

TO EVALUATE THE NEUROPROTECTIVE ACTIVITY OF CATHARANTHUS ROSEUS AGAINST 3-NITROPROPIONIC ACID INDUCED HUNTINGTON'S DISEASE IN WISTAR RATS

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disorder characterized by progressive neuron loss, particularly in the striatum, leading to motor dysfunction, cognitive decline, and psychiatric issues. HD is caused by an elongated CAG trinucleotide repeat in the Huntingtin gene, resulting in a mutant Huntingtin protein (mHTT) that leads to neuronal death. To standardize animal models of Huntington's disease, assess physical and behavioral parameters, and study the neuroprotective effects of alkaloids by evaluating biochemical markers like oxidative stress, mitochondrial dysfunction, and neuro-inflammation, exploring potential mechanisms and treatment effects on different brain regions. This study aimed to evaluate the neuroprotective effects of *Catharanthus roseus* against 3-nitropropionic acid (3-NP)-induced HD in Wistar rats. Biochemical analysis of lipid peroxidation, glutathione, nitrite levels, and mitochondrial function. *Catharanthus roseus* significantly reduced lipid peroxidation and nitrite levels while increasing glutathione and protein levels. Motor coordination and anxiety behavior improved significantly. The study concluded that *Catharanthus roseus* exhibits neuroprotective effects, making it a potential therapeutic candidate for HD.

Keywords: Huntington's disease, Neuronal death, *Catharanthus roseus*, 3-Nitropropionic acid.

1. INTRODUCTION

Huntington's disease (HD) is an inherited neurodegenerative disorder, transmitted in an autosomal dominant manner, characterized by progressive motor, cognitive, and psychiatric symptoms. Symptoms usually appear around age 40, with juvenile (before 20) and late-onset (after 70) forms also occurring (Craufurd and Snowden., 2002; Nance and Westphal., 2002; Rothlind *et al.*, 1993). The condition is caused by an extended CAG (cytosine, adenine, guanine) repeat (36 or more repetitions) on the short arm of chromosome 4p16.3 in the huntingtin gene (O'Donovan., 1993). The huntingtin (Htt) protein undergoes an abnormally long polyglutamine (polyQ) expansion due to the mutation. This expansion causes the mutant Htt to acquire one or more toxic activities, leading to neurodegeneration (Ross *et al.*, 2011). While brain pathology is a well-known hallmark of HD, a growing body of research suggests that peripheral tissue pathology also plays a significant role in disease progression (MacDonald *et al.*, 1993). Children of carriers of the Huntington's disease (HD) gene have a 50% chance of inheriting the condition. Intermediate numbers of repeats, ranging from 27 to 35, are typically not associated with disease expression but can expand during paternal transmission, potentially leading to the disease in descendants. Repeats of 36 to 39 are associated with reduced penetrance, meaning that some individuals in this range may develop HD while others may not (Chong *et al.*, 1997; MaatKievit *et al.*, 2001; Semaka *et al.*, 2010). The prevalence of Huntington's disease (HD) is estimated to be between 10.6 and 13.7 per 100,000 individuals in Western populations, with an average age of onset commonly in mid-life. HD is a complex disease with a broad impact on the lives of patients, families, and caregivers. Management of HD should be provided through a multidisciplinary approach, including pharmacological and non-pharmacological interventions. Other symptoms, such as weight loss, skeletal muscle wasting, and cachexia, become relevant with a decline in quality of life, increased comorbidity, and risk for mortality (McColgan and Tabrizi., 2018).

Huntingtin is involved in several crucial cellular processes, including protein trafficking, vesicle transport, postsynaptic signaling, transcriptional regulation, and apoptosis. The loss of normal huntingtin function, coupled with the toxic gain of function from the mutant protein, disrupts various intracellular pathways. This disruption is further compounded by excitotoxicity, dopamine toxicity, metabolic impairment, mitochondrial dysfunction, oxidative stress, apoptosis, and autophagy, all contributing to the progressive neurodegeneration observed in Huntington's disease (Haim *et al.*, 2015; Crotti and Glass., 2015; Ransohoff and Perry., 2009). These changes are accompanied by neurochemical alterations, which involve not only glutamate receptors but also other receptors, such as dopamine (DA) and adenosine receptors involved in motor functions. HD is a classic example of an autosomal dominant disease. The age of onset and disease severity are dictated by the extent of the HD gene mutation and by the sex of the patient. However, environmental factors and genetic modifiers can modify the variability of clinical expression (Centonze *et al.*, 2003).

A member of the Apocynaceae family, *Catharanthus roseus* is indigenous to Madagascar and is sometimes referred to as the Madagascar periwinkle. *Catharanthus roseus*, formerly known as *Vinca rosea*, is the primary source of vinca alkaloids, which are

also occasionally referred to as catharanthus alkaloids (Brun., 2001). Presently, it is cultivated in Europe, India, China, and America. It was used to cure a variety of illnesses around the world, from lung and eye infections to coughs and sore throats. Its traditional usage in treating diabetes is its most intriguing feature. (Kaushik et al., 2017). This plant produces a diverse array of secondary metabolites that are pharmaceutically important and used as chemotherapeutic agents in the treatment of several types of cancers (Senthilraja et al., 2015).

Catharanthus roseus is an herbal plant that has been shown to have neuroprotective properties, which means it can protect the brain and nervous system from damage or degeneration.

Vincamine: This is an alkaloid that is extracted from the leaves of the plant. It can improve blood flow and oxygen supply to the brain, enhancing cognitive function and memory. It can also prevent or treat conditions such as Alzheimer's disease, dementia, stroke, and vertigo (Balandrin et al., 1985)

Vinpocetine: This is a synthetic derivative of vincamine that has similar effects but is more potent and bioavailable. It can also modulate the levels of neurotransmitters such as acetylcholine, dopamine, and serotonin, which are involved in learning, mood, and motivation (Jacobs et al., 2004).

It has been reported to have numerous pharmacological actions, viz., anticancer and antitumor properties, Anti-ulcer activity, Enhancement of Memory, Antioxidant activity.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

3-NP was procured from D.P. Traders & Services D-5 Industrial Area, Phase 1 Mohali, and tetrabenazaine was purchased from a local medical shop.

2.2 Plant Material Collection and Identification

In September 2023, fresh leaves of Catharanthus roseus were harvested from G.H.G. Khalsa College of Pharmacy Guruser Sadhar, Ludhiana The Raw Material Herbarium and Museum, Delhi (RHMD), using the specimen reference number NIScPR/RHMD/Consult/2023/4589-90, identified and verified the plant material.

