Neuroprotective Effects of *Rauwolfia vomitoria* Against 3-Nitropropionic Acid Induced Motor Impairment and Oxidative Stress in Mice

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ABSTRACT

Motor dysfunction severely reduces quality of life and is frequently linked to neurodegenerative disorders. By inhibiting succinate dehydrogenase, 3-nitroproprionic acid (3-NP), a toxin, targets mitochondria and results in neurodegeneration. Neurons perish and lose energy as a result. Numerous models of neurodegenerative diseases with motor impairment have made use of this neurotoxic impact. Because of its phytoconstituent, the medicinal plant *Rauwolfia vomitoria* (RV) has neuroprotective qualities. The precise effects of RV on oxidative stress brought on by 3-NP and deterioration of motor coordination, however, are yet poorly understood. The objective of this research is to assess RV's neuroprotective benefits against oxidative stress and motor impairment brought on by 3-NP in mice. At 10 weeks of age, thirty (30) Swiss male mice weighing between 19 and 35 g were split into three groups at random (n = 10) and given the following treatment: Group II received 3-NP 15 mg/kg intraperitoneally (i.p.) for 5 days, Group III received 3-NP 15 mg/kg i.p. and RV 20 mg/kg p.o., and Group I received 2 ml of distilled water orally (p.o.) as the control. The beam walk test was used to assess motor coordination on the final day of the 21-day treatment. Following the neurobehavioral experiments, the animals were put to sleep, and blood was drawn by heart puncture. The levels of catalase, glutathione peroxidase (GPX), superoxide dismutase (SOD), and malondialdehyde (MDA) were measured in the sample. 3-NP significantly reduced motor coordination, as demonstrated by the rise in foot slips, decrease in reversals, and decrease in line crosses during the beam walk test. Concurrently, 3-NP generated oxidative stress, as demonstrated by raised levels of MDA and a decrease in levels of catalase, SOD, and GSH. Upon administration of RV, a considerable amelioration of the motor impairments and decreased oxidative stress generated by 3-NP occurred, which was indicated by an increase in the levels of catalase, SOD, and GPX and a drop in MDA. In a 3-NP-induced model of motor dysfunction, the study's results demonstrated RV's neuroprotective-like capability and antioxidant qualities. They also point to RV as a possible treatment for movement disorders.

Keywords: Neuroprotective, Rauwolfia vomitoria, 3-Nitropropionic acid, motor dysfunction

1. Introduction

The cerebellum and the basal ganglia have a major impact on movement control and coordination. Cerebellar damage causes obvious deficits in motor control and coordination, but no observable deficits in perception or emotion (1). Motor dysfunction, which is marked by deficiencies in the initiation, coordination, and execution of movement observed in Parkinson's disease, is also caused by dysfunctions of the basal ganglia (2). Although the exact origin of these disorders is still unknown, oxidative stress and mitochondrial dysfunction have been found to be important contributors to neuronal degeneration and the resulting motor deficits (3).

Animal models have been crucial in helping to clarify the fundamental causes of motor dysfunction and investigate possible treatment options. Because it can mimic important aspects of neurodegenerative diseases marked by motor deficits, the 3-nitropropionic acid (3-NP)-induced neurotoxicity model has become well-known among these models (4). 3-NP, a mitochondrial toxin, inhibits succinate dehydrogenase, resulting to energy depletion and neuronal cell death, culminating in motor impairment (5).

An imbalance between a biological system's capacity to detoxify these reactive products and the generation and buildup of reactive oxygen species in cells and tissues causes oxidative stress. The onset and progression of motor impairment in a number of neurodegenerative illnesses are significantly influenced by oxidative stress. Neuronal death and functional impairment result from reactive oxygen species' damage to mitochondria, lipids, proteins, and DNA. Neuropathological lesions and brain damage can arise from the reaction of free radicals, such as free oxygen species, with substrates such lipids, proteins, and nucleic acids (6,7). The brain is extremely vulnerable to peroxidation due to its high polyunsaturated fatty acid content, increased oxygen demand, and insufficient defences (8,9). Therefore, in order to create therapeutic strategies targeted at lowering oxidative damage and maintaining the integrity of the motor function in affected individuals, it is essential to comprehend the processes by which oxidative stress contributes to motor dysfunction. The effects of oxidative stress on motor performance may be mitigated by antioxidant treatments, mitochondrial protectors, and anti-inflammatory drugs.

