neurobehavioral and neuropathological effects during or following repeat-dose toxicity studies.

Standard neurotoxicity studies include:

- Detailed clinical observations
- Monitoring changes in skin, fur, eyes, secretions, and autonomic activity (e.g., pupil size, respiratory rate)
- Assessment of motor coordination, posture, gait, reactivity, and presence of tremors, clonic or tonic movements
- Identification of stereotypical or unusual behaviors (e.g., repetitive grooming, backward walking)

Functional assessments further evaluate:

- Sensory function (e.g., sensory irritation tests)
- Motor function (e.g., grip strength, foot splay)
- Cognitive function (e.g., learning and memory tests)

The Functional Observational Battery (FOB) standardized protocol is extensively applied for the detection of alterations in behavior, neuromuscular, sensory, and autonomic function. The FOB, with its validation by known neurotoxicants, supports general toxicity endpoints. In addition, the EPA classifies neurotoxic endpoints into structural, neurophysiological, neurochemical, behavioral, and developmental categories [9].

1.6.2 In Vitro Models

In light of the cost, ethics, and time concerns surrounding animal testing, considerable effort is aimed at the development of in vitro models of neurotoxicity. While good tools for first-level screening, these models are not yet established as alternatives to in vivo research. According to EPA guidelines, in vitro data alone for neurotoxicity is insufficient but can augment in vivo results when combined.

Common in vitro models include:

- Primary cultures (e.g., rat astrocytes, cerebellar granule neurons, spinal cord motor neurons)
- Organotypic brain slices (e.g., hippocampal slices)
- Cell lines (e.g., C6 glioma, PC12, NIE115, neuro-2a, IMR32, chromaffin cells)

These models enable assessment of specific neurotoxic endpoints, such as:

- Morphological alterations and neurite outgrowth
- Myelin integrity (CNS/PNS)
- Disruption of axonal transport

- Electrophysiological responses
- Blood-brain barrier integrity
- Calcium homeostasis
- Neurotransmitter and hormone levels

Although promising uses, additional validation and standardization are required to provide reliability and predictive precision of in vitro systems. Combination of both in vivo and in vitro data improves the strength of neurotoxicity evaluations and facilitates regulatory safety assessments [10].

2. REVIEW OF LITERATURE [Rhodiola rosea Linn.]

2.1.Plant Description and Geographical Source

Rhodiola rosea, or Golden root, is a perennial herb that grows mainly in dry, sandy environments at high elevations in the Arctic parts of Europe and Asia. It reaches a height of 30 inches (70 cm) and bears yellow flowers. It has a dense rhizome and a rose-scented smell when cut. Used traditionally in Russia, Scandinavia, and other parts of the world, its medical use was noted in 77 CE by the Greek physician Dioscorides. The species Rhodiola rosea was named by Linnaeus because it has a rose-like scent.

Figure 3: Rhodiola rosea[11]

2.2.Chemical Composition

Rhodiola rosea is rich in different bioactive compounds, such as essential oils, sterols, glycosides, organic acids (oxalic, citric, malic, gallic, succinic), phenolics (tannins), and proteins. Its chief active compounds are rosavin, rosarin, salidroside, and tyrosol. The extracts are standardized for rosavins (3%) and salidroside (0.8-1%) in clinical trials.

2.3.Toxicity

Rhodiola rosea is of low toxicity. The lethal dose (LD50) in rat experiments is estimated at about 3,360 mg/kg, several times greater than the usual clinical dose of 200-600 mg/day, which leaves a wide margin of safety.

2.4.Medicinal Activities

Rhodiola rosea has been studied extensively for various pharmacological properties, including:

- **Adaptogenic and Anti-stress Effects:** Rhodiola rosea is known for its adaptogenic features that increase defense against a range of biological, physio-chemical challenges. It increases serotonin levels in the hypothalamus and midbrain and modulates the release of stress hormones, improving overall stress tolerance without harming the central nervous or cardiovascular systems.
- **Antitumor Activities:** In animal studies, Rhodiola rosea has shown antitumor and antimetastatic effects. It inhibited tumor growth in mice with Ehrlich adenocarcinoma, with significant effects when administered orally.
- **Cardioprotective Effects:** Rhodiola rosea has cardioprotective effects, such as reducing myocardial catecholamine levels and preventing stress-induced cardiac damage. Its activation of mu-opiate receptors in heart muscle helps prevent arrhythmias.
- **Effect on Learning and Memory:** Studies suggest that Rhodiola rosea enhances rats' learning and memory abilities. A 40% ethanol extract showed positive trends in memory protection, especially in tasks involving avoidance learning.
- **Endocrine and Reproductive Effects:** Animal studies indicate that Rhodiola rosea enhances thyroid function and improves adrenal gland function. It also has anabolic effects, promoting muscle building and gonad strengthening, similar to low-dose testosterone effects.
- **Anti-inflammatory and Neuroprotective Effects:** Rhodiola rosea demonstrates neuroprotective effects, such as suppressing neurotoxicity induced by L-glutamate in neuronal cells. It reduces the expression of pro-inflammatory cytokines and iNOS, indicating potential in treating inflammation and neurodegenerative diseases [11, 12].

2.5.Research Envisaged

Acrylamide-induced neuropathy is characterized by oxidative stress and results in neurodegeneration as well as inflammation. Although some studies have exhibited potential of antioxidants in preventing the condition, so far no one has examined pharmacological assessment of Rhodiola rosea versus acrylamide-induced neuropathy.

