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Original Article

# Neuro-Preventive Effect and Elevation of Cellular Adenosine Triphosphate by PUFAs from *Pteleiosis suberosa* Stem Bark on Mercury Sub-Acute Exposed Rats

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**Objectives:** Occupational/industrial exposure and experimental intoxication of mercury can produce neurological effects but *Pteleiosis suberosa* stem bark extract (PTSSBE) might be useful in the treatment of brain disorders because it's anti-ulcer, anti-inflammatory and antioxidant effects had been documented.
**Methods:** The present study was therefore designed to investigate some phenolic constituents, evaluate its antioxidant properties and examine its reversal effects of PTSSBE on sub-acute mercury-induced brain toxicity. Rats were divided into five groups of 10 animals each. Group I was given distilled water; group II, III, IV and V was orally administered with mercury at a dose of 3.75 mg/kg body weight. Group III, IV and V were co-treated with PTSSBE of 25, 50 and 100 mg/ kg body weight respectively, for 10 days.
**Results:** The results revealed that the stem bark extract exhibited high presence of antioxidants. Experimental exposure of rats to mercury significantly decreased the activities of catalase (CAT), lactate dehydrogenase (LDH), and the level of reduced glutathione (GSH), while the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the formation of malondialdehyde (MDA) were increased. These effects were reversed by co-administration with PTSSBE in mercury-induced brain toxicity in rats.
**Conclusion:** The protective effects of *Pteleiosis suberosa*, during mercury exposure suggest that these phenolics and PUFAs may be helpful in treating neurological disorders and other related cerebral toxicity implicated in depleted cellular ATP and oxidative stress.

**Key words:** *cellular ATP, mercury, neuro-protection, PTSSBE, PUFAs, sub-acute exposure*

## Introduction

Mercury is a unique chemical specie found in nature.[1] It is exists as liquid with high vapour pressure at room temperature and consequently released into the environment as mercury vapour.[2] It is practically impossible for humans to avoid exposure to some form of environmental mercury.[3] It was reported that 80% of the mercury vapours inhaled was retained in the human body.[4] Recent investigation had reported that Mercury (Hg) is a highly toxic metal that causes adverse neurologogical disorders, renal dysfunctions, hypertension, carotid atherosclerosis, myocardial infarction, genotoxicity and coronary heart disease.[5]

Brain disorder particularly neurological alteration is emerging as one of the greatest public health risks. It is suggested that 50% of adults in 85 people are afflicted by Alzheimer's disease, the most com-

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mon type of dementia.[6] Also, in 2013, it was estimated that more than 24 million people were suffering from Alzheimer's disease, with 4.6 million new cases each year.[7] In terms of costs, cause, cure and care, this disease is of great challenge in public health for modern societies and regulatory agencies. Meanwhile, crucial efforts are been made each year to find cures to brain related problems. Due to the high cost of neurodegenerative disease drugs in both developed and developing countries, many alternatives to the treatment of neurodegenerative diseases have been advocated.[7,8] Therefore, in spite of the millions of chemical therapies currently available for preventing and curing ailments, natural products from plant origin remain an imperative source of new drugs.[9] The increase in neurodegenerative diseases has led to the resurgence of interest in herbal products as sources of novel compounds to reverse brain related problems. Efforts are also being made to minimize the mercury toxicity by antioxidant agents such as fish oil, selenium, boron compounds, melatonin,[10] curcumin[11] and Allium sativum L. extract.[12] Additionally, *Pteleiosis suberosa* stem bark extract (PTSSBE) might be useful in the treatment of brain disorders because its phytochemicals, antibacterial, antifungal, anti-ulcer, anti-inflammatory and antioxidant effects had been previously documented.[13,14] In view of this, it is therefore very important to search for effective but of low cost and reliable traditional therapeutic agents which could cure various diseases in which PTSSBE has been applied traditionally for decades with limited scientific basis of its action.