Natural chemicals have gained significant attention in the quest for innovative medicinal agents. Among these is Rauwolfia vomitoria (RV), a plant with a long ethnomedical history that has shown neuroprotective qualities in early research. The Nigerian rainforest shrub Rauwolfia vomitoria has clusters of small flowers and oval leaves with straight venations (10, 11). Common names include swizzle stick, African snake root, African serpent wood, and deadly devil's pepper. In Yoruba, it is known as Asofeyeje, Ira in Igbo, and wadda in Hausa; in Efik and Ibibio, it is either mmoneba ebot or utoenyin, respectively (11). It is also possible to powder the root of RV and consume it with pap or as a decoction (12). According to other reports, RV may have antipsychotic and anticonvulsant effects (13). Due in large part to its extensive phytochemical makeup, RV is well known for its wide range of pharmacological characteristics. Numerous bioactive substances, such as alkaloids, flavonoids, terpenoids, and saponins, are present in the plant. These substances add to the overall pharmacological profile of the plants, with alkaloids being the most common class that gives them their medicinal properties (14). These alkaloids have been shown to have anti-inflammatory, hypotensive, and neuroprotective effects (15). This study intends to explore the potential neuroprotective effects of Rauwolfia vomitoria against 3-NP-induced motor dysfunction and oxidative stress in mice, but its precise effects on motor coordination deficits caused by neurotoxins are still poorly understood. This study offers insights into the antioxidant mechanisms of action of the plant and its potential as a therapeutic agent for neurodegenerative disorders characterised by motor impairments.

2. Materials and Methods

2.1. Drugs and Chemicals

ELISA Abcam reagent kits were acquired from Randoux Laboratory in the United Kingdom, while 3-nitroproprionic acid (3-NP) was acquired from Sigma Aldrich LTD in Canada. Every chemical and reagent employed in this investigation was of analytical quality.

2.2. Extraction Preparation

The roots of Rauwolfia vomitoria were acquired at the University of Calabar's botanical garden in Calabar. The plant sample was verified using the voucher number -Bot/Herb/UCC/0576. The roots were cleaned of sand and grime using flowing tap water. They scraped off its bark and dried the roots in the fresh air. An electric kitchen blender was used to powder the scraped root bark, yielding 700 g of powdered plant material. Five litres of distilled water were used to fully submerge 700 grammes of Rauwolfia vomitoria powered root bark, and the mixture was then agitated briskly. For a whole day, it was let to stand at room temperature, with periodic stirring. A rotatory evaporator was used to allow the filtrate to concentrate after a 24-hour period, and an Astell Hearson oven was used to further dry the concentrate at 40–450 degrees Celsius. The extract was filtered multiple times, particularly using Number 01 Whatman filter paper with pore sizes of 0.45 micrometres and a funnel. This was done to guarantee that the extract completely evaporated and turned into the crude extract, a pasty brown residue. A spatula was then used to gather the RV root bark extract into a container, and an electric weighing scale was used to measure the amount. After processing, 17.5 g of Rauwolfia vomitoria paste was prepared and stored in a refrigerator until it was needed for research.

2.3. Animals

The study employed thirty (30) Swiss male mice, weighing between 19 and 35 g, who were 10 weeks of age. The University of Calabar's Zoology Department is where they were bought. The animals were housed in normal cages at the University of Calabar's Physiology Department. Their cages were regularly cleaned, and they were given commercial feed and unlimited access to tap water. The animals were given a week to become used to their new surroundings before the experimental treatments started. The University of Calabar committee on animal use and care granted ethical clearance for the experiments (Approval No. FARE C/PA/[UC/049]/181PHY122), and they were conducted in compliance with the regulations controlling the use of laboratory animals.