Objective:

This study aims to evaluate the effects of Rhodiola rosea on acrylamide-induced neuropathy in mice through behavioral and biochemical assessments, including:

- Hind limb impairments
- Coordination and movement ability under high-altitude stress
- Grip strength
- Locomotor activity

- Thermal sensitivity
- MDA, GSH, and NO levels in the brain
- Histopathological changes in the brain

This investigation will provide insights into the potential therapeutic effects of Rhodiola rosea on acrylamide-induced neuropathy.

3. MATERIALS AND METHOD

3.1.Experimental Animals:

Swiss Albino Mice were purchased from NIN, Hyderabad. They were placed individually in clean, transparent polypropylene cages with free access to food and water with 12: 12 hr dark/light cycle is followed. They were adapted for a while of one week and divided into experimental groups.

Every study technique was conducted in compliance with with the committee for the purpose of control and supervision of experiments (320/CPCSCEA dated 03-01-2001) on animals.

3.2.Chemicals:

Acrylamide (ACR) (≥99%), Thiobarbituric acid (TBA) were obtained from Sigma Aldrich. procured as gift sample from sanata products Limited, new delhi, India. Tricholoro acetic acid (TCA) and Ellman's reagent were obtained from High media. Butanol, pyridine, Hydrogen peroxide, EDTA, and other chemicals were obtained locally.

3.3.Study Design:

To evaluate the potential improvement in the behavioral and biochemical features of ACR induced mice treated with Rhodiola rosea.

3.3.1 Experimental design

Swiss Albino mice were acclimatized to laboratory conditions and divided into 4 groups (n=6).

- Group 1 (Normal control): received Normal water and vehicle (1% CMC, p.o) for 3 weeks.
- Group 2 (Disease control): ACR (70mg/kg, s.c., 5days/week) and Vehicle for 3 week.
- Group 3 & 4 (Disease treated): Rhodiola rosea was co-administered p.o at the doses (100 & 200 mg/kg) along with ACR.

All the experiments were performed in the light phase between 09:00 and 15:00. During the treatment period behavioral tests were carried out on 7th, 10th and 14th day. Rats from every group were sacrificed under ether anesthesia and the cerebellum and spinal cord were excised for biochemical and histological analysis. Detailed experimental design was depicted (Fig 4) [13].

