 Effects of *Allanblackia floribunda* stem-bark Extracts on Oxidative stress and Mitochondrial Dysfunction in Rats Exposed to Crude Oil

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The development of degenerative diseases has been linked to oxidative stress and mitochondrial dysfunction. Additionally, it has been demonstrated that the changes caused by crude oil exposure contribute to the onset of degenerative diseases. There is little or no information on the anti-degeneration properties of *Allanblackia floribunda*. This study was carried out to investigate brain mitochondrial tissues exposed to crude oil and the effects of *A. floribunda* as a preventive and therapeutic intervention against oxidative stress and mitochondrial dysfunction. Twenty-five Wistar rats were divided into five groups: the control group received distilled water, the second group received ethanol extracts of *A. floribunda*, the third group received crude oil (5 mL/kg), the fourth and fifth groups received crude oil and *A. floribunda* extract concurrently (200 and 400 mg/kg, respectively). The results showed that crude oil treatment caused a significant increase in brain mitochondrial MDA levels and induced significant alterations in brain mitochondrial antioxidant enzyme activities compared with control. Treatment with the extract alone revealed a significant increase in protein carbonyl, conjugated dienes, and ATPases compared with crude oil-treated rats. This indicates that *A. floribunda* inhibited crude oil-induced mitochondrial lipid peroxidation and improved ATPase and antioxidant status in the rat brain mitochondria, which further suggests that *A. floribunda* possesses potential and may efficiently inhibit crude oil-induced oxidative damage and improve mitochondrial functions.

**Keywords:** *Allanblackia floribunda*; Crude Oil Toxicity; Mitochondrial Dysfunction; Degenerative Disorders; Oxidative Stress.

1. Introduction

Oxidative stress and mitochondrial malfunction result in the irreversible opening of the Mitochondrial Permeability Transition (MPT) pore, which is associated with various degenerative and neurodegenerative diseases (Oyedjei, Akobi, Onireti, & Olorunsogo, 2018, p. 25). Oxidative stress occurs when there is an imbalance between the production and accumulation of reactive oxygen species (ROS), which are metabolic by-products that can function as a double-edged sword in biological systems (Sies & Jones, 2020, p. 370). They may act primarily as signalling molecules under carefully controlled settings but may also cause cell harm when created at large levels, as they are capable of oxidising all major macromolecules, including nucleic acids (DNA and RNA), proteins, and lipids (Arimon *et al.*, 2015, p. 110). Reactive oxygen species (ROS) are oxygen free radicals and non-radical oxygen derivatives that are chemically reactive. A partial reduction of oxygen produces both radical and non-radical oxygen species, including superoxide radical anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (HO), nitric oxide (NO), and peroxynitrite (ONOO$^-$). When ROS generation is increased or the antioxidant system’s defences are compromised, the cell may experience oxidative stress (Misrani, Tabassum, & Yang, 2021, p. 617590).

The brain is susceptible to oxidative damage due to its high oxygen consumption rate, poor antioxidant levels, elevated polyunsaturated fatty acid levels and relatively high redox transition metal ion levels (Llanos-Gonzalez *et al.*, 2019, p. 6450; Cassidy *et al.*, 2020, p. 102297). ROS and oxidative stress appear to be significant risk factors for the development of a wide variety of degenerative and neurological illnesses, including atherosclerosis, cancer, and ageing ( Valko *et al.*, 2007, p. 50; Misrani *et al.*, 2021, p. 617590). Numerous studies have established that lipid peroxidation, in which reactive oxygen...
species (ROS) attack lipids to form lipid peroxidation products via a free radical chain reaction mechanism, is increased under disease circumstances (Galbusera et al., 2004, p. 105; Misrani et al., 2021, p. 617590). Malondialdehyde (MDA) is the most extensively researched lipid peroxidation product. Antioxidant levels or enzyme activity have also been employed as biomarkers for degenerative illnesses. Additionally, oxidative stress-induced cell damage have been associated with an oxidative imbalance and a significant rise in its by-products. A significant source of free radicals is mitochondrial-resident oxidative phosphorylation, which is triggered by electron leakage from the mitochondrial electron transport chain (ETC) (Ray, Huang, & Tsuji, 2012, p. 985). Oxidative stress is also associated with mitochondrial function, not because mitochondria produce reactive oxygen species (ROS) but because ROS can impair mitochondrial function. This is why lowering ROS levels through techniques such as diet and antioxidant medications may help protect brain mitochondria from oxidative damage, hence lowering the risk of neurodegeneration (Misrani et al., 2021, p. 617590).