2.4. Experimental Design

Three groups of thirty (30) male Swiss mice, each consisting of ten animals, were randomly allocated and given the following treatment: Group II received 3-NP 15 mg/kg intraperitoneally, Group III received 3-NP 15 mg/kg intraperitoneally, and Group I received Rauwolfia vomitoria 20 mg/kg orally. Group I was also given 2 ml of distilled water as a control. Motor coordination was assessed using the beam walk test and the open field test on the final day of the 21-day course of therapy. The animals were put to sleep in a closed chamber using a chloroform inhaler approach following the neurobehavioral examinations. When the animals were completely debilitated, they were put in a supine position. The heart was then found by making an incision along the Linea alba on their belly. Blood was collected via cardiac puncture with the use of a syringe. To keep it from clotting, the obtained specimen was kept in EDTA sample bottles before being utilised to test for indicators of oxidative stress.

2.5. Neurobehavioral Studies (Beam Walk Test)

Fine motor coordination and balance were tested utilising the beam walking test. This exercise is very helpful in identifying modest balance and motor skill deficiencies that other motor tests might miss. The mouse must remain upright during this test in order to traverse an elevated beam and reach a secure platform (16, 17). The beam walk equipment is raised roughly 50 cm above the ground and measures 1 to 2 cm in width and 50 to 100 cm in length. There is a goal box with food at the end of the beam, and the test environment is peaceful and distraction-free. First, the mice spent sixty seconds getting used to the goal box and beam. During testing, each mouse is placed at the starting location and permitted to walk across the beam. Foot slips (occurrences of paws slipping off the beam), reversals (changes in walking direction), and line crossings (number of preset segments traversed) are important parameters noted. An observer recorded these measurements, and they were also captured on video for further examination.

2.6. Tissue Antioxidant Assessment

After adding 1.15% potassium chloride to 100 millilitres of tissue homogenate in 5 mm Tris-HCl buffer (pH 7.4), the mixture was centrifuged for 15 minutes at 10,000 rpm and 4°C. Using hydrogen peroxide as the substrate, the supernatant was collected for the catalase (CAT) test. Reduced glutathione (GSH) was measured at 340 nm using the technique of Luchese et al. (18). Hydrogen peroxide was used as the substrate for the glutathione peroxidase (GPX) test (18). The technique outlined by Misra and Fridovich (19) was used to measure superoxide dismutase (SOD). Choudhary et al. (20) and Okhawa et al. (21) provided an explanation of how malondialdehyde (MDA) was measured in thiobarbituric acid-reacting compounds (TBARS). Following that, 0.2 ml of 8.1% sodium dodecyl sulphate was produced by the combined reaction. To 0.2 ml of 10% (w/v) homogenate, 1.5 ml of a 20% acetic acid solution that was matched to PH 3.5 with sodium hydroxide and 1.5 ml of a 0.8% water solution of thiobarbituric acid were added. Using distilled water, the mixture was increased to 4.0 millilitres and cooked for 60 minutes at 95 degrees Celsius. After adding around 1.0 ml of distilled water and 50 ml of the n-butanol and pyridine mixture (15.1 v/v), the mixture was centrifuged at 4000 rpm with ice chilling. After discarding the crude layer, absorbance at 532 nm was added to the findings derived from MDA standards. Absorption measurements were used to compute the concentrations as normal absorption.

2.7. Statistical Analysis

The results were analysed using a one-way Analysis of Variance (ANOVA) and a post-hoc multiple comparison test to compare the level of significance between the control and other groups. The data were expressed as Mean ± Standard Error of Mean (M ± S.E.M.). The analysis was conducted using Microsoft Excel and SPSS version 20, with a significance level of $P<0.05$.