## Materials and Methods

### Sample selection

Fresh sample of stem bark of *Pteleiosis suberosa* stem bark extract (PTSSBE) was purchased from the local market, in Malete metropolis, Kwara State, Nigeria (Table 1). Authentication of the plant was carried out in the Department of Biology, Obafemi Awolowo University Ile-Ife, Nigeria. Adult male wistar strain albino rat was purchased from the Biochemistry Department animal colony, University of Ilorin, Nigeria and maintained *ad libitum* on commercial diet and water.

### Chemicals and reagents

DPPH (1,1-diphenyl-2-picrylhydrazyl), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), GSH, hydrogen peroxide, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK). All the kits used for the bioassay were sourced from Randox Laboratories Ltd. (Crumlin, Dublin, Northern Ireland, UK).

**Table 1.** Quantitative characterization of phytochemicals of *Pteleiosis suberosa* stems bark extract

| Phytochemical | Extract (mg/100 g) |
|---|---|
| Total phenols | 78.12 ± 0.33 |
| Tannins | 106.05 ± 0.08 |
| Saponins | 60.9 ± 0.27 |
| Ascorbic acids | 4.69 ± 0.09 |
| Flavonoids | 9.72 ± 0.09 |

The values are in Mean ± SD, n = 2.

### Stem bark extraction and preparation

The bark of the stem was thoroughly washed in distilled water to remove any contaminant, chopped into small pieces before being milled. The methanol extract of the stem was subsequently prepared by soaking the grinded sample (10 g) in methanol (200 mL) for about 24 h at 37°C; the mixture was filtered and the filtrate was concentrated by rotator evaporator and stored in the refrigerator for subsequent analysis.

### Determination of relevant phenolic compunds in PTSSBE

The total phenol content of PTSSBE was determined according to the Folin-Ciocalteu method used Chan et al.[15] Total flavonoid was assessed as reported by Kale et al.[16] Determination of tannin content was done using the method of Padmaja.[17] Also, total saponins of PTSSBE was determined by the method of Hiai et al.[18] and modified by Makkar et al.[19] Vitamin C level of PTSSBE was estimated using the method of Benderitter et al.[20]

### Determination of free radical scavenging ability

The free radical scavenging ability of the (PTSSBE) against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated.[21] Briefly, appropriate dilu-

tion of the extracts (1 mL) was mixed with 1 mL of 0.4 mM methanolic solution containing DPPH radicals. The mixture was kept in the dark for 30 min and the absorbance was taken at 516 nm.

### Degradation of deoxyribose (Fenton's reaction)

The ability of the methanol extract stem bark of *Pteleiosis suberosa* (PTSSBE) to prevent $Fe^{2+}/H_2O_2$ induced decomposition of deoxyribose was carried out using the method of Halliwell.[22] Briefly, freshly prepared methanol extract (0-200 µl) was added to a reaction mixture containing 120 µl of 20mM deoxyribose, 400 µl of 0.1 M phosphate buffer (pH 6.9), 40 µl of 500 µm of FeSO4, and the volume were made up to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30minutes and the reaction was the stopped by the addition of 0.5 mL of 2.8% trichloro acetic acid (TCA). This was followed by the addition of 0.4 mL of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 1hr. The absorbance was measured at 532 nm in a spectrophotometer.

### Animal protocol

Fifty adult male wistar rats weighing approximately 200-220 g obtained from the Department of Biochemistry, University of Ilorin, Nigeria were randomly assigned into 5 groups of 10 animals per group. They were housed in an iron suspended cage placed in a well-ventilated rat house, provided rat pellets and water *ad libitum*, and subjected to a natural photoperiod of 12 h light and 12 h dark cycle. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. Ethic regulations have been followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments.[23] Rats in Group I served as control and were administered distilled water. Animals in Groups II, III, IV and V received 3.75 mg/kg body weight of mercuric chloride. Group III, IV and V were co-treated orally with *Pteleiopsis suberosa* bark extract (PTSSBE) at doses of 25 mg/kg, 50 mg/kg and 100 mg/kg body weight respectively, and the experiment last for a week. The animals were fasted overnight and sacrificed by decapitation 24 h after the last treatment, brains were removed and cleared of adhering tissues, washed in ice-cold 1.15% potassium chloride and dried with blotting paper. The blood was collected via retino-ocular technique.

