Polyherbal Formulation Enhanced Sensorimotor Function in Oxidative Stress Induced by Unpredicted Mild Chronic Stress in Wistar Rats

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ABSTRACT

Stress is a mental strain resulting from adverse circumstances. One of the main predictors of the onset of a major depressive episode is chronic mild stress. Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to remove them through antioxidant defenses. This imbalance in sensorimotor function may have a substantial effect on both motor output and sensory processing. This study evaluates the impact of polyherbal formulation (PHF), on sensorimotor function in unpredicted mild chronic stress (UCMS). 25 adult Wistar rats (120-150 g), were divided at random into five groups consisting of five animals each. Rats in Group 1 received 1 mL of distilled water each, Group 2 was exposed to UCMS, Group 3 was exposed to UCMS and treated with Imipramine (25 mg/kg), 4 and 5 were exposed to UMCS and received PHF extract (250 mg/kg and 750 mg/kg) respectively. All groups received oral treatment once daily for 21 days. Animals were subjected to a Beam-walking task to assess sensorimotor function following 21 days of treatment. Following behavioral tests, the animals' cervical dislocation was followed by histological examination of the cerebellum and biochemical estimation of the activities of corticosterone, malondialdehyde, and catalase. Using Lorke's method, the LD50 of PHF was determined to be 2500 mg/kg. A significant improvement in motor deficits was suggested by the treatment groups' significantly lower beam walking time ($p < 0.05$), significantly lower levels of corticosterone and malondialdehyde expression ($p < 0.05$), and significantly higher levels of catalase ($p < 0.05$). Furthermore, moderate healing with active Purkinje cells and mild degeneration of the granular cells in the histological section of the treated groups was observed. Conclusively, treatment with PHF enhanced sensorimotor functions and mitigated oxidative damage due to stress.

Keywords: Catalase; Corticosterone; Malondialdehyde; Polyherbal formulation; Sensorimotor

1. Introduction

Mental illnesses are a global health issue (1). WHO statistics indicate that approximately 450 million people worldwide suffer from mental health issues (2). With mood disorders as a whole constituting the most commonly diagnosed condition, major depressive disorder (MDD) is the most prevalent psychiatric condition (3). An estimated 264 million people worldwide suffer from MDD, which is the leading cause of suicide and contributes to the global burden of disease (4). The prevalence of depression increased by 18 % worldwide between 2005 and 2015, and the number of cases is growing at an exponential rate (3).

One of the main predictors of the onset of a major depressive episode is chronic mild stress (CMS), which is commonly mentioned in the literature (5). The pathophysiology of MDD includes alterations in the oxidative and inflammatory pathways (6, 7). Myeloperoxidase (MPO), which increases expression is increased in depressed individuals, and interleukin 6 (IL-6) are two molecules linked to oxidative and inflammatory processes. The use of quetiapine therapies alters MPO activity. These changes might be temporary, with a decrease in amygdala activity, or with a reduction in hippocampus and prefrontal cortex function (6). Interleukin levels in arthritic mice were also assessed and found quetiapine to possess anti-inflammatory effect (8).

Sensorimotor function—the integration of sensory and motor output—constitutes a fundamental aspect of human cognition and behavior. It is how the nervous system acquires, interprets, and uses sensory information for the control of motor processes (9). Integration of sensory information and motor information includes several levels of the nervous system. Cerebellar structures, primary sensorimotor cortices, and more. Higher-order motor planning areas are some of the most critical areas where pathological alterations may be observed. Because sensorimotor operations are so central to early life, this type of involvement is developmentally significant.

. Infants learn primarily through perceptual exploration and motor interactions with their surroundings during the sensorimotor stage (0–2 years), according to Piaget's developmental theory (10).

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to remove them through antioxidant defenses. This imbalance in sensorimotor function may have a substantial effect on both motor output and sensory processing (11).

The goal of the current study is to examine how PHF affects sensorimotor function in Wistar rats under oxidative stress caused by unpredicted chronic stress. The knowledge gained from this study significantly improved occupational medicine, healthcare delivery, and key mechanisms of stress-related motor dysfunction (12).