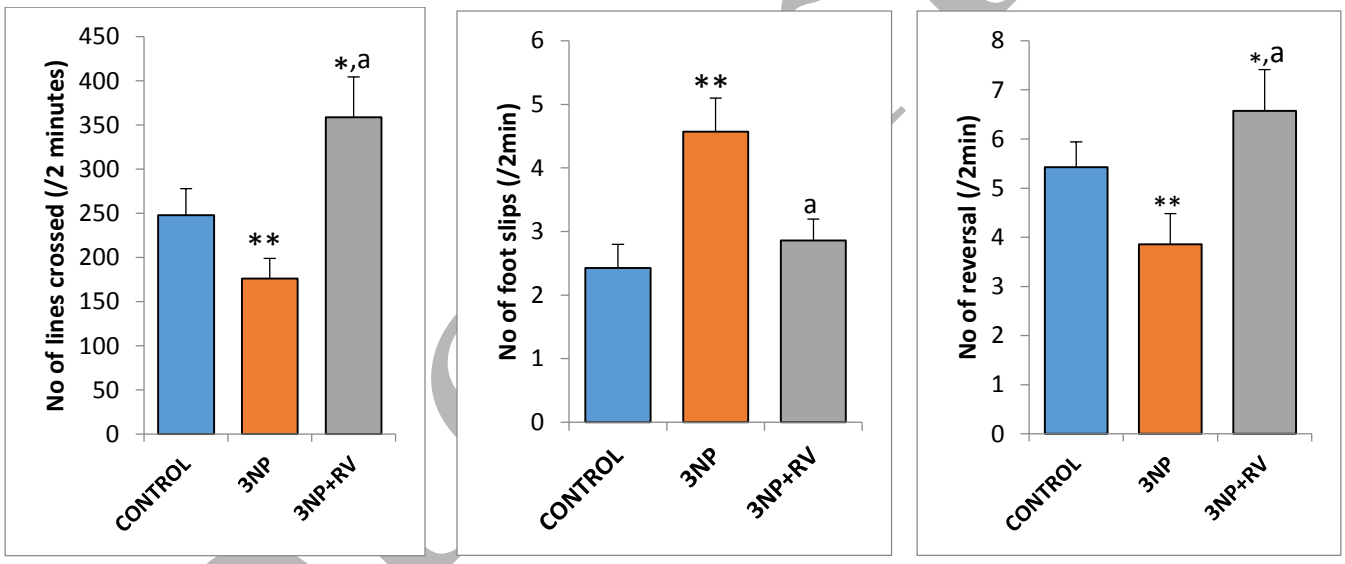
3. Results

3.1. Effect of 3-Nitropropionic Acid and *Rauwolfia vomitoria* on Motor Coordination

Prior research has confirmed that performance on the balance beam is a valuable indicator of balance and fine coordination. The beam test can reveal motor deficiencies due to age, central nervous system injuries, genetic and pharmacological alterations in young and older animals (17). The effects of administering 3NP and RV with 3NP on motor coordination are depicted in Figures 1A, 1B, and 1C, respectively. In comparison to the control group, mice administered with 3NP saw a substantial (**$p<0.01$) increase in foot slips and a decrease in line crossing and reversals during the beam-walking test. However, as demonstrated in Figures 1A, 1B, and 1C, when compared to the control and 3NP groups, the co-administration of RV with 3NP (3NP+RV group) led to a significant (a, * <p0.05) decrease in the number of foot slips and an increase in the number of line crossings and reversals in the mice's beam-walking test.

3.2. Effect of 3-Nitropropionic Acid and *Rauwolfia vomitoria* on Biomakers of Oxidative Stress

The effect of 3-Nitropropionic acid (3NP) and *Rauwolfia vomitoria* (RV) on oxidative stress and antioxidant enzyme levels was assessed by measuring the concentrations of Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), and Malondialdehyde (MDA) in the different groups. Treatment with 3NP resulted in a significant ($p<0.001$) decrease in the levels of CAT, SOD, and GPX, and a significant increase in MDA levels when compared to the control group. Specifically: The 3NP group showed a marked reduction in CAT levels compared to the control (***$p<0.001$). The co-administration of RV with 3NP (3NP+RV group) significantly (a $p<0.05$) increased CAT levels compared to the 3NP group (Figure 1A). Similar to CAT, the SOD levels were significantly reduced in the 3NP group compared to the control (***$p<0.001$). The 3NP+RV group exhibited a significant ('a $p<0.05$) increase in SOD levels compared to the 3NP group (Figure 1B). The GSHPX levels were also significantly lower in the 3NP group compared to the control (***$p<0.001$). The administration of RV significantly ('a $p<0.05$) improved GSHPX levels compared to the 3NP group (Figure 1C). In contrast, the MDA levels, were significantly elevated in the 3NP group compared to the control (***$p<0.001$). The 3NP+RV group showed a significant (a $p<0.05$) reduction in MDA levels compared to the 3NP group, suggesting an (Figure 1D).