Mitochondria are critical for the myriad metabolic reactions that ensure the survival of cells in all animals. It is the primary source of energy (adenosine triphosphate; ATP) and performs respiratory functions. It is also involved in a variety of processes critical for the cell's survival and death, including the regulation of second messenger levels such as calcium ions (Ca$$^{2+}$$) and reactive oxygen species (ROS) (Giorgi, Marchi, & Pinton, 2018, p. 720; Misrani et al., 2021, p. 617590). External stresses directly affect mitochondrial morphology, which rapidly responds by altering its shape and metabolic state via mitochondrial dynamics (fission and fusion processes), hence maintaining mitochondrial quality and homeostasis. This external stressor could be pathogenic illness or chemical or environmental pollutants such as heavy metals or crude oil (petroleum). Numerous sorts of studies have shown harmful symptoms (oxidative stress) in experimental animals' tissues and organs. The administration of the majority of medicinal plants (Olasantan, Areola, Ayannuga, & Babalola, 2015, p. 140; Olubodun, Fayemi, & Osagie, 2021b, p. 70) have also been shown to protect against oxidative stress, hepatotoxicity, neurotoxicity, and DNA breakage caused by the environment (Misrani et al., 2021, p. 617590; Olubodun et al., 2021b, p. 70). This is because plants have been shown to contain phytochemicals that serve as hepatoprotective and antioxidant agents and have the capacity to mitigate the toxicity of biological macromolecules (Ngueguim et al., 2016, p. 340).

Allanblackia floribunda, often known as Oliver or tallow-tree, is a medicinally significant plant native to Africa. It is distributed across Nigeria but is most prevalent in the south-eastern regions. It is a member of the Clusiaceae family. It may operate as an antioxidant, assisting in the destruction of free radicals and preventing oxidative damage in a variety of health problems that have oxidative stress as a factor in their pathophysiology (Marwa et al., 2015, p. 20; Olubodun et al., 2021b, p. 70). A. floribunda contains phytochemicals that have been demonstrated to have antitumor, antibacterial, anti-inflammatory, and antioxidant properties (Borut, & Rok, 2014, p. 671539; Olasantan et al., 2015, p. 140; Olubodun, Eze, & Eriyamremu, 2021a, p. 55).

Over the last few decades, efforts have been undertaken to examine the effect of numerous forms of mitochondrial dysfunction on the aetiology of degenerative diseases, including excessive reactive oxygen species (ROS) generation, ATP deficiency, and problems in mitochondrial dynamics and transport. Recent research indicates that restoring mitochondrial activity through an antioxidant diet and therapeutic interventions may help delay the onset and progression of degenerative illnesses. The research is aimed to determine the protective effect of ethanol extracts of A. floribunda stem bark as an antioxidant diet against crude oil-induced mitochondrial dysfunction and oxidative stress in Wistar rats' brains.

2. Materials and Methods

2.1 Study area

The study was carried out in the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria (Latitude 6° 23' 44"; Longitude 5° 36' 49"; Altitude 360 feet).

2.2 Collection of Crude oil, Animal Feeds, Plant Materials and Wistar rats

The crude oil was obtained from Warri Refinery and Petrochemical Company, Nigeria. Male Wistar rats were obtained from the Department of Animal Science, Faculty of Agriculture, University of Benin, Edo State, Nigeria, and rat pellets from a Nigerian manufacturer. A. floribunda's fresh stem bark was gathered from a woodland location in Edo State, Nigeria. The plant was recognised and authenticated by a
botanist at the University of Benin's Department of Plant Biology and Biotechnology, and a voucher specimen number UBHA361 was deposited in the Department's Herbarium for reference.

2.3 Preparation of Crude oil, Plant Materials and Animals

The stem bark was washed thoroughly under running water, air dried, macerated and sieved through a micropore sieve. The macerated form of *A. floribunda* stem bark was soaked in ethanol for 72 hours with occasional stirring. The extracts were filtered using a double-layered muslin cloth, and the filtrate was concentrated to dryness with a rotary evaporator at reduced pressure. The crude extract was stored in a refrigerator until required. Twenty-five male albino Wistar rats (average weight 180 g) were maintained in the Laboratory Animal Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Benin. They were fed with a standard diet and water ad libitum. They were acclimatised for two weeks. The study was approved by the ethics committee of the University of Benin, Nigeria and was handled in accordance with the guidelines on the care and wellbeing of research animals.

2.4 Animal Grouping and Treatments

The rats were divided into three (3) groups. The first group made of five (5) rats received distilled water, the second group made of five (5) rats received the ethanol extracts of *A. floribunda*, and the third group made of fifteen (15) rats received crude oil (5 mL/kg) for seven (7) days. At the end of the seventh day, the first and second groups were maintained, and the third group was divided into three (3) groups of five (5) rats each: one group of the crude oil-treated rats received concomitantly crude oil and distilled water (10 mL/kg) and the second groups of crude oil-treated rats received concomitantly crude oil and *A. floribunda* extract at the doses of 200 and 400 mg/kg respectively.

2.5 Experimental Design

Acute toxicity test of crude oil was carried out using Wistar albino rats. Rats were grouped into four with five rats per group and treated orally with 2, 3, 5, and 10 ml/kg body weight of crude oil, respectively (Ujowundu, Kalu, Nwaoguikpe, Okechukwu, & Ihejirika, 2012, p. 90). The rats were observed for 24 hr for nervousness, dullness, weight loss, in.coordination and/or death. Increased dullness and weight loss were concentration-dependent. Although there was no recorded death, the range of concentration used for the study was 5 ml/kg body weight. A total of twenty (25) male Wistar albino rats weighing (160 to 180) g were divided into five groups, with each group containing five rats. The rats were housed in steel cages and allowed to acclimatise. Crude oil of 5 mL/kg body weight was administered by oral dosing needle (oral gavage) to induce oxidative stress in the rats.