2. Materials and Methods

2.1 Research Design

Twenty-five healthy female Wistar rats, weighing between 120 and 150 g, were obtained from the Alex Ekwueme Federal University animal farm in Ndufu Alike, Ebonyi State, Nigeria (AE-FUNAI) at the age of six months. After being housed in ventilated wire cages for 14 days, the rats were divided into five treatment groups at random. All the animals were kept in carefully regulated lab settings with a 12-hour day-night cycle, 23 ± 2 °C ambient temperature, and 50 ± 5% relative humidity. Rodents' standard chow (Vital Feeds Nigeria Ltd. Water and Jos) was freely available. The experiment was done according to the guidelines of the Institute of Laboratory Animal Resources (USA) (13) for the care and use of laboratory animals.

2.2.1 Plant Collection and Identification

On November 17, 2024, Thyme (*Thymus vulgaris*), Rosemary (*Salvia rosmarinus*), Beetroot (*Beta vulgaris*), *Praxelis clematidea*, and *Lantana Camara* were all gathered and identified from a local market in Abakaliki, Ebonyi State, Nigeria. A plant taxonomist at the University of Uyo's Department of Pharmacy confirmed the botanical identity of Lantana camara leaves using reference specimens kept in the university herbarium (Voucher number: UUPH17AI).

2.2.2 Preparation and Extraction of the Plant Extract

After being washed in running water, the plants were chopped into smaller pieces to aid in drying. Using a mechanical machine, the freshly chopped plants were ground into powder after being reduced and allowed to dry at room temperature in the shade with active ventilation. The Mettle Toledo electronic scale was employed for weighing 100 g portions of fine powdered *Thymus vulgaris, Salvia rosmarinus, Beta vulgaris, Praxelis clematidea, and Lantana camara* (Table 1). Moreover, the Mettle Toledo electronic scale was used to weigh a 250 g portion of finely ground Lantana camara powder. After combining the finely ground ingredients in a 2:1:1:1 ratio, they were dissolved in 60 ml of distilled water and 2 points 5 L of 70% methanol. After a 72-hour maceration in a solvent, extraction was done, and homogenous filtrates were obtained by filtering through Whatman Grade 1 filter paper. There were two phases to the concentration process: primary rotary evaporation at 45 °C and secondary evaporation in open dishes on a water bath with temperature control. The final stock solution was made with distilled water as the solvent and had a concentration of 100 mg/ml (1g/10ml).

Table 1: Composition of Polyherbal formulation

Botanical name	Family	Part used	Weight
Thymus vulgaris	*Lamiaceae*	Flower	100 g
Salvia rosmarinus	*Lamiaceae*	Leaves	100 g
Beta vulgaris	Amaranthaceae	Root	100 g
Praxelis clematidea	*Asteraceae*	Leaves	100 g
Lantana camara	Verbenaceae	Leaves	250 g

2.3 Acute Toxicity Study (LD50)

By Lorke's established protocol (14), 15 animals were divided into five treatment groups (3 animals per group) for dose-range testing. PHF was given in oral doses ranging from 1000 to 5000 mg/kg. Daily observations for 14 days were conducted after ongoing monitoring for immediate reactions (the first hour after dosing). At any dose level administered, the study found no toxicological or mortality symptoms.

2.4 Unpredictable Chronic Mild Stress

By changing the stressors, we modified Duccoted's chronic stress protocol. Eight different modalities were included in our stress battery: physical restraint, forced swimming in warm water (30 °C), food deprivation, acoustic stimulation, damp bedding exposure, water restriction, and undisturbed control condition. To avoid habituation, the presentation of stressors was done according to a randomized schedule with varying durations (15).

2.5 Experimental Design

Throughout the experimental period, daily oral administration was done for the following groups:

Group 1: Vehicle control (distilled water, 1 mL, oral)

Group 2: UCMS-only group (untreated)

Group 3: UCMS + standard drug (imipramine 25 mg/kg, oral)

Group 4: UCMS + PHF (250 mg/kg, oral)

Group 5: UCMS + PHF (750 mg/kg, oral).

2.6 Narrow Beam-walk

On day 21 (8:00–10:00 AM), 60 minutes after the final PHF administration, the beam walking test was carried out. Following predetermined procedures, animals were trained to cross a raised wooden beam 1 m high and 2 points 5 cm wide (16, 17). Measurements of (a) traversal latency (time to cross) and (b) foot faults (occurrences where limbs contacted beam sides or slipped off the surface) were made for each session, which consisted of three consecutive trials. Faults were defined as any departure from typical plantar stepping, and final scores were the average values across all trials.