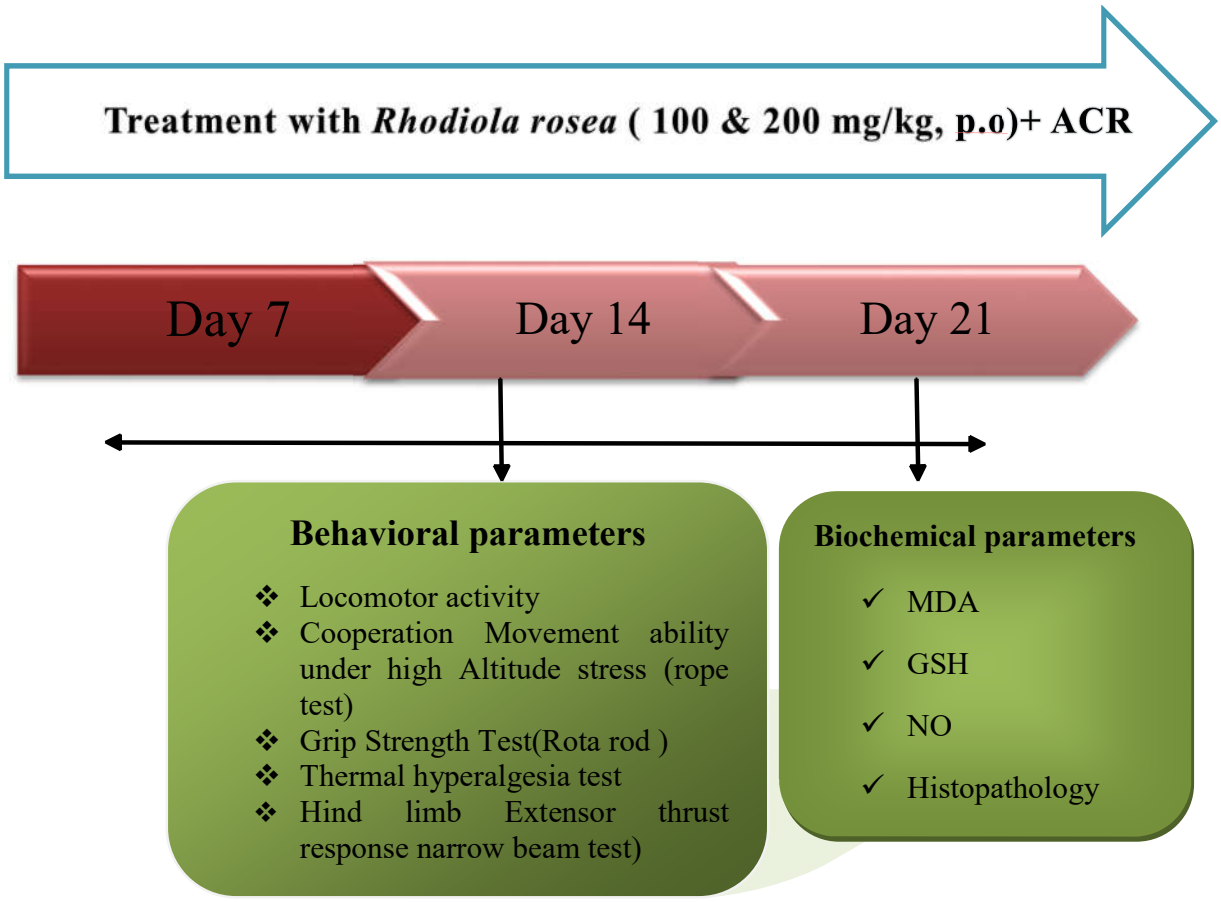

Figure 4: Experimental Design

3.4 Behavioral studies:

3.4.1 Narrow beam test

Narrow beam test was recorded to measure hind-limb impairments. According to a platform at the end to the animal's home cage at the other, which was positioned horizontally 100 cm above the floor, the animals were trained to walk across a 180 cm long a wooden beam that was separated into three segments (1, 2, and 3) of 60 cm each (Fig 5). It was noted how many times a foot slipped onto an overhanging ledge.

Score 0 – Rat traverse through the beam without falling.

Score 1 – Rat fell off in the third segment.

Score 2 – Rat fell in second segment.

Score 3 – Rat fell in first segment.

Score 4 – Rat fail even to sit/ balance the beam.

Each mice was tested three times. The averages of scores for three trials per mice were taken [14].

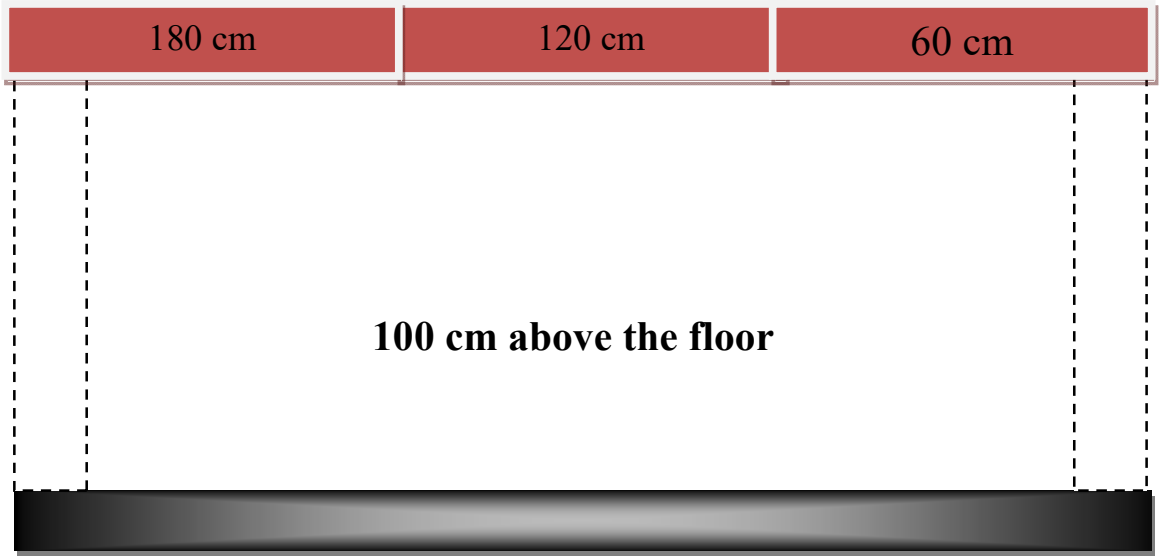

Figure 5: Narrow beam test

3.4.2 Grip strength (motor coordination)

The rota rod was used for the study of muscular coordination, degree of motor dysfunction and balance that requires an unaltered central function. The speed of the rotarod was set at 10 cycles per min and cut off time was 180s. The test was repeated three times at 60 min interval for each mouse and the averaged times were used for statistical comparisons [15].

3.4.3 Hyperalgesia

Thermal sensitivity is determined using Eddy's Hot Plate, the hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms. In this experiment, each animal was put on a hot plate (Eddy's Hot Plate) that was set to 45 ± 1°C. The cut-off time was 12 s to prevent injury to paws 16, 17. The latency to the first indication of paw licking or the leap response to avoid the heat was used as an indicator of the pain threshold.

3.4.4 Rope test

Crawling along a rope measures the animal's Cooperation Movement Ability Under High Altitude Stress. Mice were placed on a 2-m-long rope, the distance of which was about 1.5 m from the ground, and then the behaviors of the mice were observed for 3 min. The mice which could grasp and crawl along the rope were deemed to have "succeeded" in the test, and those mice that could either not grasp a rope tightly or grasped the rope tightly but could not crawl along "failed" in the test [15].

3.4.5 Locomotor Activity

It allows to track the movement of the animal during treatment induced disturbances in sleep/wake patterns serving as an index for underlying general toxicity. Activity was measured using the equipment, Actophotometer where the movement of the animals cuts the light beam showing as locomotor score. Animals were placed individually in the chamber and locomotor score was recorded with a cut off time of 300sec [14].

3.5 Biochemical estimations

Preparation of 2% Tissue homogenate:

100 mg of tissue was weighed appropriately and homogenized in 5 ml of buffer solution with semi motor at a speed of 2500 rpm for 2 minutes in ice cold surrounding environment. The homogenate is centrifuged at 200 rpm for 2 minutes.