The rats were placed in five groups of five rats each as follows:

- **Group I**: Normal Control (no treatment) throughout the period of the study.
- **Group II**: *A. floribunda* Control
- **Group III**: Crude oil Control (5mL/kg crude oil)
- **Group IV**: Treatment group: (5mL/kg crude oil + 200mg/kg *A. floribunda*), and
- **Group V**: Treatment group: (5mL/kg crude oil + 400mg/kg *A. floribunda*).

Water was provided *ad libitum* to all groups. The experiment lasted for twenty-eight (28) days, and rats were sacrificed on the twenty-ninth (29th) day. The brain was recovered for the analyses.

2.6 Collection of Brain Sample and Isolation of Mitochondria

The rats were sacrificed on the 29th day, 24 hours after the last day of crude oil gavage. Isolation of mitochondria from the rat brain was according to the method described by Johnson & Lardy (1967) (p. 95) and modified by Lapidus & Sokolove (1993) (p. 250). The brain was excised, weighed and washed thrice in ice-cold homogenising Buffer C (210 mM mannitol, 70 mM sucrose, one mM EGTA, five mM HEPES-KOH, (pH 7.4) and minced with a pair of scissors. A standardised 10% brain homogenate was prepared by homogenising rat brain in ice-cold homogenising Buffer C in a homogeniser. Centrifugation of the homogenate was carried out at 4°C for 5 min at 2,300 rpm. The nuclear fraction and cellular debris sedimented were discarded, while the supernatant was centrifuged at 13,000 rpm for 10 min to pellet mitochondria. Pelleted mitochondria were washed twice with washing buffer D (210 mM mannitol, 70 mM sucrose, 0.5% BSA, 5 mM HEPES-KOH, pH 7.4) by spinning at 12,000 rpm for 10 min. Washed mitochondria pelleted were resuspended in an appropriate volume of MSH buffer (210 mM mannitol, 70 mM sucrose and 5 mM HEPES-KOH, (pH 7.4) dispensed into Eppendorf tubes and kept at 4°C for use.
2.7 Biochemical Analyses

2.7.1 Assessment of Oxidative Stress Markers

The activity of superoxide dismutase (SOD) was determined using the method reported by Misra & Fridovich (1972) (p. 3173) and represented in units/mg of tissue weight. One unit of the enzyme was defined as the amount required to inhibit 50% of epinephrine oxidation to adrenochrome in one minute. The specific activity of catalase (CAT) was calculated using Sinha's (1971) method and expressed as units/g wet tissue. When heated in the presence of H$_2$O$_2$, dichromate in acetic acid is converted to chromic acetate, with the creation of perchromic acid as an unstable intermediate (p. 390). A unit of activity was defined as the amount of perchromic acid produced. The activity of peroxidase (POX) was evaluated using the Nyman (1959) method and was represented as units/mg of protein (para. 3). The enzyme's activity unit was defined as the amount of purpurogallin generated during the oxidation of pyrogallol to purpurogallin by peroxidase at 200 degrees Celsius. The amounts of thiobarbituric acid reactive substances (TBARS)/malondialdehyde (MDA) were assessed using the thiobarbituric acid (TBA) reaction as a lipid peroxidation marker (Gutteridge & Wilkins, 1982, p. 330), whilst total proteins were determined using the Lowry, Rosebrough, Farr, & Randall, method (1951) (p. 200).

2.7.2 Assessment of Protein Carbonyl Content

Protein carbonyl content was assessed according to the method described by Ardestani & Yazdanparast, (2007) and was expressed as nmol/mg protein (p. 25).

2.7.3 Assessment of Conjugated Dienes

The conjugated dienes were measured in brain tissue according to the method described by Slater, (1984), with slight modification (p. 285). The brain tissues were homogenised separately in ice-cold phosphate buffer (pH 7.4) at a tissue concentration of 50 mg/ml. The tissues were also homogenised in the same buffer at a concentration of 5 mg/ml. A 0.5-ml aliquot and a chloroform-methanol mixture (2:1) were taken in a centrifuge tube. This mixture was centrifuged at 1000-g for 5 min. Chloroform was evaporated after steaming at 50°C. The lipid residue was dissolved in 1.5 ml methanol. Readings were taken at 233 nm.

2.7.4 Assessment of Mitochondrial ATPase

Mitochondrial ATPases (Na$^+$/K$^+$, Ca$^{2+}$ and Mg$^{2+}$) were measured by the method of Matsukawa and Tajikuchi (1982) (p. 715). Activity of the ATPases was assigned by measuring the amount of inorganic phosphate liberated following incubation with 25mM disodium ATP. The inorganic phosphate liberated was estimated by the method of Fiske and Subarow (1925) (p. 380).

2.7.5 Assessment of Mitochondrial Protein

The method of Lowry et al., (1951) was used to quantify mitochondrial protein. Bovine Serum Albumin (BSA) was used as standard (p. 200). All experiment was carried out in triplicate.