2.7 Animal Sacrifice and Sample Collection

On day 22 (9:00–10:00 AM), whole brains were removed after cervical dislocation euthanasia and preserved in physiological saline. Using a motor-driven Teflon homogenizer, homogenates were made in 0.1 M phosphate buffer (pH 7.0). Cold centrifugation was then performed for 15 minutes at 4 °C and 3000 rpm. Until biochemical tests were completed, the supernatant was separated and kept at -80 °C.

2.8 Biochemical Assay.

2.8.1 Determination of Lipid Peroxidation

Wills' spectrophotometric method was used to quantitatively analyze malondialdehyde (MDA), the end product of lipid peroxidation (18).

2.8.2 Determination of Catalase (CAT) Activity

The enzymatic activity of catalase (CAT) was measured by measuring the rate at which hydrogen peroxide decomposes at 240 nm using the spectrophotometric protocol developed by Aebi (19).

2.8.3 Assay of Corticosterone

Using ferric iron (Fe^{3+}) and modifying Singh and Verman's principle (20), corticosteroids were oxidized in an acidic medium. The reaction between ferrous iron (Fe^{2+}) and potassium hexacyanoferrate (III) produced color.

2.8.4 Histopathological Studies

Histopathological analysis was conducted on the brain (particularly the hippocampus and hypothalamus). In each group, one sample was collected and preserved in (10 %) formalin in which their cerebellum was separated and used for histology studies.

2.9 Statistical Analysis

Results are presented as mean ± standard error of the mean (SEM). Statistical significance was determined by one-way ANOVA with Tukey's post-hoc test (GraphPad Prism 8.0), considering p-values <0.05 as statistically significant.

3. RESULT

3.1 LD_{50} of PHF

The acute toxicity profile of PHF as established by Lorke's method is shown in Table 2. Between the highest non-lethal dose and the lowest lethal dose in the experimental series, the computed LD_{50} was 2500 mg/kg (oral administration).

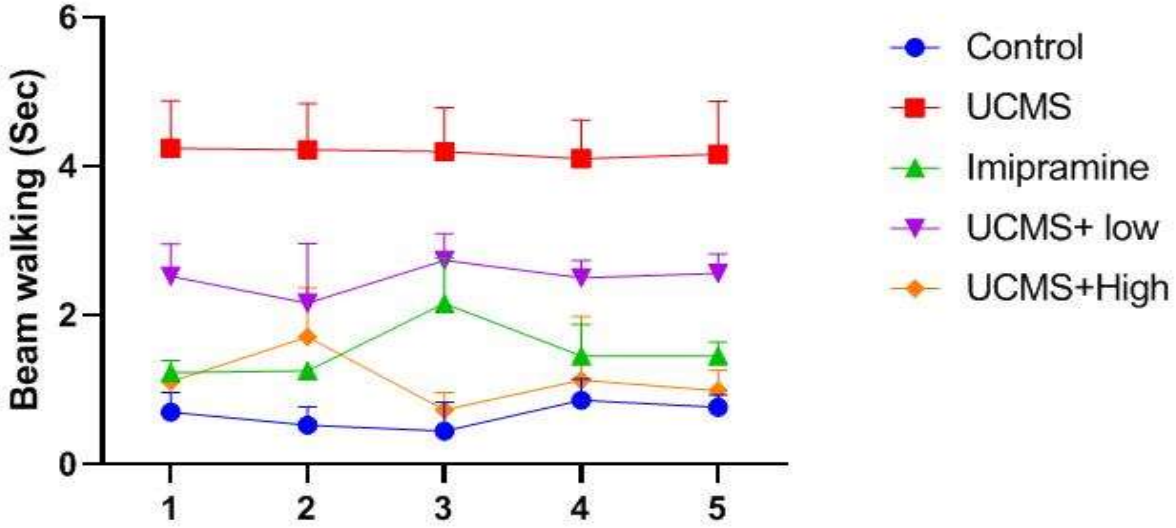
Table 2: Acute Toxicity Screening of PHF