Catharanthus Roseus Leaves: Prepare The Extracts In September 2023, fresh Catharanthus roseus leaves were gathered from Ludhiana. The plant material was carefully cleaned and allowed to dry in a regulated shade environment. A powerful grinding machine was used to reduce the dried material to a fine powder. For the extraction process, the cold maceration method was applied 1.6 letter ethanol at room temperature used to soak the 165 g of powdered plant material. The extract was vacuum-filtered through Whatman Filter Paper No. 1 after a week. After being steeped in ethanol for a further seven days, the residue was once again filtered. After combining the filtrates, ethanol was vacuum-expelled using a 45° rotating evaporator (Buchi Rotavapor R-200). The dried, green residue was redissolved in 75 milliliters of ethanol, then diluted with 75 milliliters of distilled water and acidified to pH 1 using 3% hydrochloric acid. 75 milliliters of hexane were used three times to extract this combination. The fraction of hexane was disposed of. After being cooled to 10 °C and basified with a 25% ammonia solution to a pH of 10, the residual aqueous layer was extracted using 75 mL of dichloromethane. To obtain 23.60 g, the mixed dichloromethane extract was dried over anhydrous sodium sulfate, rinsed with 25 mL of brine, and evaporated until dry (Mishra et al., 2001).

2.3 Experimental animals

In accordance with the National Guidelines on the Proper Care and Use of Animals in Laboratory Research, Indian National Science Academy, New Delhi (2000), all of the animals were authorized by the Institutional Animal Ethics Committee (Project Proposal No. G.H.G/2024/IAEC/P03/M05). Adult male Wistar rats (2–3 months old), weighing 150–200 g, were obtained and kept at the Central Animal House of the G.H.G. Khalsa College of Pharmacy in Guruser Sadhar, Ludhiana, under standard circumstances, with unlimited access to food and water *ad libitum*.

2.4 Acute oral toxicity and dose selection

In accordance with Organization for Economic Co-operation and Development (OECD) 425 points (Acute Oral Toxicity – Up-and-Down Procedure; OECD 425/2001), the acute oral toxicity and dose selection of ethanol extracts were studied in Wistar rats. Four hours before the dose, the animals were fasted. Animals were forced to eat oral methanol extracts at multiple dose levels up to 5000 mg/kg. Throughout a 21-day period, the animals were monitored every day for clinical indications of toxicity, behavioral alterations, altered movement, and death. A total of 30 Wistar rats were split into five groups at random, each group containing six Wistar rats, and treated for 21 days as follows: 3-nitropropionic acid (10 mg/kg) I.P. was administered for 21 days.

2.5 Experimental grouping

The experimental protocol was divided into two phases and consists of five groups. The experimental protocol for the phase I and II study is given below:

Phase I: In this phase, we have evaluated the effect of ethanol extract at medium dose Group I: Saline was administered to the rats.

Group II: 3-NP (10 mg/kg; i.p) (for 21 days)

Group III: Nitropropionic acid (21 days) + tetrabenazaine (1 mg/kg) p.o. (4 weeks)

Group IV: 3-NP (10 mg/kg; i.p) + Catharanthus roseus extract (250 mg/kg) p.o. (4 weeks)) Group V: 3-NP (10 mg/kg; i.p) + Catharanthus roseus extract (500 mg/kg) p.o. (4 weeks)

Phase II

Phase I involved the randomization and division of 30 rats into 5 groups (n = 6).

Before the end of Phase I, six rats from each group underwent two trials per day, six days per week. The Animals were observed daily for 21 days for clinical signs of toxicity including behavioral changes, locomotion and thiobarbituric acid reactive compounds (TBARS) and antioxidants were estimated in six rats per group.

2.6 Treatment schedule

3-NP was dissolved in normal saline (adjusted pH to 7.4) and administered intraperitoneally (i.p) at a dose of 10 mg/kg for 21 days. Ethanol extract (250 mg/kg, 500 mg/kg) were suspended in 0.5% w/v CMC (carboxy methyl cellulose) solution and administered per orally for 21 days in 3- NP treated groups, one and half hour prior to 3-NP dosing. On 0, 7th, 14th, and 21st days, behavioral parameters like body weight, narrow beam, grip strength, locomotor activity and motor coordination were assessed. On 22nd day, animals were sacrificed and striatum was isolated to carry out the evaluation of biochemical Lipid peroxidation (LPO), Glutathione (GSH), and nitrate], mitochondrial dysfunction (Complex II) and neurochemical [Dopamine (DA), Nor-epinephrine (NE), 5-hydroxy tryptamine (5-HT), GABA, glutamate and their metabolites] parameters.

2.7 Measurement of body weight

Animal body weight was recorded on the first and last days (21st day) of experiment (Kumar *et al.*, 2011). Percentage change in body weight was calculated. $\text{Change in BodyWeight} = \frac{\text{BodyWeight}(1\text{st day} - 21\text{st day})}{\text{Body Weight (1st day)}} \times 100$

2.8 Behavioral assessment

1. Groups For The Open Field Test: Five groups of rats, each comprising six rats, were employed in the present study. Group 1:

Normal Group: (Vehicle treated, p.o.)

Group 2: Nitropropionic acid (10 mg/kg, I.P.) was administered orally 30 min before the test.

Group 3: Tetrabenzaine (1 mg/kg) p.o. was administered 30 min before the test.

Group 4: Catharanthus roseus extract (250 mg/kg) p.o. was administered 30 min before the test. Group 5: Catharanthus roseus extract (500 mg/kg) p.o. was administered 30 min before the test.

Open-field test The open field equipment used in this experiment included a rectangular, brown-colored apparatus measuring 100 × 100 cm with a 40 cm-high enclosing wall. It was utilized to monitor the locomotor activity of rats. The floor of the apparatus was divided into 25 smaller squares. A 40-watt white bulb was positioned 150 cm above the test apparatus to illuminate the area. Before the actual recording on the open field equipment, the animals were given a 10-minute acclimation period to move freely. Two hours after their initial exposure to the equipment, each animal was placed in the center, and the number of squares crossed, grooming episodes, and rearing behaviors within a 10-minute period were recorded. A crossing was counted only when all four paws of the animal entered a new square. After each trial, the equipment was thoroughly cleaned. The total activity for each 10-minute session was calculated by summing the number of squares crossed, grooming episodes, and rearing behaviors exhibited by the animal (Thangarajan *et al.*, 2014; de Lago *et al.*, 2005).

2. Groups for the Rota rod activity test: Five groups of rats, each comprising six rats, were employed in the present study. Group 1:

Normal Group: (Vehicle treated, p.o.)

Group 2: Nitropropionic acid (10 mg/kg, I.P.) was administered orally 30 min before the test. Group 3: Tetrabenzaine (1 mg/kg) p.o. was administered 30 min before the test.

Group 4: Catharanthus roseus extract (250 mg/kg) p.o. was administered 30 min before the test. Group 5: Catharanthus roseus extract (500 mg/kg) p.o. was administered 30 min before the test.

The motor coordination and grip strength of rats were evaluated using rotarod equipment. Before the actual recording on the rotarod, the animals were given a prior training session for acclimation on the apparatus, which operated at a constant speed of 25 rpm with a rod diameter of 7 cm. The cut-off time was set at 180 seconds, and each rat performed three separate trials with a 5-minute interval between each trial. The average time to fall was recorded for each rat (Kulkarni., 1987; Kumar *et al.*, 2014).

3. Groups for the elevated plus maze test: Five groups of rats, each comprising six rats, were employed in the present study. Group 1:

Normal Group: (Vehicle treated, p.o.)

Group 2: Nitropropionic acid (10 mg/kg, I.P.) was administered orally 30 min before the test. Group 3: Tetrabenzaine (1 mg/kg) p.o. was administered 30 min before the test.