### Biochemical assay

The brains were homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl and the homogenate was centrifuged at 10,000 g for 15 min at 4°C.The supernatant was collected for the estimation of catalase (CAT) activity using hydrogen peroxide ($H_2O_2$) as substrate according to the method of Clairborne.[24] Protein concentration was determined by the method of Lowry et al.[25] The supernatant (plasma) was removed from the blood and used for the determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).[26]

### Toxicological analysis

The activity of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[26] were measured using available commercially kits (Randox Laboratories Kits, St Louis, MO, USA)

### Reduced glutathione (GSH) assay

Reduced glutathione (GSH) was determined at 412 nm using the method described by Jollow et al.[27]

### Lipid peroxidation assay

Lipid peroxidation was quantified by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation as malondialdehyde (MDA) according to the method described by Ohkawa et al.[28] and expressed as µmol/mg tissue.

### Lactate dehydrogenase (LDH) assay

The brain homogenate was assayed for lactate dehydrogenase (LDH) activity using commercially available kit (Randox Laboratories UK). Assay was carried out according to the manufacturer's instructions.[29]

### Statistical analysis

The results of the replicates were pooled and expressed as mean ± standard deviation. A one way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple test was used for the post hoc.[30] Statistical package for Social Science (SPSS) 17.0 for windows was used for the analysis

and the least significance difference (LSD) was accepted at $p < 0.05$.

# Results

## DPPH scavenging free radical

The ability of PTSSBE to scavenge DPPH (1,1-diphenyl-2-picyhydrazyl) stable radical is presented in Fig. 1. It was discovered that PTSSBE significantly ($p < 0.05$) scavenged DPPH stable radical ($IC_{50}$ = 100 µg/mL) by 39.38%, 48.35%, 56.94% and 64.79% respectively, when compared with the control.

## Inhibition of hydroxyl radical (OH•)

The inhibitory effect of PTSSBE on hydroxyl radical (OH•) production-in vitro are presented in Fig. 2. PTSSBE significantly ($p < 0.05$) inhibited the hydroxyl radical (OH•) content in a dose-dependent manner ($IC_{50}$ = 155 µg/mL) by 49.09%, 38.99%, 30.16% and 12.3% respectively when compared with the control.

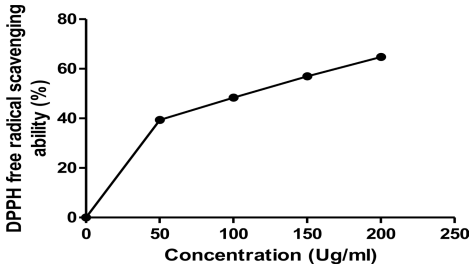


**Fig. 1.** Methanol extract of *Pteleiosis suberosa* stem bark (PTSSBE) scavenged stable free radical (DPPH) in dose-dependent manner. The percentage scavenging ability of DPPH stable radical production was expressed in 100% ($IC_{50}$ = 100 µg/mL). Significance was accepted at $p < 0.05$.

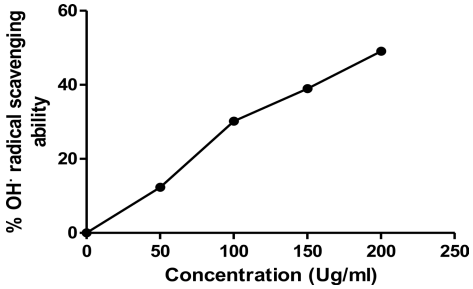


**Fig. 2.** *Pteleiosis suberosa* stem bark extract (PTSSBE) scavenged free OH• radical production in dose-dependent manner. The percentage scavenging ability of hydroxyl radical production was expressed in 100% ($IC_{50}$ = 155 µg/mL). Significance was accepted at $p < 0.05$.