Group (n=3)	Dosage (mg/kg)	Mice Mortality
Group 1	3000	0/3
Group 2	4000	0/3
Group 3	4500	0/3
Group 4	5000	3/3

Key: 0 = number of death, 3 = number of mice used for test

3.2 Neurobehavioral Evaluation of Beam Walking Time

Significant changes in beam walking crossing times between experimental groups are shown in Figure 1. Crossing times were noticeably longer in the UCMS-exposed group ($p<0.05$ compared to control). When compared to controls, the imipramine-treated and high-dose PHF groups showed noticeably shorter latencies ($p<0.05$). Significantly better performance was shown by all treatment groups (imipramine, low- and high-dose PHF) in comparison to the UCMS group ($p<0.05$).



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195 **Figure 1:** Effect of PHF on Beam Walking Time in Beam Walking Task.

196 **3.3 Evaluation of Catalase Activity (CAT)**

197 In Figure 2, catalase activity showed notable group differences: compared to controls,
198 the UCMS, imipramine, and high-dose PHF groups had lower CAT activity ($p<0.15$). PHF at
199 low doses showed higher CAT activity than controls ($p<0.05$). CAT activity was increased in
200 comparison to UCMS by imipramine and high-dose PHF treatments ($p<0.05$).

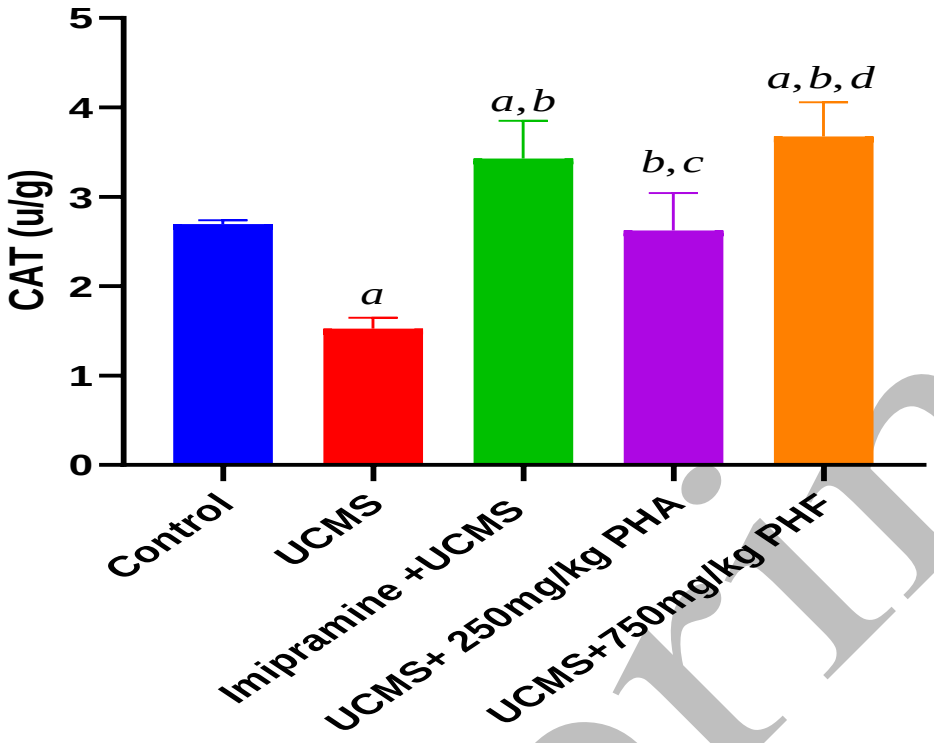


Figure 2: Effect of PHF on Catalase Activity**.** a = ($p<0.05$, Positive Control) b = ($p<0.05$, UCMS group), c = ($p<0.05$, Imipramine group) d = ($p<0.05$, PHF low dose) using one-way ANOVA followed by Turkey post-test. Each data represented mean SEM (n =5).

3.4 Evaluation of Corticosterone Level

Corticosterone levels changed significantly between experimental groups, as shown in Figure 3. Compared to controls, the UCMS group's corticosterone levels were noticeably higher ($p<0.05$). Although the corticosterone levels of all treatment groups (imipramine, low- and high-dose PHF) were higher than those of controls ($p<0.05$), they also showed significant decreases when compared to the UCMS group ($p<0.05$).