3.5.1 Estimation of Malondialdehyde / Thiobarbituric acid reactive substances:

TBARS level in tissue is a measure of lipid peroxidation. It was measured in cerebral cortex and cerebellum tissue homogenate using biodiagnostic and research reagents.

Principle: Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm.

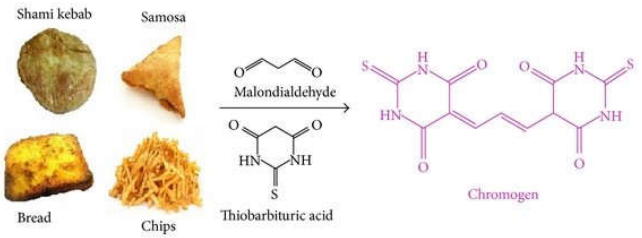

Figure 6: Reaction of TBA with Malondialdehyde to yield pink colored compound [22]

Procedure: This approach involved adding 3 milliliters of phosphoric acid (1%) and 1 milliliter of TBA (0.6%) to a 10% KCl brain tissue homogenate.

- The mixes were boiled in a boiling water bath for forty-five minutes.
- 4 ml of n-butanol was added to the mixture once it had cooled, and it was vortexed for one minute before being centrifuged for ten minutes at 3000 g.
- After removing the organic layers and moving them to a new tube, the absorbance at 532 nm was measured.
- MDA concentrations were given as nmol/g of tissue.

Calculation: Malondialdehyde in sample:

$$\text{Tissue} = \frac{\text{A sample} \quad 10}{\text{A standard} \quad \text{g of tissue use}} * \text{n mol/g.tissue}$$

3.5.2 Estimation of Glutathione

Using the Ellman (1959) approach, the reduced glutathione (GSH) level in the spinal cord and cerebellum was assessed.

Principle: The sulfahydryl group of GSH easily reduces the disulfide chromogen 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) to a bright yellow substance. As shown in Figure 7, the absorbance of the reduced chromogen at 412 nm is exactly proportional to the GSH content.

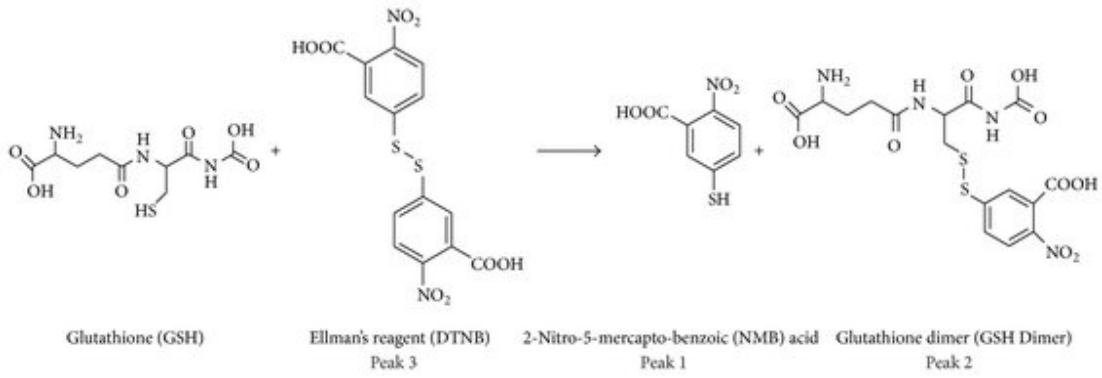

Figure 7: Reaction of DTNB with GSH yielding yellow colored compound[23]

Procedure:

- Tissue was taken and homogenized in 0.1M phosphate buffer pH 7.4.
- The homogenate was added with equal volume of 20% Tricholoro acetic acid containing 1mM EDTA to precipitate the tissue proteins.

- Mixture was allowed to stand for 5 minutes.
- Centrifugation was done at 200 rpm for 10 minutes.
- Supernatant (200 µl) was taken in fresh tubes
- 1.8ml of the Ellman's reagent (0.1mM) was prepared in 0.3M phosphate buffer with
- 1% sodium citrate solution. The test tubes were made up to 2 ml.
- After completion of total reaction, solution was read at 412 nm against blank.

Reagents	**Sample**	**Blank**
Phosphate buffer solution	1.9 ml	1.9 ml
Supernatant	0.1 ml	0.1 ml
H_2O_2	1 ml	---------

Calculations:

- The concentration of GSH = $\frac{Absorbance}{L} * \frac{1}{\varepsilon} * D$
- L: Light bath (cm)
- ε : Extinction coefficient (14150 M^{-1} .Cm^{-1})
- D: Dilution factor.

Units: µmol / mg protein.

3.5.3 Determination of total nitrite levels

Nitrite estimation in erythrocytes was done in by using Griess reaction.

Principle: A reddish-violet (λmax≈540 nm) water-soluble azo dye (HO3SC6H4–N =N–C10H7NH2) is produced when nitrite reacts with sulfanilic acid (HO3SC6H4NH2) in an acidic environment to form a diazonium cation (HO3SC6H4–N N+), which then couples to the aromatic amine 1-naphthylamine (C10H7NH2) at para-position. This reaction was initially described by Johann Peter Griess in 1879 as a method of nitrite (NO2−).