Group 4: Catharanthus roseus extract (250 mg/kg) p.o. was administered 30 min before the test.

Group 5: Catharanthus roseus extract (500 mg/kg) p.o. was administered 30 min before the test

Elevated Plus Maze The Elevated Plus Maze (EPM) test is primarily used to measure anxiety in rodents and can also be modified to evaluate spatial learning and memory. The EPM consists of two opposite open arms (50 cm × 10 cm) and two closed arms of the same size with 40 cm high walls, all connected by a central square (10 cm × 10 cm). The entire maze is elevated 50 cm above the ground. At the end of the experimental period, animals are tested for acquisition memory. Each rat is placed individually at one end of the open arm, and the time taken to move from the open arm to the closed arm is recorded as the initial transfer latency (ITL). After recording the ITL, the rats are allowed to explore the maze for 20 seconds before being returned to their home cages. On the last day, the retention of memory is assessed by placing the rat in an open arm again and noting the retention latency, which measures

how long it takes for the animal to move from the open arm to the closed arm. This method effectively measures both anxiety-related behavior and cognitive functions such as learning and memory in rodents (Pawlak *et al.*, 2012).

2.9 Brain tissue collection and homogenization

On the 21st day, animals from each group were randomly divided into three sub-groups: one for biochemical estimations, the second for neuroinflammatory marker estimation, and the third for neurochemical estimation immediately after the behavioral assessments. Prior to euthanasia, rats were anesthetized via intraperitoneal injection of a mixture of thiopental (0.1 g/kg) or by CO₂ narcosis. The rats were transcardially perfused with 0.9% ice-cold saline, and the whole brain was isolated. The hippocampus, striatum, and cortex were dissected using sterilized surgical blades and ophthalmic forceps. Sectioned brain parts were then kept immediately on dry ice before being stored in a deep freezer at -80 °C. To prepare brain homogenate, the brain samples were homogenized in ice-cold phosphate buffered saline (PBS) (10% w/v) on ice. Homogenized brain samples were then centrifuged at 12,000 g for 15 minutes at 4 °C. The supernatant was collected and used for the following biochemical analysis (Kaur *et al.*, 2015).

2.10 Biochemical Assessment (Measurement of oxidative stress parameters)

1. Measurement of lipid peroxidation: To 0.5 ml of supernatant, 0.5 ml of Tris HCl was added and incubated at 37 °C for 2 hours. After incubation, 1 ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 10000 g for 10 min. To 1 ml of supernatant, 1 ml of 0.067% thiobarbituric acid was added, and the tubes were kept in boiling water for 10 min. After cooling, 1 ml of double-distilled water was added, and the amount of malondialdehyde (MDA), a measure of lipid peroxidation, was measured by reaction with thiobarbituric acid at 532 nm using a UV-1700, Shimadzu, Japan (Binawade *et al.*, 2013).

2. Estimation of nitrite: 100 µl of sample or standard (100, 200, 400, 800, 1000 µg/ml) was added to 400 µl of distilled water. 500 µl of Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride + 1% sulphanilamide + 5% phosphoric acid) was added to the above solution and kept at room temperature for 5 min. Absorbance was measured at 546 nm, and the values of nitrite concentration were obtained from the sodium nitrite standard curve and expressed in µg/m (Binawade *et al.*, 2013).

3. Estimation of glutathione The mixture of homogenate and 4% sulfosalicylic acid was kept at 4 °C for one hour. Then the mixture was centrifuged at 1200 × g for 15 min at 4 °C. The supernatant was collected, and phosphate buffer (0.1 M, pH 8) and DTNB (0.4% w/v in 0.1 M phosphate buffer, pH 8) were added to it. Absorbance was measured immediately spectrophotometrically at 412 nm (Kaur *et al.*, 2015).

4. Protein estimation: To 0.1 ml of sample or standard (10 µg, 20 µg, 40 µg, 80 µg, and 100 µg/ml), 0.1 ml of 2 N NaOH was added and hydrolyzed at 100 °C for 10 min in a heating block or boiling water bath. Hydrolysate was allowed to cool down, and 1 ml of freshly mixed complex-forming reagent [2% (w/v) Na₂CO₃ in distilled water + 1% (w/v) CuSO₄·5H₂O in distilled water + 2% (w/v) sodium potassium tartrate in distilled water] was added to it. The solution was kept at room temperature for 10 minutes. To the above solution, 0.1 ml of Folin reagent was added, and the mixture was kept at room temperature for 30–60 min (do not exceed 60 min). Absorbance was noted at 660 nm. A standard curve of absorbance vs. protein concentration was plotted and used to determine the unknown protein concentrations (Hariharan *et al.*, 2014).

5. Complex II (SDH activity): Potassium ferricyanide oxidizes succinic acid to fumaric acid. The reaction was started by adding mitochondrial preparation (0.05 ml) to the reaction mixture consisting of 1.5 ml of phosphate buffer (0.2 M, pH 7.8), 0.2 ml of Succinic acid (0.6 M, pH 7.8), 0.3 ml of BSA (1%, w/v), and 0.1 ml of potassium ferricyanide (0.3 M). A decrease in absorbance was recorded at 420 nm for 3 minutes against water as a blank. Results were expressed as nmol succinate oxidized/min/mg protein (Hariharan *et al.*, 2014).