2.8 Statistical Analysis

Statistical evaluations of all data were done using one-way analysis of variance (ANOVA) to test for differences in groups. All analysed results were presented as mean ± standard error of mean (SEM) and Duncan's multiple comparisons test was used to determine significant differences between means. Instant-Graphpad Software, San Diego, California, USA, was used for this analysis. A p-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Results

3.1.1 Lipid Peroxidation Level and Enzymatic Antioxidant Status in the Brain Mitochondria

The results in Table 1 recorded the effects of crude oil, A. floribunda and their combinations on oxidative stress markers in control and experimental Wistar rats. MDA levels in the crude oil treated group were significantly (p < 0.05) higher than in the control group. MDA levels in the A. floribunda stem bark extract-treated group were significantly (p < 0.05) lower than that in the crude oil-treated group. Significant variations were observed in enzymatic antioxidant status [superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX)] in the brain mitochondria of the rats. CAT activity in the crude oil-treated group was significantly (p < 0.05) higher than in the control group. CAT levels in the A. floribunda stem bark extract-treated group were significantly (p < 0.05) lower than that in the crude oil-treated group. The rats exposed to crude oil-induced significant (p < 0.05) reduction in the activity of SOD and POX but activated significant (p < 0.05) increase in

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CAT activity and MDA levels, while the groups treated with *Allanblackia floribunda* stem bark extract maintained statistically comparable values to normal control in a dose-dependent manner. Statistically significant lower SOD and POX activities (p < 0.05) were observed in rats from the crude oil treated group compared to the normal control group (Table 1). Compared to the standard control group, treatment with oil significantly decreased the SOD and POX activity in the brain mitochondria (44.33% and 60.57%, respectively).

### Table 1: Effects of crude oil, *A. floribunda* stem bark extracts and their combinations on brain mitochondrial oxidative stress markers in control and experimental Wistar rats.

| Group / Assay | Group 1 Control | Group 2 E only | Group 3 Co only | Group 4 Co+E200mg/kg | Group 5 Co+E400mg/kg |
|---------------|----------------|---------------|----------------|----------------------|---------------------|
| Catalase      | 3.54 ± 0.47<sup>a</sup> | 3.23 ± 0.21<sup>a</sup> | 5.84 ± 0.38<sup>b</sup> | 3.32 ± 0.37<sup>a</sup> | 4.15 ± 0.36<sup>a</sup> |
| POX           | 3.50 ± 0.35<sup>a</sup> | 2.86 ± 0.15<sup>a</sup> | 1.38 ± 0.33<sup>a</sup> | 2.94 ± 0.45<sup>a</sup> | 3.01 ± 0.25<sup>a</sup> |
| SOD           | 4.94 ± 0.25<sup>a</sup> | 4.04 ± 0.50<sup>a</sup> | 2.75 ± 1.09<sup>b</sup> | 4.38 ± 0.58<sup>a</sup> | 4.60 ± 0.86<sup>a</sup> |
| Protein       | 54.6 ± 2.90<sup>a</sup> | 57.6 ± 2.00<sup>a</sup> | 46.0 ± 1.60<sup>b</sup> | 54.7 ± 2.30<sup>a</sup> | 58.6 ± 2.40<sup>a</sup> |
| MDA           | 8.02 ± 1.05<sup>a</sup> | 7.94 ± 1.02<sup>a</sup> | 15.82 ± 1.24<sup>b</sup> | 7.76 ± 1.04<sup>a</sup> | 7.89 ± 0.58<sup>a</sup> |

The values are expressed as mean ± SEM of determinations from five samples. Means with different superscripts are significantly different (p < 0.05) across the row. Catalase activity is expressed as unit/mg protein. SOD = Superoxide dismutase activity is expressed as unit/mg protein. POX = Peroxidase activity is expressed as unit/mg protein. MDA = Malondialdehyde level is presented in µmole MDA/g tissue.

### 3.1.2 Protein Carbonyl Content and Conjugated Dienes

The changes in the levels of hepatic protein carbonyl content and conjugated dienes in normal control and experimental rats are shown in Table 2. The levels of protein carbonyl content and conjugated dienes were significantly increased (p < 0.05) in crude oil-treated rats when compared with normal control rats. The combined administration of *A. floribunda* extract and crude oil significantly lowered the levels of protein carbonyl content and conjugated dienes in the brain of rats when compared to crude oil-treated rats.

### Table 2: Effects of crude oil, *A. floribunda* stem bark extracts and their combinations on brain mitochondrial protein carbonyl and conjugated diene levels in control and experimental Wistar rats.

| Group / Assay | Group 1 Control | Group 2 E only | Group 3 Co only | Group 4 Co+E200mg/kg | Group 5 Co+E400mg/kg |
|---------------|----------------|---------------|----------------|----------------------|---------------------|
| Protein carbonyls | 2.49 ± 0.05<sup>a</sup> | 3.70 ± 0.21<sup>a</sup> | 5.27 ± 0.32<sup>c</sup> | 3.91 ± 0.36<sup>b</sup> | 4.01 ± 0.42<sup>c</sup> |
| Conjugated dienes | 8.74 ± 1.05<sup>a</sup> | 14.26 ± 1.2<sup>c</sup> | 19.86 ± 0.98<sup>c</sup> | 15.05 ± 0.74<sup>b</sup> | 15.64 ± 0.58<sup>c</sup> |

The values are expressed as mean ± SEM of determinations from five samples. Means with different superscripts are significantly different (p < 0.05) across the row. Protein carbonyl and conjugated diene levels are expressed as nmol/mg protein.