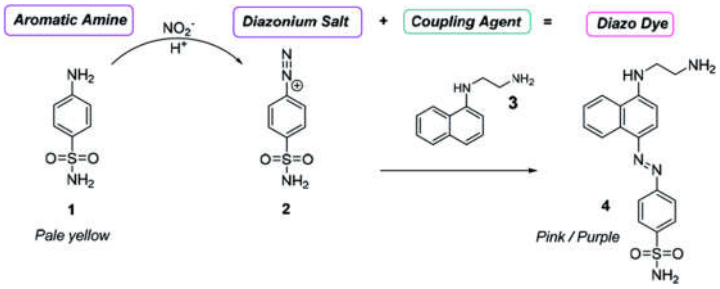

Figure 8: The Griess reaction[22]

Both in batch and automated Griess assays, the modified Griess assay yields the sum of nitrite and nitrate without distinguishing nitrite from nitrate. The Griess reaction, which

is the basis for nitrate analysis by assay, necessitates reducing nitrate to nitrite before diazotization. Chemicals and the enzyme nitrate reductase from various sources, including Aspergillus species, can be used to do this. b Vanadium chloride (VCl3 or VCl3/VCl4) and cadmium (Cd) in powder, sponge, wire, and activated columns are examples of chemical reductors. Vanadium chloride reduction is carried out at HCl-acidic conditions, or 1 N HCl.

Preparation of Reagents:

- VCl3 solution: Dissolve 40 mg of VCl3 (vanadium trichloride) in 5 ml of 1 N HCl.
- Griess reagent: Available preparation in market contains Sulfanilic acid, 2-Naphthylamine, Acetic acid.

Procedure:

- Take 100µl of tissue homogenate, add 100µl of VCl3 and 100µl of griess reagent solution, mix thoroughly.
- Mix and incubate at room temperature (37ºC) for 3 minutes.
- Measure the absorbance at 540 nm.

Reagents	Sample	Blank
Supernatant	100 µl	-
VCl_3	100 µl	-
Griess reagent	100 µl	100 µl

Calculation:

- Nitrite concentration was assessed from standard curve prepared with solutions of KNO2 ranging from 0 to 200 µM.
- Concentration calculated according to formula: NO (mM) = -2.015+164.75 * optical density at 540nm.

Units: mMol / L blood [18].

3.6 Histopathology

Following animal sacrifice, the brains were promptly separated, processed, and embedded in paraffin after being placed in a 10% neutral formalin solution. Haemotoxylin and eosin (H&E) was used to stain sagittal sections of the cerebellum, which were 5 µm thick. The sections were then examined under a light microscope and photographed with a camera.

3.7 Statistical analysis

Data expressed as Mean ± SEM were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test as a post hoc by using Graph Pad prism software (5.03 version) and Chi-square test. p values less than 0.05 were considered as statistically significant.

4. RESULTS

4.1.Behavioral Studies

4.1.1 Effect of Rhodiola rosea on altered locomotor activity test in Acrylamide induced neuropathic mice:

Locomotor activity serves as an indicator of underlying general toxicity by enabling the tracking of the animals' movements throughout treatment-induced disruptions in sleep/wake patterns. When compared to the normal control group, the ACR-treated group showed a statistically significant ($p<0.001$) drop in locomotor activity. When compared to the ACR control group, R. rosea significantly corrected the ACR-induced decrease in locomotor activity at doses of 100 and 200 mg/kg ($p<0.001$). Table 1 tabulates the results.

S.No	Groups	ACTOPOTOMETER COUNT (5 min)		
		7^{th} day	14^{th} day	21^{st} day
1	Normal Control	190±0.00	190±0.00	190±0.00
2	Acrylamide Treated (ACR)	$98 \pm 0.91^{\alpha}$	$56.77 \pm 1.44^{\alpha}$	$22.81 \pm 0.61^{\alpha}$
3	ACR+Rhodiola rosea(100mg/kg)	$145 \pm 0.27^{\beta,a}$	$156.14 \pm 2.27^{\gamma,a}$	$159.76 \pm 0.40^{\gamma,a}$
4	ACR+Rhodiola rosea(200mg/kg)	173.19 ± 1.23^{a}	181.33 ± 3.28^{a}	187.25 ± 2.09^{a}

Table 1: Effect of Rhodiola rosea (100 & 200 mg/kg) on Acrylamide induced altered Locomotor score

Data are expressed as Mean ± SEM. Data analyzed by one way ANOVA followed by Tukey's test.

$\alpha p<0.001$, $\beta p<0.01$, $\gamma p<0.05$ when compared to Normal Control.

$ap<0.001$ when compared to ACR treated group

4.1.2 Effect of Rhodiola rosea on Hind Limb Impairment in Acrylamide induced neuropathic mice

The narrow beam assay is a metric used to identify modest impairments in balance, coordination, and motor skills, primarily in the rear limbs. When compared to normal control, administration of ACR has demonstrated a substantial increase in hind limb disability from day 14 to day 21. When compared to groups treated with ACR, treatment with R. rosea (100 mg/kg) considerably decreased hind limb impairment; however, R. rosea (200 mg/kg) had a noticeable effect from day 14 to day 21 and returned the levels to normal. Table 2 tabulates the results.