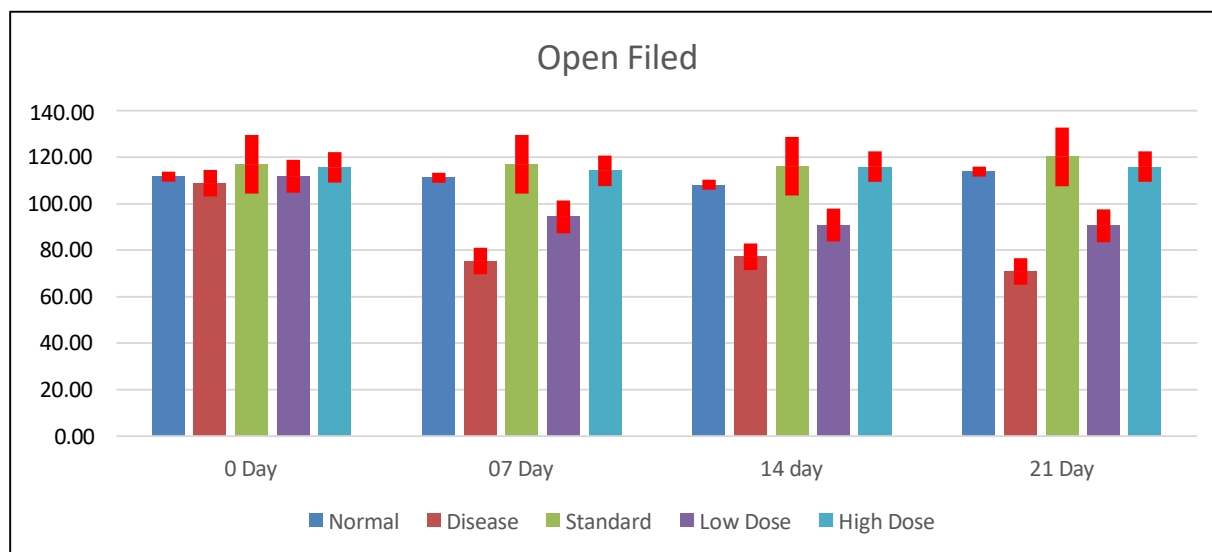
3. RESULTS

3.1 Effect of *Catharanthus roseus* extracts on 3-NP Induced changes in Open field test

Ethanol extracts of *Catharanthus roseus* were prepared and tested in 3-NP-treated rats at doses of 250 mg/kg p.o. and 500 mg/kg p.o. In the present study, ethanol extract showed more promise. Based on these findings, we further preceded with lower (250 mg/kg; p.o.) and higher doses (500 mg/kg; p.o.) of ethanol extract and checked their activity in 3-NP-treated rats. Systemic 3-NP (10 mg/kg/day; i.p.) treatment produced a significant (p<0.05) gradual decrease in locomotor activity on the 1st week (day 0), 2nd week (day 7th), 3rd week (day 14th), and 4th week (day 21st) as compared to the normal control group. Pretreatment with ethanol extracts of *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day; p.o.) significantly (p<0.05) prevented the impairment in locomotor activity as compared to 3-NP-treated rats. The effect of EECR (500 mg/kg/day; p.o.) was more significant (p<0.05) as compared to EECR (250 mg/kg/day; p.o.).

Table.1\Showing data expressed Mean±SD of Open field

Treatment	Activity time (sec)			
	0 Day Mean±SD	07 Day Mean±SD	14 day Mean±SD	21 Day Mean±SD
Normal	111.67±2.16	111.17±5.71	108.17±6.37	113.83±5.38
Disease	108.83±5.19	75.33±6.09	77.17±4.07	70.83±5.53
Standard	117.00±12.62	117.00±9.82	116.17±7.63	120.17±14.36
Low Dose	111.83±7.08	94.33±4.37	90.83±4.07	90.50±3.78
High Dose	115.67±6.56	114.17±2.64	116.00±3.63	116.00±2.76



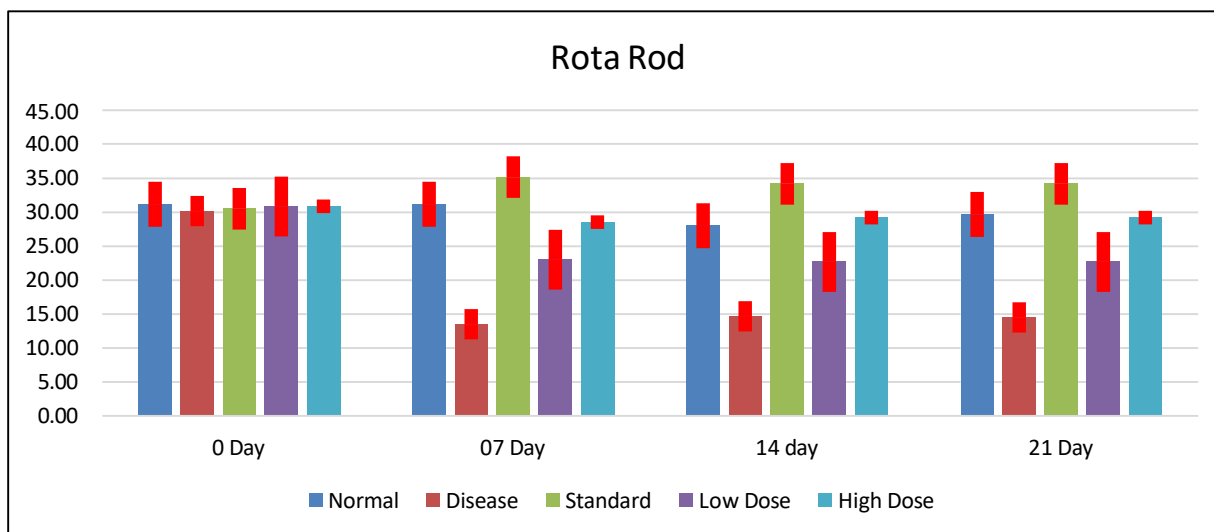
Graph:1.1 Effect of *Catharanthus roseus* extracts on 3-NP induced changes in locomotor activity: Data expressed as Mean $\pm$ SD: $p_a < 0.05$ v/s 3-NP (10mg/kg), $p_b < 0.001$ v/s Standard drug, $p_c < 0.05$ v/s EECR (250), $p_d < 0.01$ v/s EECR (500). 3-NP= 3-Nitropropionic acid, EECR = ethanolic extract *Catharanthus roseus*.

3.2. Effect of *Catharanthus roseus* extracts on 3-NP Induced changes in Rota rod Apparatus

Ethanol extracts of *Catharanthus roseus* were prepared and tested in 3-NP-treated rats at doses of 250 mg/kg p.o. and 500 mg/kg p.o. In the present study, ethanol extract showed more promise. Based on these findings, we further preceded with lower (250 mg/kg; p.o.) and higher doses (500 mg/kg; p.o.) of ethanol extract and checked their activity in 3-NP-treated rats. Systemic 3-NP (10 mg/kg/day; i.p.) treatment produced a significant ($p < 0.05$) gradual decrease in grip strength on the 1st week (day 0), 2nd week (day 7th), 3rd week (day 14th), and 4th week (day 21st) as compared to the normal control group. Pretreatment with ethanol extracts of *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day; p.o.) significantly ($p < 0.05$) prevented the impairment in grip strength as compared to 3-NP-treated rats. The effect of EECR (500 mg/kg/day; p.o.) was more significant ($p < 0.05$) as compared to EECR (250 mg/kg/day; p.o.).

Table.2\ Showing data expressed Mean $\pm$ SD of Rota rod Apparatus

Treatment	Fall time (sec)			
	0 Day Mean $\pm$ SD	07 Day Mean $\pm$ SD	14 day Mean $\pm$ SD	21 Day Mean $\pm$ SD
Normal	31.17 $\pm$ 3.31	31.17 $\pm$ 3.31	28.00 $\pm$ 5.29	29.67 $\pm$ 6.31
Disease	30.17 $\pm$ 2.23	13.50 $\pm$ 3.56	14.67 $\pm$ 3.61	14.50 $\pm$ 1.87
Standard	30.50 $\pm$ 2.66	35.17 $\pm$ 3.06	36.17 $\pm$ 3.31	34.17 $\pm$ 3.31
Low Dose	30.83 $\pm$ 2.48	23.00 $\pm$ 2.90	22.67 $\pm$ 4.41	22.67 $\pm$ 3.54
High Dose	30.83 $\pm$ 2.48	28.50 $\pm$ 2.43	29.17 $\pm$ 4.40	29.17 $\pm$ 3.76