### 3.1.3 Brain Mitochondrial ATPase Activity

The effects of crude oil, *A. floribunda* stem bark extracts and their combinations on brain mitochondrial ATPase activity in control and experimental Wistar rats are presented in Table 3. Crude oil significantly (p < 0.05) reduced ATPases in the brain mitochondrial tissues when compared with the control. The combined treatment of crude oil with *A. floribunda* significantly (p < 0.05) increased the activities of ATPases to levels comparable with the control.

### Table 3: Effects of crude oil, *A. floribunda* stem bark extracts and their combinations on brain mitochondrial Adenosine triphosphatase (ATPase) activity in Wistar rats.

| Group / Assay | Group 1 Control | Group 2 E only | Group 3 Co only | Group 4 Co+E200 mg/kg | Group 5 Co+E400mg/kg |
|---------------|----------------|---------------|----------------|----------------------|---------------------|
| Ca<sup>2+</sup> ATPase | 22.4 ± 1.40<sup>a</sup> | 52.5 ± 0.06<sup>b</sup> | 10.4 ± 0.09<sup>c</sup> | 18.3 ± 0.07<sup>d</sup> | 20.1 ± 0.07<sup>e</sup> |
| Mg<sup>2+</sup> ATPase | 07.5 ± 0.01<sup>a</sup> | 35.8 ± 0.09<sup>b</sup> | 04.3 ± 0.03<sup>c</sup> | 10.1 ± 0.05<sup>d</sup> | 14.7 ± 0.06<sup>e</sup> |
| Na<sup>+</sup>/K<sup>+</sup> ATPase | 32.5 ± 2.50<sup>a</sup> | 60.6 ± 4.50<sup>b</sup> | 16.5 ± 6.84<sup>c</sup> | 17.1 ± 0.07<sup>d</sup> | 06.2 ± 0.08<sup>abe</sup> |
| Total ATPase | 112.4 ± 0.08<sup>a</sup> | 198.9 ± 0.04<sup>a</sup> | 81.2 ± 0.01<sup>a</sup> | 95.5 ± 0.10<sup>a</sup> | 100.2 ± 0.22<sup>a</sup> |

Values are mean ± SEM (n=5). Means carrying different notations are statistically different at p < 0.05. Total protein unit is in mg/ml, ATPase (= Adenosine triphosphatase) activity is expressed as µmole of free phosphate (Pi) released min<sup>-1</sup> (mg protein)<sup>-1</sup>.
3.2 Discussion

3.2.1 Lipid Peroxidation Level and Enzymatic Antioxidant Status in the Brain Mitochondria

Malondialdehyde (MDA) levels are mainly used as a biomarker of free radical-mediated lipid peroxidation damage. The activity of antioxidant enzymes may be increased or inhibited under chemical stress depending on the intensity and duration of the applied stress and the susceptibility of the exposed species (Mahmoud, Shalahmetova, & Deraz, 2011, p. 2011). Catalase (CAT), SOD and POX are essential in protecting cells against oxidative stress and damage. The observed significant decrease in the activities of SOD and POX could be due to their involvement in anti-oxidative functions, which may have resulted in the formation of pro-oxidants and a relative decrease in the antioxidant status of the brain cells. At the same time, the increase in CAT may be a physiological adaptation for the elimination of generated ROS. Other researchers also reported a decrease in antioxidant enzymes (Mahmoud et al., 2011, p. 2011; Ujowundu et al., 2012, p. 90; Al-Rubai, Mohammad, & Ali, 2014, p. 1240; Olubodun et al., 2021a, p. 55). This result agrees with the report of other researchers who used other treatment protocols (Vasanth et al., 2012, p. 1700; Ujowundu, Ogbede, Igwe, & Nwaoguikpe, 2016, p. 1630). Since oxidative stress due to the toxicants is usually indicated by increased levels of products of oxidative damage (MDA) and subsequent increase in defence enzymes (POX, SOD and CAT) in response to the stress (Doherty, Ogunkuade, & Kanife, 2010, p. 360) or decreased due to overwhelming effect of the pollutants (Faramobi, Adewole, & Ajimoko, 2007, p. 160), we inferred that the decrease in the activity of SOD is due to the overwhelming impact of the toxicants from the crude oil where the system used the SOD to detoxify the resulting superoxide radicals. The non-significant reduction or relative maintenance of POX activity in the A. floribunda treated group suggests the anti-oxidative and neuro-ameliorative ability of the stem bark extract (Ottemenyn & Uguru, 2006, p. 112). The increase observed in the levels of MDA (lipid peroxidation product) confirms the induction of oxidative stress (since ROS are produced as by-products of mitochondrial respiration) in the rats exposed to crude oil only (Mahmoud et al., 2011, p. 2011; Ujowundu et al., 2012, p. 90). The inhibitory effects of the ethanol stem bark extracts may depend on the antioxidant properties of the extracts. Several studies have demonstrated that plants possess antioxidant properties in flavonoids and polyphenols, which act as potent inhibitors of ROS (Oyedeji et al., 2018, p. 25; Olubodun et al., 2021a, p. 55).