## Hepatic biochemical indices

The effect of on hepatic biochemical indices are presented in Figs. 3-4. Sub-acute exposure of mercury to rat significantly ($p > 0.05$) caused a significant ($p < 0.05$) increase in the plasma activity of ALT by 27.71% when compared with the control group (Fig. 5). Co-treatment of mercury with PTSSBE significantly ($p < 0.05$) decreased the activity of this enzyme

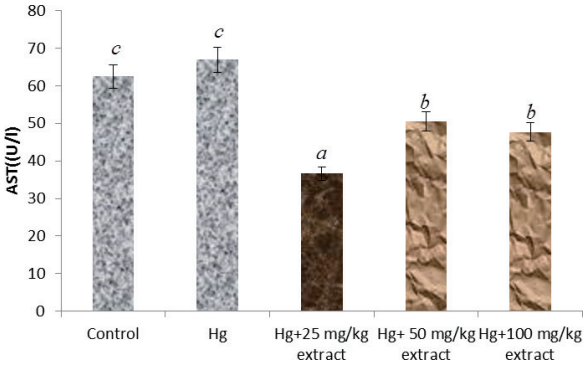


**Fig. 3.** Effect of PTSSBE on plasma activity of aspartate aminotransferase in mercury-induced toxicity. Values represent mean ± standard deviation, n = 10. Values with different (superscript letters) colour graphs are significantly ($p < 0.05$) different. $^{a}p < 0.05$; $^{b}p < 0.05$; $^{c}p < 0.05$; "a, b, c" analyzed by ANOVA; ANOVA = analysis of variance.

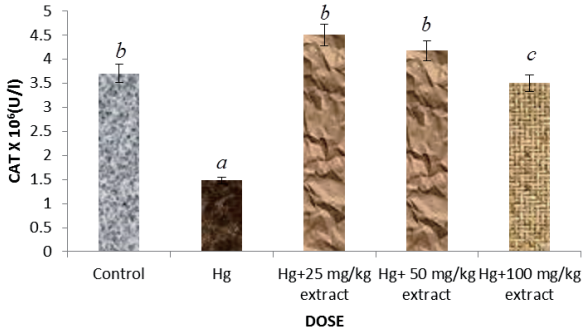


**Fig. 4.** Effect of PTSSBE on activity of catalase in mercury-induced brain toxicity. Values represent mean ± standard deviation, n = 10; Values with different (superscript letters) colour graphs are significantly ($p < 0.05$) different. $^{a}p < 0.05$; $^{b}p < 0.05$; $^{c}p < 0.05$; "a, b, c" analyzed by ANOVA; ANOVA = analysis of variance.

by 49.12%, 50.44%, and 60.35% respectively (Fig. 5). Similarly, mercuric treatment non-significantly ($p > 0.05$) increased the plasma activity of AST by 7.2%, relative the control group (Fig. 3). Whereas co-administrations of mercury with PTSSBE significantly ($p < 0.05$) decreased the activity of AST by 45.37%, 24.63% and 28.81% respectively (Fig. 3).

## Antioxidant status in the brain

In order to explore the possibility that sub-acute exposure to metallic mercury interferes with antioxidant defense system and thereby induces oxidative damage to rat brain, the antioxidant level and marker of oxidative stress were evaluated. The activity of CAT in the post-mitochondrial fraction of rat brain was decreased significantly in the mercury-treated rats ($p < 0.05$) by 60.08% (Fig. 4). Co-administration of PTSSBE significantly ($p < 0.05$) increased the activity of CAT by 204.6%, 182.3% and 136.97%, respectively (Fig. 4). Correspondingly, administration of mercury caused a non-significant decrease ($p < 0.05$) by 11.11% in brain GSH, antioxidant protein, when compared with the control animals (Fig. 6). Whereas co-treatment of mercury with different concentrations of PTSSBE significantly ($p > 0.05$) elevated the GSH to normal by 17.5%, 50%, and 56.66% respectively (Fig. 6). In addition, co-treatment of mercury with different concentrations of PTSSBE significantly ($p > 0.05$) exercabated the protein synthesis (Fig. 7)