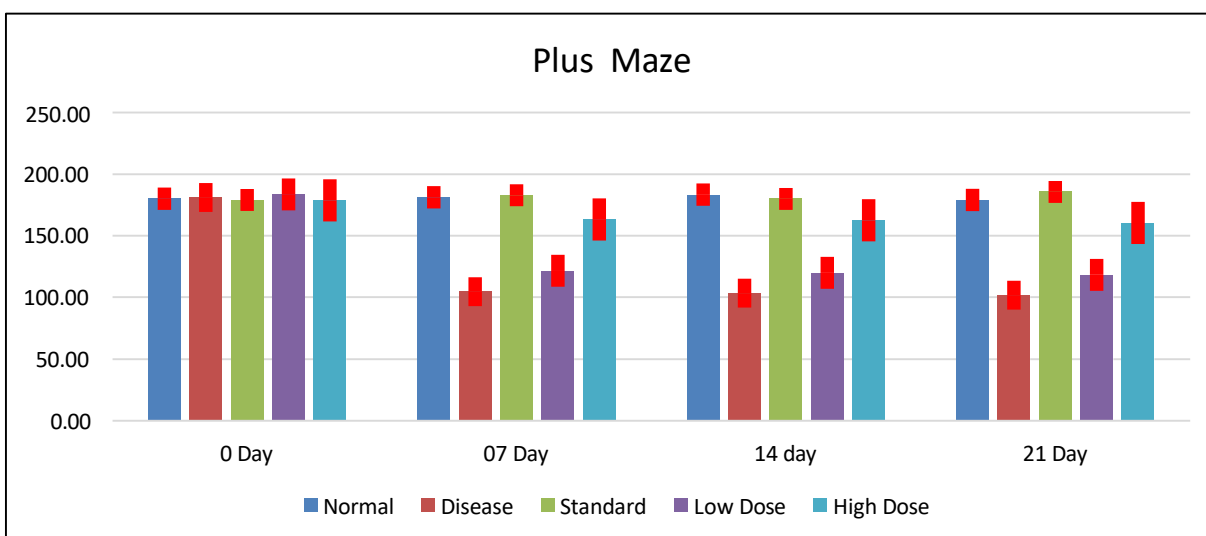
Graph:1.2 Effect of *Catharanthus roseus* extracts on 3-NP induced changes in rotarod activity: Data expressed as Mean $\pm$ SD: $p_a < 0.05$ v/s 3-NP (10mg/kg), $p_b < 0.001$ v/s Standard drug, $p_c < 0.05$ v/s EECR (250), $p_d < 0.01$ v/s EECR (500). 3-NP= 3-Nitropropionic acid, EECR = ethanolic extract *Catharanthus roseus*.

3.3. Effect of *Catharanthus roseus* extracts on 3-NP Induced changes in Elevated plus maze

Ethanol extracts of *Catharanthus roseus* were prepared and tested in 3-NP-treated rats at doses of 250 mg/kg p.o. and 500 mg/kg p.o. In the present study, ethanol extract showed more promise. Based on these findings, we further preceded with lower (250 mg/kg; p.o.) and higher doses (500 mg/kg; p.o.) of ethanol extract and checked their activity in 3-NP-treated rats. Systemic 3-NP (10 mg/kg/day; i.p.) treatment produced a significant ($p < 0.05$) gradual increase in anxiety-like behaviors in rats on the 1st week (day 0), 2nd week (day 7th), 3rd week (day 14th), and 4th week (day 21st) as compared to the normal control group. Pretreatment with ethanol extracts *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day; p.o.) significantly ($p < 0.05$) prevented the impairment in anxiety-like behaviors as compared to 3-NP-treated rats. The effect of EECR (500 mg/kg/day; p.o.) was more significant ($p < 0.05$) as compared to EECR (250 mg/kg/day; p.o.).

Table.3\ : Showing data expressed Mean $\pm$ SD of Elevated plus maze

Treatment	Time spent in open arm(sec)			
	0 Day Mean $\pm$ SD	07 Day Mean $\pm$ SD	14 day Mean $\pm$ SD	21 Day Mean $\pm$ SD
Normal	180.17 $\pm$ 9.06	181.33 $\pm$ 8.69	183.50 $\pm$ 10.17	179.17 $\pm$ 9.95
Disease	181.17 $\pm$ 11.72	104.67 $\pm$ 10.46	103.50 $\pm$ 10.07	101.83 $\pm$ 11.37
Standard	179.17 $\pm$ 9.28	183.00 $\pm$ 8.90	180.00 $\pm$ 12.77	185.67 $\pm$ 13.62
Low Dose	183.67 $\pm$ 12.96	121.67 $\pm$ 5.01	120.00 $\pm$ 10.45	118.33 $\pm$ 7.55
High Dose	178.83 $\pm$ 11.44	163.33 $\pm$ 10.71	162.67 $\pm$ 17.11	160.50 $\pm$ 13.98

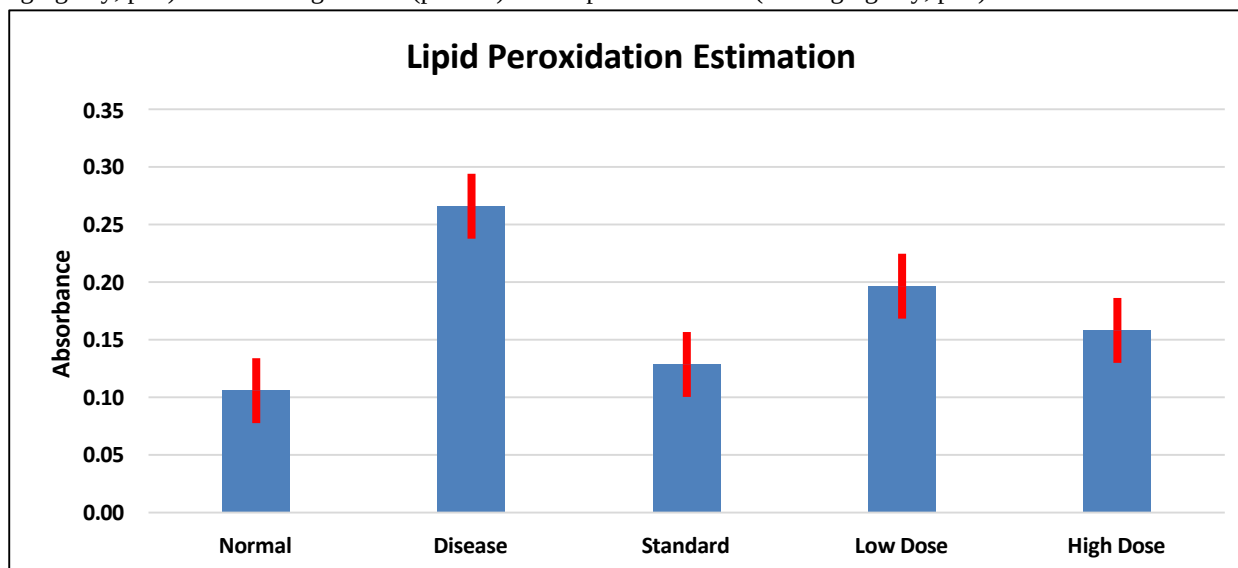


Graph:1.3 Effect of *Catharanthus roseus* extracts on 3-NP induced changes in EPM activity: Data expressed as Mean $\pm$ SD: $p_a < 0.05$ v/s 3-NP (10mg/kg), $p_b < 0.001$ v/s Standard drug, $p_c < 0.05$ v/s EECR (250), $p_d < 0.01$ v/s EECR (500). 3-NP= 3-Nitropropionic acid, EECR = ethanolic extract *Catharanthus roseus*.