High levels of ROS destroy biomolecules within the cell and the mitochondria, leading to the permeabilisation of the mitochondrial membranes, loss of membrane potential and the release of proapoptotic proteases such as cytochrome c apoptosis-inducing factors. High levels of ROS have also been reported to be a critical regulator of cell death and mitochondrial dysfunction in neurodegenerative conditions. Since the extracts could inhibit lipid peroxidation and thus ROS, we suggest that it may be a potential target for treating neurodegenerative disorders (Oyedeji et al., 2018, p. 25).

The alteration in oxidative stress parameters induced by crude oil did not appear in the rats orally given A. floribunda extract alone. The antioxidants in A. floribunda extract are likely to counteract or minimise the undesirable effects induced by crude oil. Marwa et al. (2015) reported that Allium sativum reduced the toxicity of the pesticide deltamethrin in haematology and erythrocyte antioxidant defence systems in rats (p. 20).

The significant decrease in total protein in the crude oil group may be attributed to the decrease in the function of the brain as a result of the crude oil. However, the non-significant decrease or near control values of total protein concentrations in the treatment group may indicate ameliorative effects of the stem bark extracts. This observation is in agreement with the studies of the researchers who reported the protective potential of O. gratissimum and G. latifolium against ethanol-induced and CCl4-induced liver injury in albino rats, respectively (Ujowundu et al., 2012, p. 90; Ujowundu, Nwaoguikpe, Okwu, & Ene, 2014, p. 647).

3.2.2 Protein Carbonyl Content and Conjugated Dienes

Protein carbonyl content, in addition to lipid peroxidation, may also serve as a marker for protein oxidation for proteins containing amino acid residues like lysine, arginine, proline, threonine and glutamic acid. The significant increase in protein carbonyl levels and conjugated dienes in the brain mitochondrial of crude oil exposed rats confirm the oxidative damage in the brain tissues. The increase of protein oxidation and the protection by the
ethanol extract is in accordance with the findings of Ajiboye, Adeleye, Salau, Ojewuyi, & Adigun (2014), who reported that phenol extract of Parkia biglobosa fruit pulp decrease significantly the hepatic protein oxidation in aflatoxin B treated rats (p. 670). The increased levels of conjugated dienes (a mutagenic product of lipid peroxidation) in crude oil-treated rats indicate the state of redox imbalance and may lead to oxidative stress. A. floribunda extracts significantly decrease brain mitochondrial conjugated dienes in crude oil-treated rats, which prevented the peroxidation of lipids. This may be due to the antioxidant properties in the extracts.

### 3.2.3 Brain Mitochondrial ATPase Activity

Mitochondria is not just the energy house of the cell; it is also involved in several metabolic pathways. It is essential for both the survival as well as the death of a cell. Energy is generated by the mitochondria in the form of ATP by ATP synthase. ATP synthase has a dual role and can act in a reverse direction as mitochondrial ATPase to hydrolyse ATP under conditions of mitochondrial dysfunction (Oyedeji et al., 2018, p. 25). Table 3 shows the effects of crude oil, A. floribunda stem bark extracts and their combinations on brain mitochondrial ATPase activity in control and experimental Wistar rats. Crude oil significantly (p < 0.05) reduced ATPases in the brain mitochondrial tissues compared to the control. Crude oil may have negatively affected the neuronal cells in the brain by compromising energy supply and antioxidant status, causing disruption in mitochondrial function (mitochondrial dysfunction) (Cunnane et al., 2020, p. 620; Misrani et al., 2021, p. 617590). Also, mitochondrial dysfunction may contribute to reduced ATP production and ROS generation. The combined treatment of crude oil with A. floribunda significantly (p < 0.05) increased the activities of ATPases to levels comparable with the control.

When ROS levels are elevated within the cell and mitochondria, the resulting lipid peroxidation damages the biological membranes due to changes in the microviscosity and kinetic characteristics of the biological membranes (Asagba & Eriyamremu, 2007, p. 306). The shift in microviscosity and kinetic characteristics affect the biomembrane’s ultrastructure and integrity, resulting in the loss of membrane-bound enzymes. ATPases are membrane-bound enzymes, and their activity may be reduced in response to chemical/environmental stresses that affect membrane integrity. Numerous more ways for chemical-induced reduction of ATPase activity have been proposed. One of these strategies relies on stressors’ capacity to decouple oxidative phosphorylation (Asagba & Eriyamremu, 2007, p. 306). Given that the various ATPases require energy, a reduction in ATP concentration should impair their activity and may result in bioenergetic failure. This bioenergetics failure is frequently accompanied by programmed cell death, as seen in certain degenerative diseases (Nesci, 2020, p. 813).