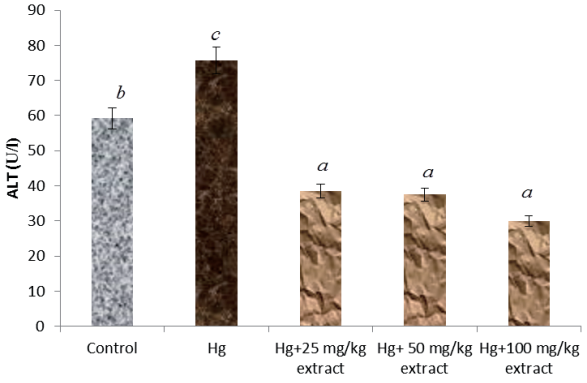


**Fig. 5.** Effect of PTSSBE on plasma activity of alanine aminotransferase in mercury-induced toxicity. Values represent mean ± standard deviation, n = 10. Values with different (superscript letters) colour graphs are significantly ($p < 0.05$) different. $^{a}p < 0.05$; $^{b}p < 0.05$; $^{c}p < 0.05$; "a, b, c" analyzed by ANOVA; ANOVA = analysis of variance.

## Marker of brain oxidative damage

The levels of MDA, a maker of lipid peroxidation, decreased significantly ($p < 0.05$) in rat brain treated with PTSSBE in vitro in a dose-dependent manner (Fig. 8) by 76.625%, 67.829%, 50.537% and 40.299% respectively. Also, the level of MDA was increased in group of animals sub-acutely exposed to mercury in vivo (Fig. 9) by 86.36%. Whereas increased MDA levels were markedly ($p < 0.05$) reversed by the administration of PTSSBE to the

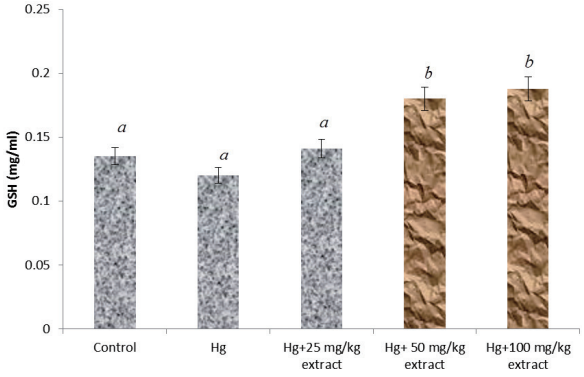


**Fig. 6.** Effect of PTSSBE on reduced glutathione in mercury-induced brain toxicity. Values represent mean ± standard deviation, n = 10; Values with different (superscript letters) colour graphs are significantly ($p < 0.05$) different. $^{a}p < 0.05$; $^{b}p < 0.05$; "a, b" analyzed by ANOVA; ANOVA = analysis of variance.

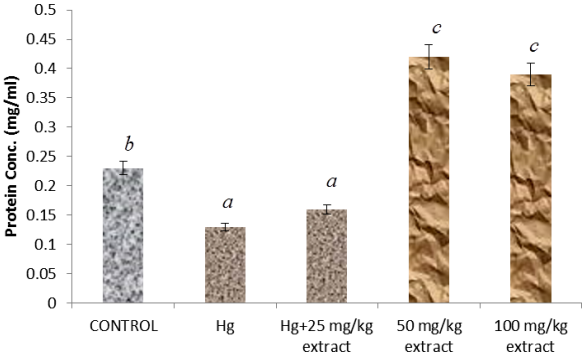


**Fig. 7.** Effect of PTSSBE on total protein synthesis in mercury-induced brain toxicity. Values represent mean ± standard deviation, n = 10; Values with different (superscript letters) colour graphs are significantly ($p < 0.05$) different. $^{a}p < 0.05$; $^{b}p < 0.05$; $^{c}p < 0.05$; "a, b, c" analyzed by ANOVA; ANOVA = analysis of variance.