3.4 Effect of *Catharanthus roseus* extracts on

3.4.1 Lipid peroxidation Estimation

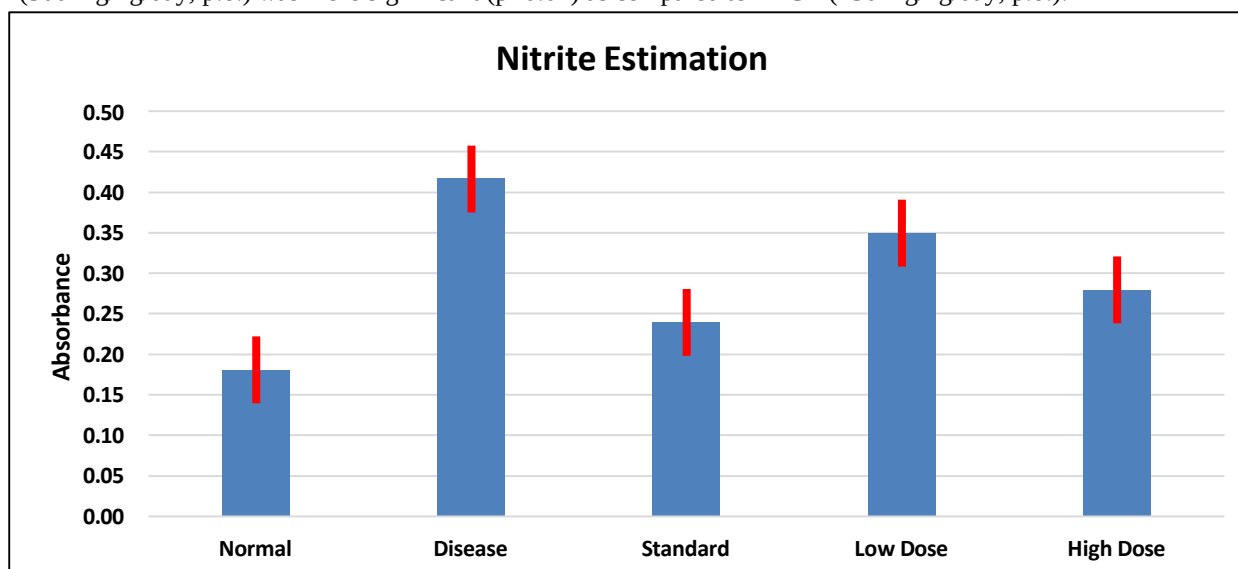
Systemic administration of 3-NP (10 mg/kg/day; i.p.) significantly ($p < 0.05$) increased lipid peroxidation in the striatum as compared to the normal control group. Pretreatment with an ethanolic extract of *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day; p.o.) significantly ($p < 0.01$) attenuated lipid peroxidation as compared to the 3-NP-only-treated group. The effect of EECR (500 mg/kg/day; p.o.) was more significant ($p < 0.01$) as compared to EECR (250 mg/kg/day; p.o.).



Graph:1.4 Effect of *Catharanthus roseus* extract on Lipid peroxidation, levels in 3-NP administered rats :Data expressed as Mean±SD: Effects produced by EECR (500 mg/Kg/day;p.o) in decreasing lipid peroxidation level were significant ($p < 0.01$) as compared to EECR (250mg/kg/day;p.o) which have mainly shows the antioxidant effect.

3.4.2 Nitrite Estimation

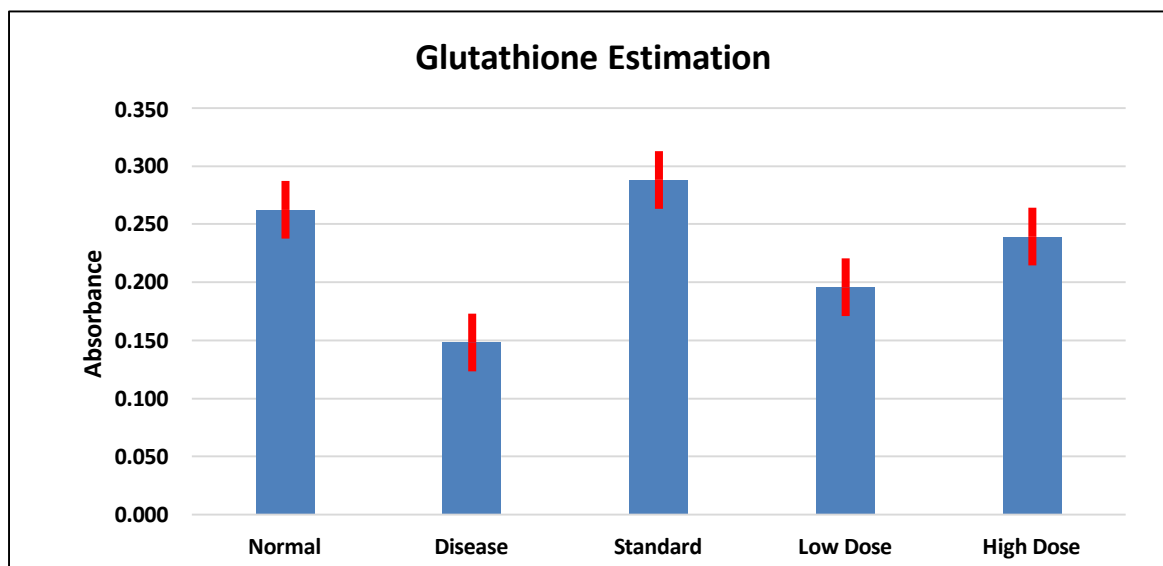
Systemic administration of 3-NP (10 mg/kg/day; i.p.) significantly ($p < 0.05$) increased nitrite concentration in the striatum as compared to the normal control group. Pretreatment with an ethanolic extract of *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day; p.o.) significantly ($p < 0.01$) attenuated nitrite concentration as compared to the 3-NP alone treated group. The effect of EECR (500 mg/kg/day; p.o.) was more significant ($p < 0.01$) as compared to EECR (250 mg/kg/day; p.o.).



Graph:1.5 Effect of *Catharanthus roseus* extracts on Nitrite level in 3-NP administered rats: Data expressed as Mean ± SD : Effects produced by EECR (500 mg/Kg/day;p.o) in decreasing nitrite concentration level were significant ($p < 0.01$) as compared to EECR (250mg/kg/day;p.o). That have mainly shows the antioxidant effect to reduced the ischemic effect of brain.