The considerable suppression of ATPases in the brain mitochondria of rats exposed to crude oil may be due to crude oil's interaction with the thiol groups of key proteins and enzymes (Asagba & Eriyamremu, 2007, p. 306). Increased ATPase activity was detected in the brain mitochondria of rats exposed to crude oil and A. floribunda, with the 40mg/kg extract having a more pronounced effect. Increased ATPase activity in the brain mitochondria of treated rats compared to crude oil-treated animals (Table 3) may reflect an attempt by the brain mitochondrial tissues to adapt to possible crude oil stress. Increased ATPase activity may increase cell viability, as the resting membrane potential required for optimal cell function would be maintained (Asagba & Eriyamremu, 2007, p. 306). The increased ATPase activity could result from a compensatory increase in viable cells, which may have been required to counteract a hypothetical input of water into brain cells. This could be an adaptive response on the part of the brain to guarantee that regular neuronal functioning is not jeopardised.

### 4. Conclusion

The study showed that oral administration of ethanol extract of A. floribunda stem bark could alter crude oil-induced oxidative stress, protein carbonyls, conjugated dienes, antioxidant enzymes, and ATPases, suggesting that it protects the brain mitochondria from mitochondrial dysfunction as a result of crude oil exposure. The probable mechanism could be attributed to its strong free radical scavenging activity due to a significant amount of the phytochemical compounds reported to be present in the plant.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Ajiboye, T. O., Adeleye, A. O., Salau, A. K., Ojewuyi, O. B. & Adigun, N. S. (2014). Phenolic extract of Parkia biglobosa fruit pulp stalls aflatoxin B – mediated oxidative rout in the liver of male rats. Rev. Bras. Farmacogn. 24: 668-676. https://doi.org/10.1016/j.bjp.2014.10.010

Al-Rubaei, Z.M.M., Mohammad, T.U. & Ali, L.K. (2014). Effects of Local Curcumin on Oxidative Stress and Total Antioxidant Capacity in vivo Study. Pakistan Journal of Biological Sciences, 17; 1237-1241. DOI:10.3923/pjbs.2014.1237.1241

Asagba S.O. & Eriyamremu, G.E. (2007). Oral Ardestani, A., & Yazdanparast, R. (2007). Antioxidant and free radical scavenging potential of Achillea santolina extracts. Food Chem. 104: 21-29. https://doi.org/10.1016/j.foodchem.2006.10.066

Arimon, M., Takeda, S., Post, K.L., Svirsky, S., Hyman, B.T., …Berezovska, O. (2015). Oxidative stress and lipid peroxidation are upstream of amyloid pathology. Neurobiol. Dis. 84, 109–119. DOI: 10.1016/j.nbd.2015.06.013

Asagba S.O. & Eriyamremu, G.E. (2007). Oral Cadmium Exposure and Levels of Superoxide Dismutase, Catalase, Lipid Peroxidation and ATPases in the Eye. Research Journal of Environmental Toxicology, 1: 204-209. DOI: 10.3923/rjet.2007.204.209

Borut, P., & Rok, F. (2014). The Protective Role of Antioxidants in the Defence against ROS/RNS-Mediated Environmental Pollution. Oxidative Medicine on Cell Longevity. 2014: 671539. DOI: 10.1155/2014/671539

Cassidy, L., Fernandez, F., Johnson, J.B., Naiker, M., Owoola, A.G., …Broszczak, D.A. (2020). Oxidative stress in Alzheimer’s disease: A review on emergent natural polyphenolic therapeutics. Compl. Ther. Med. 49:102294. DOI: 10.1016/j.ctim.2019.102294

Cunnane, S.C., Trushina, E., Morland, C., Prigione, A., Casadesus, G., Millan, M. J. (2020). Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. Nat Rev Drug Discov. 19(9): 609–633. [PubMed] [PMC free article]

Doherty, V.F., Ogunkuade, O.O., & Kanife, U.C. (2010). Biomarkers of oxidative stress and Heavy metal levels as indicators of environmental pollution in some selected fishes in Lagos, Nigeria. Am. Eurasian J. Agric. Environ. Sci. 7(3):359-365. https://www.idosi.org/aejas/aejas7

Faramobi, E.O, Adewole, O.A., & Ajimoko, Y.R. (2007). Biomarkers of oxidative stress and Heavy metals levels as indicators of environmental pollution in African Catfish (Clarias gariepinus) from Nigeria Ogun River. Int. J. Environ. Res. Public Health. 4(2):158-165. DOI: 10.3390/ijerph2007040011

Fiske, C.H., & Subarrow, Y. (1925). The colorimetric determination of phosphorus. J. Biol. Chem. 66:375–400. https://doi.org/10.1016/S0021-9247(37)99999-2

Galbusera, C., Facheres, M., Magni, F., Galimberti, G., Sala, G., …Tremolada, L. (2004). Increased susceptibility to plasma lipid peroxidation in Alzheimer’s disease patients. Curr. Alzheimer Res. 1, 103–109. DOI: 10.2174/1567205043332171

Giorgi, C., Marchi, S., & Pinton, P. (2018). The machineries, regulation and cellular functions of mitochondrial calcium. Nat. Rev. Mol. Cell Biol. 19:713-730. DOI: 10.1038/s41580-018-0052-8

Gutteridge, JMC, & Wilkins, C. (1982). Copper dependent hydroxyl radical damage ascorbic acid formation of a thiobarbituric reactive products. FEBS Lett. 137:327-331. DOI: 10.1016/0014-5793(82)80377-0