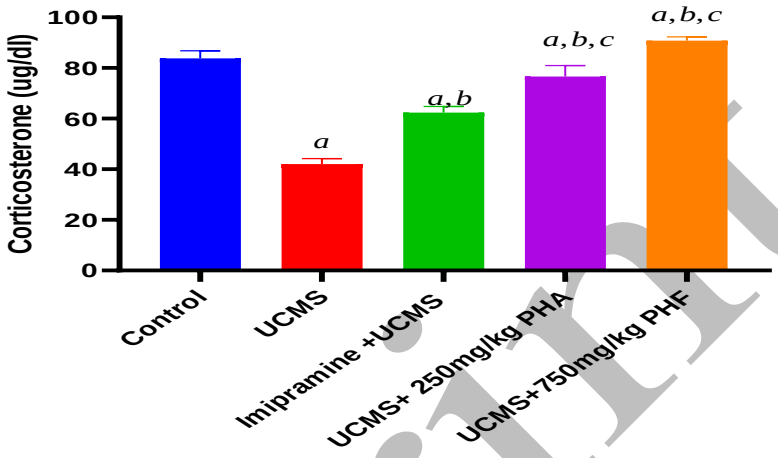


Figure 3: Effect of PHF on Corticosterone Level. a =($p<0.05$, Positive Control) b =($p<0.05$, UCMS group), c =($p<0.05$,Imipramine group) using one-way Anova followed by Turkey post test. Each data represented mean SEM (n =5).

3.5 Evaluation of Malondialdehyde Activity (MDA)

Malondialdehyde activity was investigated in Figure 4. The result demonstrated a significant ($p<0.0.5$) decrease in the MDA levels in the UCMS and PHF high-dose groups respectively when compared with the control group. There was a significant ($p<0.05$) increase in MDA level in Imipramine, PHF low and high dose groups respectively when compared with the UCMS group.