S.No	Groups	NARROW BEAM TEST SCORE		
		7th day	14th day	21st day
1	Normal Control	0±0	0±0	0±0
2	Acrylamide Treated (ACR)	$0.5\pm0.22^{\beta}$	$2.33\pm0.33^{\alpha}$	$4\pm0.33^{\alpha}$
3	ACR+Rhodiola rosea(100mg/kg)	$0.33\pm0.21^{\gamma,c}$	$1.66\pm0.42^{\beta,a}$	$0.36\pm0.04^{\gamma,a}$
4	ACR+Rhodiola rosea(200mg/kg)	0 ± 0^{a}	0 ± 0^{a}	0.16 ± 0.02^{a}

Table 2: Effect of Rhodiola rosea (100 & 200 mg/kg) on Acrylamide induced altered Hind Limb Impairment

Data are expressed as Mean ± SEM. Data analyzed by one way ANOVA followed by Tukey's test.

$\alpha p<0.001$, $\beta p<0.01$, $\gamma p<0.05$ when compared to Normal Control.

$ap<0.001$, c $p<0.05$ when compared to ACR treated group.

4.1.3 Effect of Rhodiola rosea on Acrylamide induced altered Grip Strength in mice

The rota rod test evaluates an animal's motor clumsiness. The animal's balance, coordination, physical fitness, and motor planning are all evaluated by how long it remains on the rotating rod. When compared to the normal control group, the ACR-treated group showed a significant ($p<0.001$) decrease in latency to fall off the rod. While treatment with R. rosea (200 mg/kg) significantly restored the levels to normal by the 21st day, administration of R. rosea significantly increased the latency to fall from the rod during the 7th to 14th day and the impact became more evident ($p<0.001$) from the 14th to the 21st day. Table 3 shows the outcomes of the calculation.

S.No	Groups	GRIP SCORE (3 min)		
		7th day	14th day	21st day
1	Normal Control	180±0	180±0	180±0
2	Acrylamide Treated (ACR)	$119.33\pm0.9^{\gamma}$	$56.77\pm1.47^{\alpha}$	$12.81\pm0.6^{\alpha}$
3	ACR+Rhodiola rosea(100mg/kg)	$141.77\pm.87^{\beta,b}$	$141.24\pm2.27^{\gamma,a}$	$145.76\pm0.40^{\beta,a}$
4	ACR+Rhodiola rosea(200mg/kg)	148.29 ± 1.73^{a}	151.33 ± 3.28^{a}	158.25 ± 2.09^{a}

Table 3: Effect of Rhodiola rosea (100 & 200 mg/kg) on Acrylamide induced altered Grip Strength

Data are expressed as Mean ± SEM. Data analyzed by one way ANOVA followed by Tukey's test.

$\alpha p<0.001$, $\beta p<0.01$, $\gamma p<0.05$ when compared to Normal Control.

$a p<0.001$, b $p<0.01$ when compared to ACR treated group.

4.1.4 Effect of Rhodiola rosea on Cooperation Movement Ability under high Altitude Stress in Acrylamide induced neuropathic mice

Crawling along a rope test evaluates an animal's capacity for cooperative movement under high altitude stress. By the end of the trial, a larger percentage of the mice treated with ACR were either unable to grasp a rope firmly or were able to grab the rope but were unable to crawl along it; the ACR-treated group's grip strength was significantly reduced in comparison to the normal control group. When compared to the ACR-treated group, the animals' gripping strength increased significantly and dose-dependently with R. rosea treatment, indicating that they spent a larger portion of their time on the rope. Table 4 tabulates the results.

S.No	Groups	No. of Mice Crawling along the Rope		
		7th day	14th day	21st day
1	Normal Control	6	6	6
2	Acrylamide Treated (ACR)	5^{β}	3^{α}	0^{α}

3	ACR+Rhodiola rosea(100mg/kg)	$5^{\beta,c}$	4^{a}	5^{a}
4	ACR+Rhodiola rosea(200mg/kg)	6^{b}	6^{a}	6^{a}

Table 4: Effect of Rhodiola rosea on Cooperation Movement Ability under high Altitude Stress in Acrylamide induced neuropathic mice

Data are expressed as Mean ± SEM. Data analyzed by one way ANOVA followed by Tukey's test.

αp<0.001, βp<0.01, when compared to Normal Control.

ap<0.001, b p<0.01 , c p<0.05 when compared to ACR treated group.

4.1.5 Effect of Rhodiola rosea on Hot Hyperalgesia test in Acrylamide induced neuropathic mice

Using Eddy's Hot plate test (45±1°C–Non-noxious Stimuli), thermal hyperalgesia was evaluated. It enables the evaluation of mice's aberrant pain sensitivity. Compared to normal control, mice treated with ACR showed a statistically significant decrease in hind paw licking latency. While treatment with R. rosea (200 mg/kg) resulted in an increase in hind paw licking latencies from day 14 to day 21, showing similar effects to the normal control mice, administration of R. rosea considerably increased the latency to hind paw licking. Table 5 tabulates the results.

		FIRST HIND PAW LICKING (12 s)		
S.No	**Groups**	7^{th} day	14^{th} day	21^{st} day
1	Normal Control	11.67±0.61	11.67±0.61	11.67±0.67
2	Acrylamide Treated (ACR)	$8.50±0.34^{\alpha}$	$6.00±0.36^{\alpha}$	$4.66±0.21^{\alpha}$
3	ACR+Rhodiola rosea(100mg/kg)	$7.50±0.22^{b,\alpha}$	$7.33±0.33^{a,\alpha}$	$8.50±0.42^{a,\alpha}$
4	ACR+Rhodiola rosea(200mg/kg)	$9.00±0.25^{a,\alpha}$	$10.00±0.25^{a,\beta}$	$11.0±0.36^{a}$

Table 5: Effect of Rhodiola rosea (100 & 200 mg/kg) on altered Hyperalgesia (45°C) in Acrylamide induced neuropathic mice

Data are expressed as Mean ± SEM. Data analyzed by one way ANOVA followed by Tukey's test.