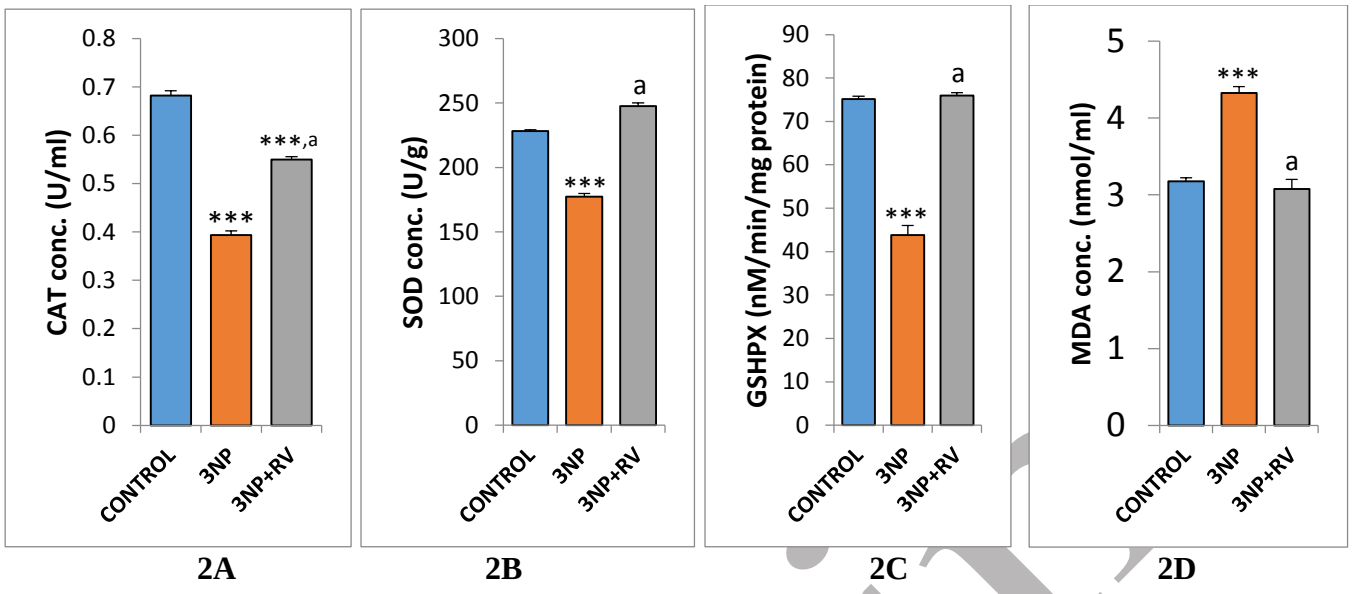


1A 1**B** **1C**

Figure 1A: Number of lines crossed in the beam-walking test in mice treated with 3-nitropropionic acid (3NP) and *R. vomitoria* (RV). *Significant at $p<0.05$ vs. control; **Significant at $p<0.01$ vs. control; a significant at $p<0.05$ vs. 3NP group.

Figure 1B: Number of foot slips in the beam-walking test in mice treated with 3-nitropropionic acid (3NP) and *R. vomitoria* (RV). *Significant at $p<0.05$ vs. control; **significant at $p<0.01$ vs. control; 'a significant at $p<0.05$ vs. 3NP group.

Figure 1C: Number of reversals in the beam-walking test in mice treated with 3-nitropropionic acid (3NP) and *R. vomitoria* (RV). *Significant at $p<0.05$ vs. control; **significant at $p<0.01$ vs. control; 'a significant at $p<0.05$ vs. 3NP group.