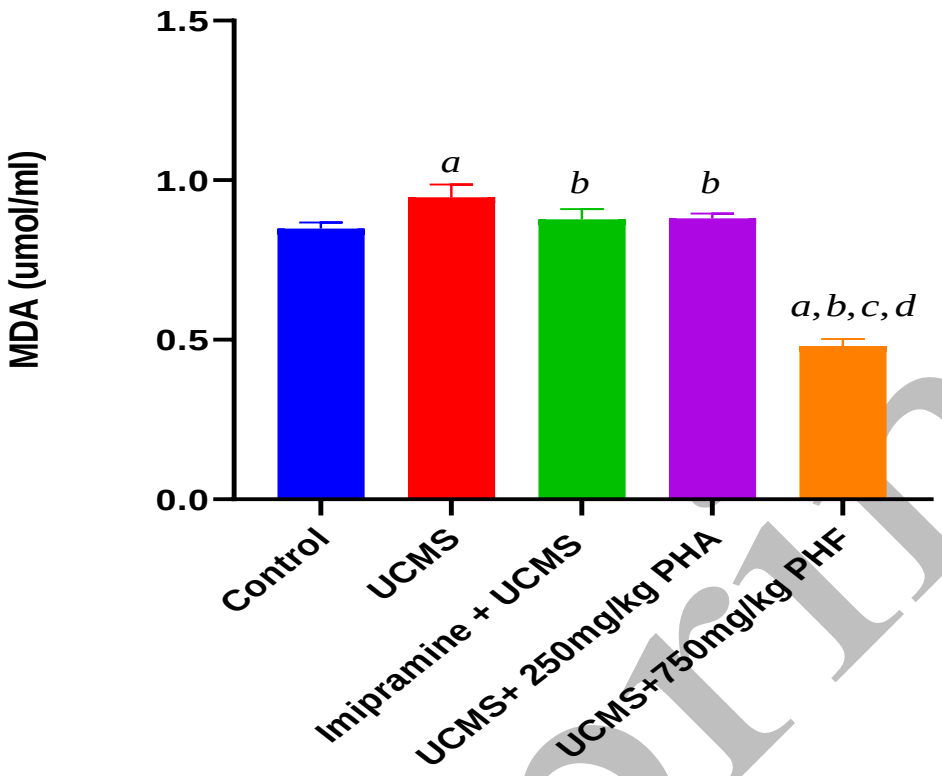
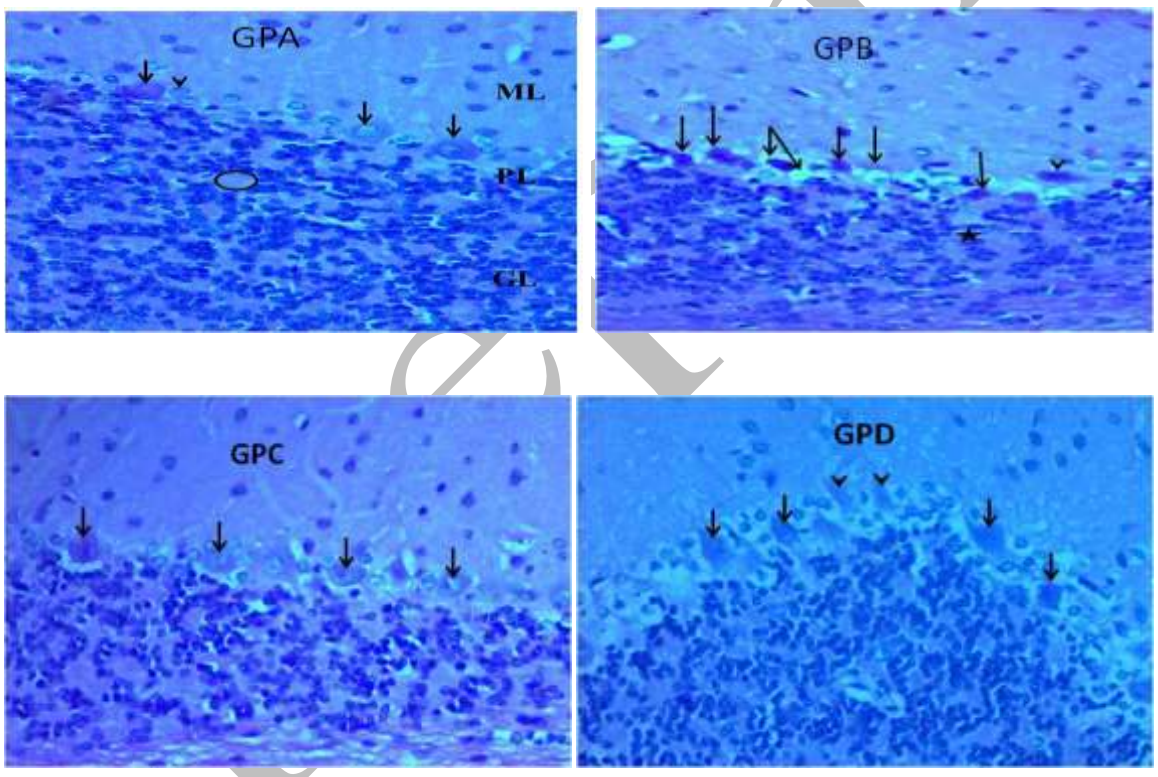


Figure 4: Effect of PHF on Malondialdehyde Level. a =($p<0.05$, Positive Control) b =($p<0.05$, UCMS group), c =($p<0.05$, Imipramine group) d =($p<0.05$, PHF low dose) using one-way ANOVA followed by Turkey post-test. Each data represented mean SEM (n =5)

3.6 Histology of the Cerebellum

Figure 5 shows the histological staining of the cerebellum. Photomicrograph of cerebellum of control animals given feed and water shows, well-defined cerebellar cytoarchitecture including molecular layer (**ML**), Purkinje layer (**PL**), granule cell layer (**GCL**), cerebellar glomerulus (**circle**), glial cell of Bergmann (**arrowhead**) and Purkinje cells (**arrows**). Photomicrograph of cerebellum of group 2 animals exposed to stress shows hypoplastic Purkinje cells (**arrows**), distorted cerebellar architecture (**double arrow**), spindle-shaped Purkinje cell (**arrowhead**) and granule cell population reduction (**star**). Photomicrograph of cerebellum of group 3 animals

exposed to stress plus 25mg/kg of standard drug shows regularly shaped Purkinje cells (**arrows)**. Photomicrograph of cerebellum of group 4 animals exposed to stress plus 250mg/kg of extract (low dose) shows regularly shaped Purkinje cells (**arrows**), though with few spindle-shaped Purkinje cells (**arrowhead**). Photomicrograph of cerebellum of group 6 animals exposed to stress plus 750mg/kg of extract (high dose) showing, regularly shaped Purkinje cells in the Purkinje cell layer (**arrows**).