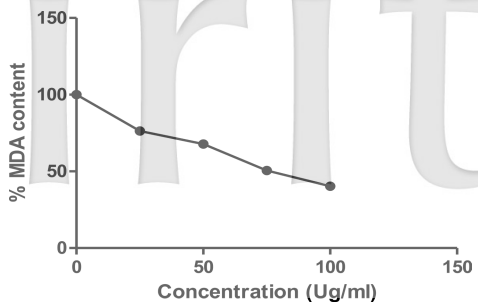


**Fig. 8.** Methanol extracts of *Pteleiosis suberosa* stem bark extract (PTSSBE) inhibits mercury (pro-oxidant) induced lipid peroxidation dose-dependently in rat cerebellum. The percentage inhibition of MDA (Malondialdehyde) production was expressed in 100%. Significance was accepted at $p < 0.05$.

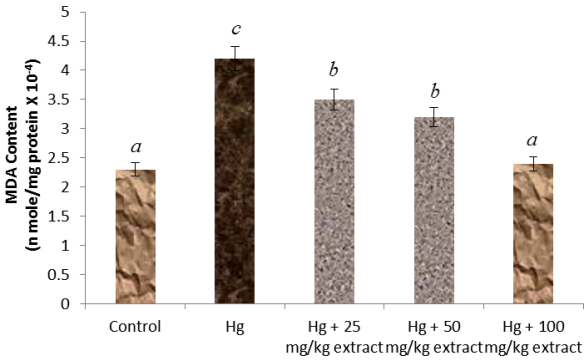


**Fig. 9.** Effect of PTSSBE on Malondialdehyde level in mercury-induced brain toxicity. Values represent mean ± standard deviation, n = 10. Values with different (superscript letters) colour graphs are significantly ($p < 0.05$) different. $^{a}p < 0.05$; $^{b}p < 0.05$; $^{c}p < 0.05$; "a, b, c" analyzed by ANOVA; ANOVA = analysis of variance.

mercuric-treated rats (Fig. 9) by 14.63%, 21.95% and 36.95% respectively.

### Cellular adenosine triphosphate in the brain

Mercury-treated group of animals significantly ($p < 0.05$) depleted the activity of lactate dehydrogenase (LDH), key marker attributed to the production of adenosine triphosphate (ATP) in the brain by 97.72%, when compared to the corresponding control group (Fig. 10). The depleted activities of LDH were increased by co-administration of PTSSBE to the mercury-treated animals (Fig. 10) by 350%, 299.9% and 3,949.95% respectively.

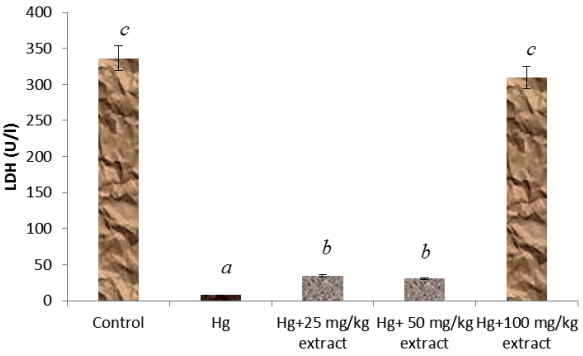


**Fig. 10.** Effect of PTSSBE on activity of lactate dehydrogenase in mercury-induced brain toxicity. Values represent mean ± standard deviation, n = 10. Values with different (superscript letters) colour graphs are significantly ($p < 0.05$) different. $^{a}p < 0.05$; $^{b}p < 0.05$; $^{c}p < 0.05$; "a, b, c" analyzed by ANOVA; ANOVA = analysis of variance.