3.4.3 Glutathione level Estimation

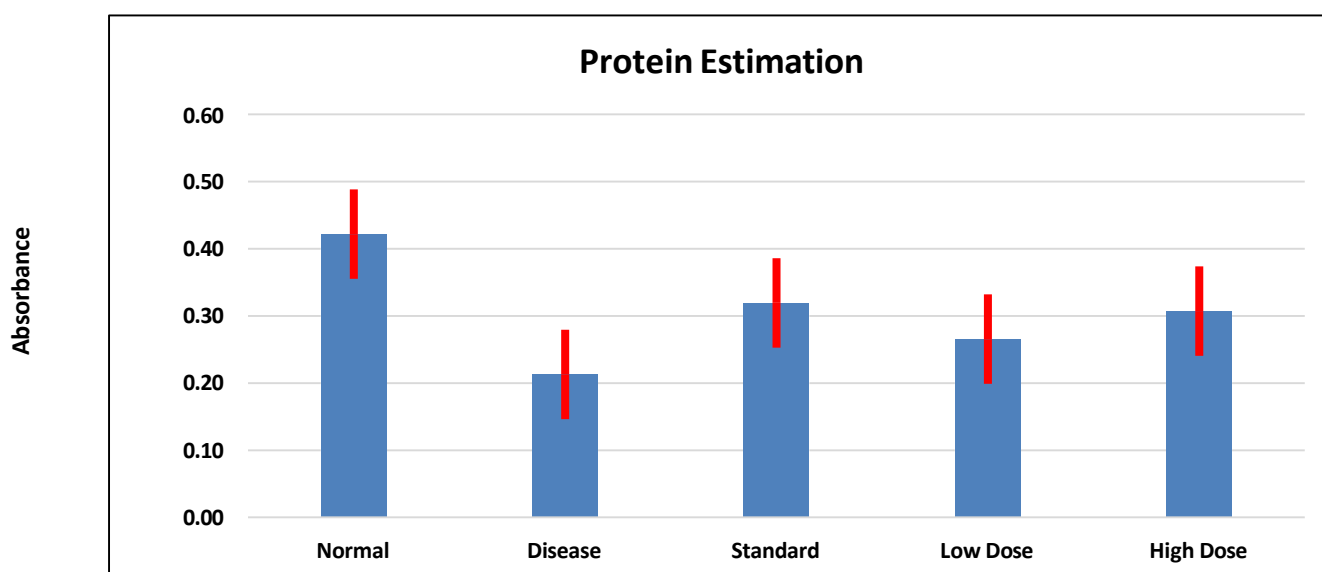
Systemic administration of 3-NP (10 mg/kg/day; i.p.) significantly ($p < 0.05$) increased depleted glutathione enzyme activity in the striatum as compared to the normal control group. Pretreatment with an ethanolic extract of *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day p.o.) significantly ($p < 0.01$) attenuated restored levels of the antioxidant enzyme glutathione as compared to the 3-NP-only-treated group. The effect of EECR (500 mg/kg/day; p.o.) was more significant ($p < 0.01$) as compared to EECR (250 mg/kg/day; p.o.).



Graph:1.6 Effect of *Catharanthus roseus* extracts on Glutathione level in 3-NP administered rats: Data expressed as Mean±SD : Effects produced by EECR (500 mg/Kg/day;p.o) in restoring glutathione levels were significant ($p<0.01$) as compared to EECR (250mg/kg/day;p.o) Which have essential for the cellular detoxification of the ROS species of brain cells, i.e show the antioxidant property.

3.4.4 Protein Estimation

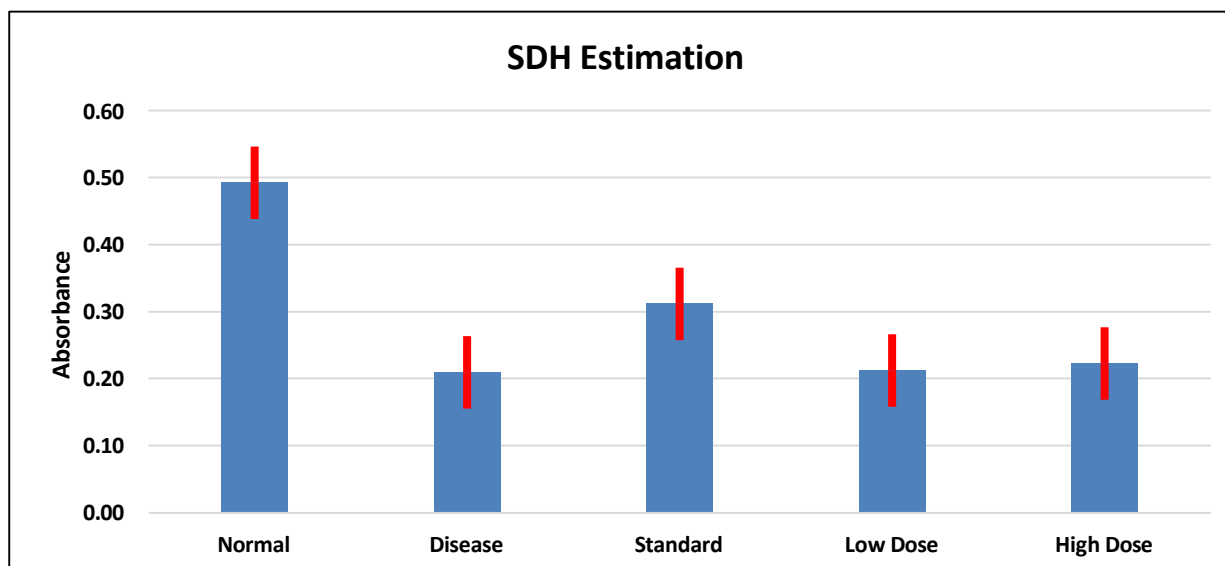
Systemic administration of 3-NP (10 mg/kg/day; i.p.) significantly ($p<0.05$) increased protein levels in the striatum as compared to the normal control group. Pretreatment with an ethanolic extract of *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day; p.o.) significantly ($p<0.01$) balanced the level of protein compared to the 3-NP-only-treated group. The effect of EECR (500 mg/kg/day; p.o.) was more significant ($p<0.01$) as compared to EECR (250 mg/kg/day; p.o.).



Graph:1.7 Effect of *Catharanthus roseus* extracts on Proteins level in 3-NP treated rats: Data expressed as Mean±SD : Effects produced by EECR (500 mg/Kg/day;p.o) that balanced the level of protein.

3.4.5 Succinate dehydrogenase(SDH)

Systemic administration of 3-NP (10 mg/kg/day; i.p.) significantly ($p<0.05$) increased succinate dehydrogenase in the striatum as compared to the normal control group. Pretreatment with an ethanolic extract of *Catharanthus roseus* (250 mg/kg/day; p.o.) and (500 mg/kg/day; p.o.) significantly ($p<0.01$) attenuated succinate dehydrogenase as compared to the 3-NP-only-treated group. The effect of EECR (500 mg/kg/day; p.o.) was more significant ($p<0.01$) as compared to EECR (250 mg/kg/day; p.o.).



Graph :1.8 Effect of *Catharanthus roseus* extracts on mitochondrial enzyme complex II levels in 3-NP treated rats: Data expressed as Mean±SD : Effects produced by EECR (500 mg/Kg/day;p.o) that increasing the activity of SDH can control lipid overloading and excitotoxicity and NMDA dependent signaling in neurodegenerative disorders that were significant ($p < 0.01$) as compared to EECR (250mg/kg/day;p.o).

3.4.6 : Comparison between the various biochemical estimations

The test drug *Catharanthus roseus* shows a significant decrease in lipid peroxidation (LPO), nitrite, and a significant increase in glutathione (GSH), succinate dehydrogenase, and protein levels. This drug shows an increase in motor **learning and enhances balance** and **motor coordination** in rotarod activity. and decrease anxiety in elevated plus maze (EPM) activity. It also enhances the number of entries and time spent in the open field chamber.