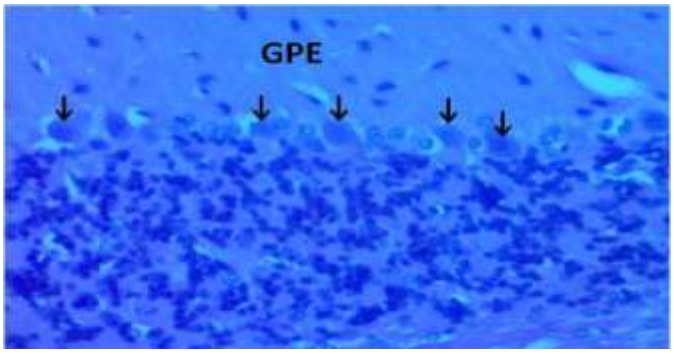


Figure 5: Photomicrograph of cerebellum (**H&E**, 40x magnification).H&E; Haematoxylin and eosin. Group 1 (GPA), Group 2 (GPB), Group 3 (GPC),Group4 (GPD),Group 5 (GPE).

4. Discussion

In the present study, we have evaluated the effect of PHF on sensorimotor function in oxidative stress induced by chronic unpredicted stress in Wistar rats. Herbal medicines were utilized for this study owing to their long-standing use in treating neuropsychiatric and oxidative stress disorders (21). The plants employed in the current study show that *Thymus vulgaris*, *Salvia rosmarinus*, *Beta vulgaris*, *Praxelis clematidea*, and *Lantana camara* exhibit antioxidant, anti-inflammatory, and neuroprotective effects as reported by traditional systems of medicine. Since oxidative imbalance and neuroinflammation play a central role in MDD pathogenesis, polyherbal formulations (PHFs) with potent antioxidant phytochemicals can be therapeutically beneficial (22). Herbal components' phytochemicals act synergistically through more than one mechanism and therefore they are suitable for complex conditions like depression and sensorimotor dysfunction. The LD_{50} is one way to measure the acute toxicity of a substance. An acute toxicity study was done to evaluate the safety of PHF methanol extract and its fractions. The study performed an acute toxicity test (LD_{50}) in accordance with Lorke's method, and arrived at the conclusion that the oral LD_{50} of PHF is 2500 mg/kg. Based on this, two doses (750 mg/kg and

250 mg/kg) were selected for this study. It was found that no manifestation of toxicity and mortality was observed in mice receiving PHF extract up to 2500 mg/kg.

The beam walking test evaluates sensorimotor function and balance by recording the time it takes for an animal to cross a narrow beam. The decrease in the beam walking time in the PHF high dose group suggests an improvement in impairment that causes disabilities affecting mobility and motor coordination. In models of oxidative stress or neurological damage, such as those induced by chronic stress, increased beam walking time generally indicates impaired motor coordination. *Beta vulgaris* has been studied for its potential neuroprotective effects, especially in the context of oxidative stress, due to its rich antioxidant content, particularly nitrates and betalains. Studies using *Beta vulgaris* extract have shown it can reduce oxidative damage, thus potentially improving motor functions and decreasing beam walking time. Clifford *et al*. (23) found that beetroot supplementation improved motor performance by reducing biomarkers of oxidative stress in rat models. Another study noted that beetroot supplementation in rodents improved sensorimotor performance and reduced inflammation. These findings suggest that PHF could help improve motor function by protecting neurons from oxidative stress.