2A **2B** **2C** **2D**

Figure 2A: Concentration of catalase (CAT) in mice treated with 3-nitropropionic acid (3NP) and *R. vomitoria* (RV). ***significant at p<0.001 vs. control; **a** significant at p<0.05 vs 3NP group.

Figure 2B: Concentration of superoxide dismutase (SOD) in mice treated with 3-nitropropionic acid (3NP) and *R. vomitoria* (RV). ***significant at p<0.001 vs. control; **a** significant at p<0.05 vs 3NP group.

Figure 2C: Concentration of glutathione (GSHPX) in mice treated with 3-nitropropionic acid (3NP) and R. vomitoria (RV). ***significant at p<0.001 vs. control; **a** significant at p<0.05 vs 3NP group.

Figure 2D: Concentration of malondialdehyde (MDA) in mice treated with 3-nitropropionic acid (3NP) and R. vomitoria (RV). ***significant at p<0.001 vs. control; **a** significant at p<0.05 vs 3NP group.

4. Discussion

An increase in foot slips and a decrease in line crossings and reversals in the beam-walking test as compared to the control group demonstrated the study's conclusions that 3-NP significantly impairs motor performance. These findings are consistent with earlier research showing that 3-NP's neurotoxic effects on the striatum cause motor impairment (22).

Additionally, co-treatment with RV reversed the decrease in line crossings and reversals and dramatically reduced the increase in foot slips caused by 3-NP. This implies that RV has neuroprotective qualities that can help with 3-NP-induced motor impairments.

An improvement in balance and coordination is indicated by the notable decrease in foot slips seen in the 3NP+RV group. This suggests that RV has a positive impact on cerebellar function, which is essential for motor control. Furthermore, a decrease in anxiety-like behaviour and an improvement in exploratory behaviour are suggested by the rise in queue crossings and reversals in the same group. These results lend more credence to RV's neuroprotective properties.

When compared to the control group, 3-NP dramatically raised levels of malondialdehyde (MDA), a sign of lipid peroxidation, while significantly lowering levels of antioxidant enzymes like glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). These results are in line with earlier observations that 3-NP produces reactive oxygen species (ROS) to cause oxidative stress (23).

Crucially, co-treatment with RV reduced MDA levels and greatly mitigated the 3-NP-induced decline in CAT, SOD, and GPX levels. It is possible that the extract's bioactive components' capacity to inhibit Nitric oxidase and restrict the production of reactive oxygen species in response to oxidative stress is what allows it to restore the level of GPX and the activity of the enzymes (CAT and SOD). Terpenoids, steroids, alkaloids, saponins, tannins, flavonoids, and vitamin C have all been identified through phytochemical examination of the RV (14Vitamin C is an antioxidant that either directly or indirectly reduces lipid peroxidation by regenerating vitamin E, as demonstrated by the work of (24). Inhibiting lipid peroxidation (24) allows vitamin C to scavenge free radicals from reactive oxygen species through a quick electron transfer, acting as an indirect antioxidant. The inclusion of phytochemicals such terpenoids and alkaloids, which have been shown to have antioxidant activity, may also be responsible for the observed antioxidant effect of RV (15). This implies that RV has antioxidant qualities that lessen the oxidative damage brought on by 3-NP.

The 3NP+RV group's markedly elevated levels of CAT, SOD, and GPX suggest a strengthened antioxidant defence mechanism, which is essential for shielding cellular constituents from oxidative damage and slowing the advancement of neurodegeneration. The antioxidant capability of RV and its capacity to lessen lipid peroxidation are further supported by the decrease in MDA levels in the same group.

To sum up, the current study shows that RV has antioxidant qualities, which help explain its neuroprotective effects against mice's motor impairment brought on by 3-NP. According to the research, RV may be a viable therapeutic option for the prevention and management of oxidative stress-related neurodegenerative illnesses such Parkinson's disease (25). To clarify the precise processes behind RV's antioxidant benefits and assess its effectiveness in other animal models of neurodegenerative illnesses, more research is necessary.

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Study concept and design: Esu Ukpai Enene.
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Ethics
We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Data Availability
Data supporting this study's findings are available on reasonable request from the corresponding author.

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