The present study demonstrates that *Catharanthus roseus*, at a dose of 500 mg/kg, showed a significant neuroprotective effect against 3-nitropropionic acid-induced Huntington's disease.

Table.4: Comparison between the various biochemical estimations

Treatment	LPO (n mole of MDA/mg protein)	Nitrite (µg/ml)	GSH (µ mole of GSH/mg protein)	Protein (mg/ml)	SDH (nmole/min/µL)
Normal	0.106±0.0319	0.181±0.0796	0.262±0.1769	0.422±0.0665	0.492±0.1692
Disease	0.265±0.0325	0.416±0.3136	0.148±0.1218	0.213±0.0386	0.209±0.0577
Standard	0.128±0.0305	0.239±0.0993	0.288±0.1812	0.319±0.0526	0.311±0.1898
Low Dose	0.196±0.0677	0.350±0.1584	0.195±0.1361	0.265±0.0761	0.212±0.1088
High Dose	0.158±0.0316	0.279±0.0588	0.239±0.1195	0.307±0.0328	0.223±0.1607

5. DISCUSSION

The present study, investigated the role of *Catharanthus roseus* (250mg/kg and 500mg/kg) p.o extract against 3-NP induced neurotoxicity. ethanol extracts of *Catharanthus roseus* were prepared using maceration process and activity of both the extracts was tested in 3-NP induced neurotoxicity in rats. *Catharanthus roseus*, commonly known as the Madagascar periwinkle, is a species of apocynaceae family and endemic to Madagascar (Tolambiya and Mathur., 2016). Alkaloids is the main constituents which have been beneficial effect on the CNS they are mainly reduces the neuroinflammation in the brain and also produce antioxidant effect. 3-Nitropropionic Acid (3-NPA) is a neurotoxin used to induce Huntington's disease (HD)-like symptoms in experimental animals. 3-NP crosses the BBB (blood-brain barrier) and leading to excessive production of free radicals (Upadhyay *et al.*, 2023). 3-NP involves mitochondrial inhibition, oxidative stress, and excitotoxicity, culminating in neuronal damage and cell death. These effects closely mimic certain aspects of neurodegenerative diseases like Huntington's disease (Gleichmann and Mattson., 2011).

In the present study, pre-treatment with ethanol extract significantly reversed the 3-NP induced oxidative stress. According to thin layer chromatography, Alkaloids, flavonoids have been found in ethanol extract of *Catharanthus roseus* also Well-known antioxidants are helping this extract's protective properties against oxidative stress. Using an animal model of Huntington's, we observe several physical factors: like open field, rota rod apparatus and elevated plus maze.

In the open field test, tetrabenazine (1 mg/kg) significantly increased the number of squares crossed and rearings compared to the control group. Similarly, ethanol extracts of *Catharanthus roseus* (EECR) at doses of 250 mg/kg and 500 mg/kg significantly enhanced both locomotor activity and rearings. Systemic administration of 3-NP (10 mg/kg/day) resulted in a marked ($p < 0.05$) decrease in locomotor activity over four weeks. However, pretreatment with EECR at both 250 mg/kg and 500 mg/kg effectively prevented this decline. The higher dose (500 mg/kg) demonstrated a more significant ($p < 0.05$) protective effect than the lower dose (250 mg/kg).

In the rotarod test, tetrabenazine significantly improved motor coordination. *Catharanthus roseus* extract, at doses of 250 mg/kg and 500 mg/kg, also enhanced motor coordination without causing anxiety. Systemic 3-NP (10 mg/kg/day) led to a significant reduction in grip strength over four weeks. However, pretreatment with *Catharanthus roseus* ethanol extracts (250 mg/kg and 500 mg/kg) significantly ($p < 0.05$) prevented this decline, with the 500 mg/kg dose being more effective than the 250 mg/kg dose in preserving motor function and grip strength.

The elevated plus maze is a common test used to measure anxiety in rodents by assessing the time spent in open areas. More time spent in these areas indicates reduced anxiety. In this study, tetrabenazine significantly increased the time and entries into the open arm, indicating an anxiolytic effect. Similarly, *Catharanthus roseus* at both 250 mg/kg and 500 mg/kg doses increased time spent in open arms, showing reduced anxiety compared to the control group. Systemic 3-NP administration caused a significant rise in anxiety-like behaviors, but pretreatment with *Catharanthus roseus* extracts (250 mg/kg and 500 mg/kg) notably prevented this effect, with the higher dose proving more effective.

Oxidative stress plays a key role in the progression of neuronal damage in Huntington's disease (HD). It occurs due to an imbalance between reactive oxygen species (ROS) production and the body's ability to detoxify these harmful byproducts. In HD, the mutant huntingtin (mHTT) protein aggravates oxidative stress. Studies show HD patients have reduced antioxidant levels, such as glutathione, which is crucial for neutralizing ROS. This deficiency contributes to oxidative damage in neurons, worsening the disease's effects and leading to cell death. In the present study, the disruption of mitochondrial function by 3-NP results in the generation of reactive oxygen species (ROS) and oxidative stress. Excessive ROS production can damage cellular components, including lipids, proteins, and DNA, contributing to cell injury and death. Additionally, 3-NP-induced mitochondrial dysfunction can lead to dysregulation of calcium homeostasis and excitotoxicity. Elevated levels of lipid peroxidation products, such as malondialdehyde (MDA), have been found in HD patients. These products result from the oxidative degradation of lipids and can disrupt cell membrane integrity and function (Paulsen *et al.*, 2013). Oxidative stress can modify proteins, affecting their structure and function. The accumulation of oxidatively damaged proteins can interfere with cellular processes and contribute to neuronal dysfunction (Pandey and Rajamma, 2018). ROS can cause breaks in DNA strands and other modifications that impair cellular function and promote cell death. This DNA damage is a significant contributor to the neurodegenerative process in HD (Bates *et al.*, 2015).

Pretreatment with an ethanol extract of *Catharanthus roseus* significantly prevented inflammation. Previous reports from the literature showed that alkaloids present in *Catharanthus roseus* have a protective effect against neuroinflammation and have free radical scavenging properties. Alkaloid (vinorelbine) reduce inflammation in microglial cells and can be used as a potential anti-inflammatory agent in the central nervous system. We can find out that the ethanol extract of *Catharanthus roseus* shows antioxidant effects, such as decreasing the amount of LPO and nitrite, increasing the amount of SDH and GSH, and maintaining the level of protein.

6. CONCLUSION

Results obtained from the current study suggested that *Catharanthus roseus*, at a dose of 500 mg/kg, showed a significant neuroprotective effect against 3-nitropropionic acid-induced Huntington's disease. The test drug shows a significant decrease in lipid peroxidation (LPO), nitrite, and a significant increase in glutathione (GSH), succinate dehydrogenase, and protein levels. This drug shows an increase in motor learning and enhances balance and motor coordination in rotarod activity. and decrease anxiety in elevated plus maze (EPM) activity. It also enhances the number of entries and time spent in the open field chamber.

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