Catalase is an essential antioxidant enzyme in the body that breaks down hydrogen peroxide into water and oxygen, mitigating the damaging effects of oxidative stress, which is particularly relevant in tissues associated with motor and cognitive functions, thereby protecting cells from oxidative damage. In this study, there was an increase in catalase activities by the administration of PHF. PHF can enhance the expression and activity of catalase by scavenging free radicals, thus reducing oxidative stress. In sensorimotor functions, oxidative stress can damage neuronal pathways and disrupt normal neural signaling, impairing coordination, movement, and response to stimuli. Studies indicate that Salvia rosmarinus supplementation can elevate catalase activity

in neural tissues, helping reduce oxidative damage (24). Enhanced catalase activity prevents excessive buildup of reactive oxygen species (ROS), maintaining cellular integrity, which is essential for preserving motor function and overall sensorimotor performance. For example, in animal models exposed to neurotoxic agents or chronic stress, rosemary extract has been observed to improve motor coordination, reduce anxiety-like behavior, and protect sensorimotor pathways, with increased catalase levels partially explaining these protective effects (25).

Elevated corticosterone levels due to chronic stress can impair cognitive and motor functions, leading to oxidative damage in brain regions responsible for sensorimotor coordination. Research suggests that Thymus vulgaris can influence the hypothalamic-pituitary-adrenal (HPA) axis, specifically by affecting levels of corticosterone, a glucocorticoid released in response to stress in animals (analogous to cortisol in humans). Research suggests that Thymus vulgaris can influence the hypothalamic-pituitary-adrenal (HPA) axis, specifically by affecting levels of corticosterone, a glucocorticoid released in response to stress in animals (analogous to cortisol in humans). Studies indicate that Thymus vulgaris may help regulate corticosterone levels, potentially protecting against stress-induced neurotoxicity and promoting healthier sensorimotor function. In animal models, Thymus vulgaris extracts have been shown to reduce corticosterone levels under stress, likely due to its antioxidant and anti-inflammatory properties, which mitigate oxidative damage in brain regions critical for motor coordination and sensory processing (26). Lower corticosterone levels of the treatment groups administered with PHF are associated with reduced neuroinflammation and preservation of neuron health, contributing to better performance in motor tasks and sensorimotor function overall (26).

Malondialdehyde is a marker of lipid peroxidation and oxidative stress, with high levels indicating increased cell membrane damage, particularly in neural tissues. Excessive oxidative

stress can impair sensorimotor functions by damaging neurons, which are crucial for coordinating sensory inputs with motor outputs. *Praxelis clematidea*, a plant known for its anti-inflammatory and antioxidant properties, has gained attention for its potential neuroprotective effects, partly due to its impact on malondialdehyde (MDA) levels. *Praxelis clematidea* may help lower MDA levels, thus reducing oxidative stress in brain regions involved in sensorimotor control. By decreasing lipid peroxidation and reducing MDA levels, *Praxelis clematidea* could preserve neural integrity and protect against damage caused by reactive oxygen species (ROS). Animal studies consistent with the findings of this research on PHF have shown that administering *Praxelis clematidea* extracts can lead to reduced MDA levels, which correlates with improved sensorimotor functions, including enhanced balance, coordination, and responsiveness to stimuli (27).

In conclusion, the study evaluated a novel polyherbal formulation (PHF) of five plants that were not previously studied together for mental illness and oxidative stress. Whereas most herbal research observes mood or behavior, this study observes sensorimotor function in the context of oxidative stress, an under-researched aspect of depression research. The findings of this study demonstrate that PHF exerts protective effects on sensorimotor function in Wistar rats exposed to oxidative stress induced by chronic stress. By modulating the HPA axis, reducing oxidative stress, and providing neuroprotection, PHF significantly improved motor coordination and function. These results suggest that PHF could be a promising therapeutic intervention for managing oxidative stress-related motor dysfunctions and may offer a natural remedy for stress-induced neurodegeneration.

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Ethical Statement

Ethical approval for this present study was sought and obtained before the commencement of the experiment from the Animal and Ethics Committee of AE-FUNAI, Ebonyi State, Nigeria which provided the approval number: AEFUNAI 2025/00345.

Conflict of Interest

The authors declared that there is no conflict of interest.

Funding Source Declaration

The authors of this manuscript self-funded the research study.

Authors' Contribution

Conceptualization: U. A. Inwang

Methodology: U. A. Inwang and E. U. Ogwo

Formal analysis and investigation: U. A. Inwang and E. U. Ogwo

Writing - original draft preparation: U.A. Inwang

Writing - review and editing: U.A. Inwang

Supervision: U.A. Inwang

Data Availability

The corresponding author can provide the datasets created and/or examined during the current study upon reasonable request.

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