αp<0.001, βp<0.01, when compared to Normal Control.

ap<0.001, b p<0.01 when compared to ACR treated group.

4.2.Biochemical Parameters

4.2.1 Effect of Rhodiola rosea on Oxidative Parameters in Acrylamide induced neuropathic mice

Studies in homogenates showed a significant enhancement of oxidative stress after ACR administration as revealed by the significant increment of MDA, decrease of GSH & NO activity, following treatment with R.rosea (200mg/kg) levels of MDA, GSH & NO was restored to normal. The results are tabulated in **Table 6**.

S.No	Groups	OXIDATIVE PARAMETERS (BRAIN)		
		MDA	**GSH**	**NO**
1	Normal Control	8.26±0.58	2.08±0.06	53.34±0.78
2	Acrylamide Treated (ACR)	26.03±0.58$^{\alpha}$	0.53±0.06$^{\alpha}$	126.03±1.028$^{\alpha}$
3	ACR+Rhodiola rosea(100mg/kg)	15.73±0.32$^{\beta,a}$	0.95±0.02 $^{\beta,b}$	80.10±1.63 $^{\beta,b}$
4	ACR+Rhodiola rosea(200mg/kg)	8.83±0.27^{a}	1.86±0.076 $^{\gamma,a}$	66.58±0.40^{a}

Table 6: Effect of Rhodiola rosea (100 & 200 mg/kg) on Acrylamide induced altered Oxidative Parameters in Mice Brain

Data are expressed as Mean ± SEM. Data analyzed by one way ANOVA followed by Tukey's test.

αp<0.001, βp<0.01, γp<0.05 when compared to Normal Control.

ap<0.001, b p<0.01 when compared to ACR treated group.

4.3.Histopathology:

As shown in Figure 9, Histopathological changes of Acrylamide treated rats showed severe vacuolar degeneration and Large number of neuronal death (1b), Whereas the Rhodiola rosea treated rats showed Moderate inflammation with low dose(100mg/kg, 1c) and appeared normal with high dose(200mg/kg , 1d) of Rhodiola rosea.

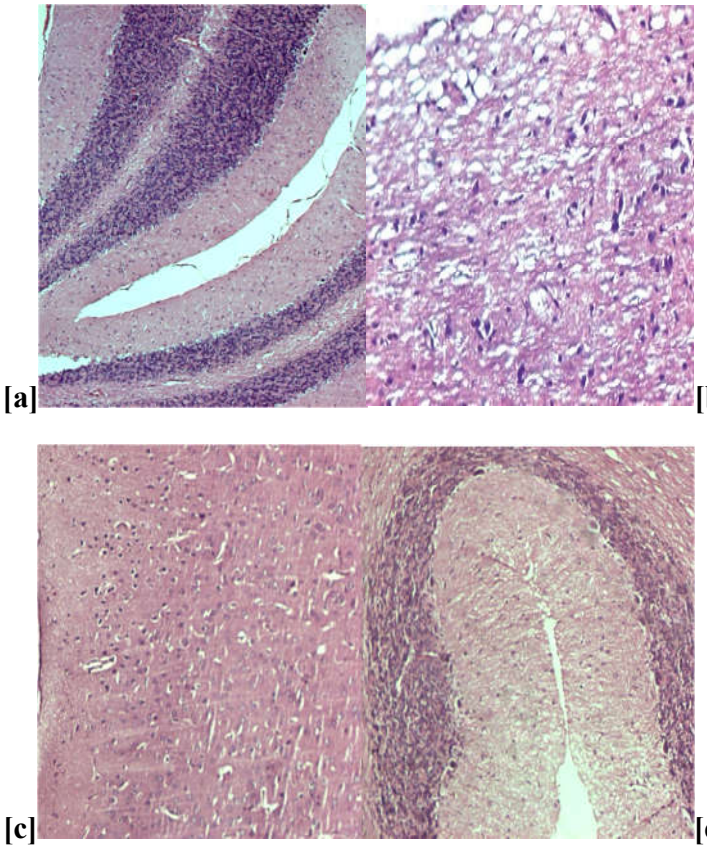

Figure 9: Sagittal sections of cerebellum stained with H&E showing Vacuolar Degeneration.

Normal Control group (a)

Disease Control group (b)

Rhodiola rosea (100 & 200mg/kg) treated group(c, d)

5. DISCUSSION

Extensive experimental evidence from mouse studies has demonstrated that exposure to ACR causes severe neurotoxicity and that oxidative stress is associated with these effects. Because of all of these factors, ACR-neurotoxicity has been transformed into an appropriate experimental paradigm for assessing the possible intervention of novel neuro-protective drugs with antioxidant properties. Therefore, assessing R. rosea's ability to prevent ACR-induced neuro-physiological problems and highlighting potential processes was the main goal of the current study. These findings demonstrated typical behavioral deficiencies, as anticipated from earlier research.