## Discussion

The present study analysed the effect of *Pteleiosis suberosa* stem bark (PTSSBE) on cerebella toxicity associated with sub-acute exposure of mercury. We observed a significant rise in the production of OH• radicals (in vitro) in the presence of pro-oxidants (1,1-diphenyl-2-picrylhydrazyl) and Fenton-induced decomposition of deoxyribose). This investigation confirmed the imbalanced metabolism and excess reactive oxygen species (ROS) generation resulting to neuronal disorders such as Alzheimer's disease, Parkinson's disease, aging and neural loss.[31] The inhibitory potential of OH• radical was linked to the presence of secondary metabolites (natural antioxidants) in the extract. Also, the scavenging capacity of the extract on DPPH induced stable radical served as significant indicator of its potential antioxidant activity. Recent report had established that the antioxidant activity of the phenolics was majorly due to their redox potentials.[32] This allows them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and highly reactive radical abstractors.[33] The high scavenging power of PTSSBE-phenolics indicates its therapeutic wellness to mammalian's brain. This result agrees with earlier reports that polyphenols from root plants promoted free-radical scavenging ability in the brain, thereby lowering the possibility of neurodegenerative diseases.[34,35] As discovered in this study, high activities of AST and ALT after exposure to inorganic mercury designate hepato-cellular damage and symptom

of liver dysfunctions. This effect was due to stable species of mercury which damage major metabolic organs especially liver-central site of metabolism.[36] The significant reduction in the activities of ALT and AST following PTSSBE treatment suggests individuals with liver malfunctions are susceptible to neural cells dysfunctions.[7,37] This investigation confirms the medicinal use of PTSSBE as hepatic healer and neuronal restorer. Its effect in lowering brain disorder was traced to synergistic outcome of tannins, saponins, total phenols, ascorbic acids, flavonoids and PUFAs (oleic acid).[38]

ROS are both mediators of chronic neuro-degeneration[39] and the excitatory agent for amino acids and neurotransmitters.[40] The depletion of both enzymatic and non-enzymatic antioxidants- CAT and GSH after mercury intoxication impaired the neuronal functions by rendering the brain to neurotoxicity.[6] In addition, the deposition of Hg across blood brain barrier (BBB) in the myelin sheath of the brain invokes inflammatory responses which trigger the recruitment of inflammatory tissue macrophage and T cells.[41] Conversely, treatment with PTSSBE caused a significant increase in endogenous enzymatic and non-enzymatic antioxidants levels. This indicated that Hg permeation to CNS which could cause serious damage to glial cells (post-mitotic cells), neurons and demyelination were prevented.[7,42] These effects were linked to antioxidants such as saponins, tannins, ascorbic acids and other possible PUFAs i.e., oleic acids.[43] More so, endogenous protein is essential to normal brain functioning. Hence, as discovered from this study, medicinal extract from PTSSBE contains active compounds which could stimulate metabolic pathways implicated in protein synthesis.[44]

Lipid peroxidation makes brain prone to initiate neurodegeneration in surrounding area of neuronal cells[6,7,45] via radical-mediated abstraction of hydrogen atoms from methylene carbons in poly unsaturated fatty acids.[46] The protective effects of PTSSBE against Hg exposure were attributed to its high antioxidant activity as reflected by its high free radical scavenging properties. It could be asserted that PTSSBE antioxidants have the potential to increase neuronal survival, glial cells formation and decrease in brain abnormality.[47]

Energetically, synergetic interaction of tannins, saponins, total phenols, ascorbic acids and flavonoids from PTSSBE potentiated LDH activity, marker of cellular ATP level[46,48] in mercury exposed rats. The prevention of the depleted LDH activity showed down-regulation of LDH activity with concomitant decrease of ATP level in unhealthy mammals and activation of LDH activity- indicator of abundant ATP.[46] This consequently enhanced the brain physiological roles. This elucidates that high activity of LDH caused effective functionality of the myelin sheath in the brain, resulting into little or no neurological problems.[49,50] Thus, this study depicted that preponderant activity of LDH could delay the progression of Alzheimer's disease, promote neuronal cell survival and prevention of demyelination in CNS.

## Conclusion

Medicinal intervention of PTSSBE inhibited OH• radical production with concomitant decrease in MDA level as well as increased LDH activity. Similarly, protein synthesis, enzymatic and non-enzymatic antioxidants were remarkably high. These could suggest and/or validate the possible mechanisms of action for the management of brain disorders in traditional medicine. However, these observed effects could be attributed to the antioxidants, PUFAs and phenolic compounds acting synergistically, collectively or additively. Therefore, further studies should focus on specific underlying molecular mechanism associated with its improvement in neuronal survival.

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