Numerous studies have indicated that the narrow beam test is a crucial metric for determining the degree of hind limb damage and motor dysfunction in experimental animals. In a dose-dependent fashion, R. rosea administration has considerably avoided

the impairment. Compared to rats given a lesser dose, those given 200 mg/kg of R. rosea showed a notable improvement.

A valid test for examining motor dysfunction that necessitates motor coordination is the rotarod paradigm. According to the current findings, mice inebriated with ACR exhibited impaired motor function. This lends credence to the idea that selective nerve terminal injury, both in the peripheral and central nervous systems, is linked to ACR intoxication. When R. rosea was administered, the motor incoordination levels returned to almost normal.

Similar to this, sensitivity to heat stimuli is a crucial factor in assessing neuropathy brought on by acrylamide. The injection of ACR considerably reduced the delay to the initial licking of the hind paw in this investigation. It is commonly known that the main causes of hyperalgesia in acrylamide-induced neuropathy were axonal degradation and a changed pain threshold at the nerve terminals. According to other research, the pathophysiological processes for the reduction in pain threshold in acrylamide-induced neuropathic mice may involve enhanced pain signaling pathways in the central nervous system (CNS) brought on by degeneration of the brain stem, spinal cord, and dendrites of purkinje cells in the cerebellum. Because of its antioxidant properties, Rhodiola rosea treatment markedly increased the altered latency to the hind paw licking.

Numerous chemically induced cell damage and neurodegenerative disorders have been shown to be largely caused by oxidative stress and mitochondrial dysfunction [45,46]. An imbalance between pro-oxidants and antioxidants that results in apoptosis is known as oxidative stress, and it is caused by increased production of reactive species and reactive nitrogen species as well as the depletion of the antioxidant defense system. It has been proposed that the mechanism linking environmental exposure to acrylamide in workplaces and those who eat fried foods high in carbohydrates is acrylamide-induced oxidative stress.

The development of neuropathy in mice treated with ACR is known to be influenced by several pathological circumstances, including elevated MDA, NO, and impaired antioxidant defense system (GSH) in the cerebellum and spinal cord. According to these findings, neuropathic mice's LPO levels significantly increased in comparison to the control group, suggesting that exposure to environmental pro-oxidants such acrylamide may cause the production of reactive oxygen species (ROS). Administration of R. rosea dramatically decreased LPO. Based on their reducing power, the polyphenols in RR have the ability to donate electrons and H+, which is thought to be the reason for the LPO chain reaction's termination. Several studies have shown that flavanoids interact with cell membranes, improving their stability, thereby protecting them from LPO.

GSH conjugation, in which acrylamide is converted to glycidamide (a reactive epoxide) and conjugated with glutathione, is the primary mechanism of ACR metabolism. ACR may raise intracellular ROS and oxidative damage by lowering the antioxidant levels of the cells through the depletion of GSH. Reduced GSH levels in the brain and spinal cord were considerably reduced after ACR was administered, according to Beiswanger et al. (1993). These findings are consistent with our results.

The maintenance of pain in neuropathic animals, which NOS inhibitors reduce, depends on the increased local production of NO. NO donor's sodium nitroprusside and NO precursor L-arginine both promote the hyperalgesia. The generation of NO rises in response to increasing NOS activity. Endothel, inducible, and neuronal NOS (eNOS, iNOS, and nNOS, respectively) are the three NOS genes that are known to have different tissue location and characteristics [47]. The conventional Ca2+-dependent activation of eNOS and nNOS is characterized by nNOS's strong coupling to the Ca2+-permeable NMDA receptor (NMDAR), which is connected to the CNS's postsynaptic densities (PSD-95) via their shared PDZ binding motifs. While iNOS can produce micromolar quantities of NO, eNOS and nNOS only produce low nanomolar concentrations.

Based on the actual results, it is likely to speculate that Rhodiola rosea treatment may effectively protect intoxicated mice through its antioxidant effects, as occurred with rutin, crocin, and thalassemia testudinum, even though the mechanism of action of such effects is outside the scope of the current work.

The histological results of mice treated with ACR in this investigation revealed significant vacuolar degeneration, indicating neuropathy. In contrast, the mice treated with rhodiola had fewer vacuoles, and the animals treated with greater doses looked nearly normal.

6. SUMMARY & CONCLUSION

In summary, in the present study, treatment with Rhodiola rosea has shown significant protective effect on acrylamide induced neuropathy characterized by,

1. Decreased hind limb impairments.
2. Altered Locomotor activity
3. Altered Cooperation Movement Ability Under high Altitude Stress
4. Increased Pain Threshold
5. Increased antioxidant profile in rat cerebellum and spinalcord.

In conclusion the present study implies that Rhodiola rosea enriched with Bioflavanoids, Poly phenols and Triterpenes may be particularly useful xenobiotics detoxifying agent as it could decrease lipid peroxidation and enhance brain Antioxidants and significantly prevent the brain from Neurotoxic Effects of Acrylamide

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ETHICAL STATEMENT

All experimental procedures involving animals were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and approved as per the

norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

COMPETING INTERESTS

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

AUTHORS' CONTRIBUTION

Joga Sarika Lead Author: Conceptualization, Methodology, Writing – Original Draft, Supervision

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A V Vasanthi Co-author 2: Writing – Review & Editing, Formal Analysis

B Medha Gayatri Co-author 3: Formal Analysis

A Thanmai Co-author 4: Critical Reviews

All authors have read and approved the final manuscript.

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