Johnson, D., & Lardy, H. (1987). Isolation of Liver and Kidney mitochondria. Methods Enzymol. 10: 246-253. DOI: 10.1016/0076-6879(87)92293-X

Lapidus, R.G., & P.M. Sokolove, (1993). Spermine inhibition of the permeability transition of isolated rat liver mitochondria: An investigation mechanism. Arch Biochem. Biophys., 306: 246-253. https://doi.org/10.1006/abbi.1993.1507

Llanos, Gonzalez, E., Henares-Chavarino, A.A., Pedrero-Prieto, C.M., Garcia-Carpintero, S., Frontinan-Rubio, J., …Sancho-Bielsa, F.J. (2019). Interplay between Mitochondrial Oxidative Disorders and Proteostasis in Alzheimer’s Disease. Front. Neurosci. 13:1444. DOI: 10.3389/fnins.2019.01444

Ojewuyi, O. B. & Adigun, N. S. (2014). Potential of Achillea santolina extracts. Pakistan Journal of Biological Sciences, 17: 204–209. DOI: 10.3923/pjbs.2014.1237.1241

Rubaei, Z.M.M., Mohammad, T.U. & Ali, L.K. (2014). Effects of Local Curcumin on Oxidative Stress and Total Antioxidant Capacity in vivo Study. Pakistan Journal of Biological Sciences, 17: 1237-1241. DOI:10.3923/pjbs.2014.1237.1241

Sancho-Bielsa, F.J. (2019). Interplay between Mitochondrial Oxidative Disorders and Proteostasis in Alzheimer’s Disease. Front. Neurosci. 13:1444. DOI: 10.3389/fnins.2019.01444

S. O. Olubodun and O. Ikponmwosa-Eweka
Effects of Allanblackia floribunda Stem-bark Extracts on Oxidative Stress and Mitochondrial Permeability Transition Pore Opening in Rat Liver.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). "Protein measurement with folin-phenol reagent," J. Biol. Chem. 193: 265 – 275. http://garfiled.library.upenn.edu/classics/1977/A1977DM02300001.pdf

Mahmoud, K., Shalhametova, T., & Deraz, Sh. (2011). Effect of Crude oil Intoxication on Antioxidant and Marker Enzymes of Tissue Damage in Liver of Rat. Int. J. Biol. Biomol. Agric., F. Biotech. Eng. 5 (11): 1-2011. DOI:10.5281/zenodo.1062600

Marwa, N., Ghada, B.S., Hassen, K., Fatma M.A., Abdelmajid, K., …. Mongi, S. (2015). Histopathological, oxidative damage, biochemical and genotoxicity alterations in hepatic rats exposed to deltamethrin: modulatory effects of garlic (Allium sativum). Canadian Journal of Physiology and Pharmacology. 1-34. DOI: 10.1139/cjpp-2015-0477

Matsukawa, R., & Takiguchi, H. (1982). Effect of indomethacin on Ca2+-stimulated adenosine triphosphatase in the synaptic vesicles of rat brain In vitro. International Journal of Biochemistry. 14:713–717. DOI: 10.1016/0020-711x(82)90007-6

Misra, H.P., & Fridovich, I. (1972). The role of superoxide ion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170 – 3175. PMID: 4623845

Misrani, A., Tabassum, S., & Yang, L. (2021). Mitochondrial Dysfunction and Oxidative Stress in Alzheimer’s Disease. Front. Aging Neurosci. 13:617588. DOI: 10.3389/fnagi.2021.617588

Nes, S. (2020). The mitochondrial permeability transition pore in cell death: A promising drug binding bioarchitecture. Medicinal Research Reviews. 40: 811-817. DOI: 10.1002/med.21635

Ngueguim, T.F., Mbatchou, A., Donfack, J.H., Dzeufiet, D.D.P., Gounoue, K.R., …. Dimo, T. (2016). Dichrocephala integrifolia (Linn.f.) O. Kuntze (Asteraceae) leaves aqueous extract prevents ethanol-induced liver damage in rats. Pharmacolog. DOI: 10.5567/pharmacologia.2016.337.343

Nyman, M. (1959). Serum haptoglobin. Scand. J. Clin. And Lab. Invest. 11, Suppl. 39. https://pubmed.ncbi.nlm.nih.gov/106589

Olantantanta O.D., Areola, J.O, Ayannuga, O.A, & Babalola, O.O. (2015). Evaluation of the gonadoprotective effects of Allanblackia floribunda Oliver (Clusiaceae) on testes and accessory organs of Wistar rats. Journal of Medical and Biological Science Research. 1 (9): 134-144. http://www.pearresearchjournals.org/journals/mjbr/archive/2015/Nov/Pdf/Olanta	nt%20et%20al.pdf

Olaboda, S.O., Eze, C.K. & Eniyemre, G.E. (2021a). Ash from palm bunch modulates enzyme functions in the root of maise (Zea mays) and cowpea (Vigna Unguiculata) grown in crude oil polluted soil. Bioscience Research Journal. 33(1): 53-62. http://www.niseb.org.ng/journals

Olaboda, S.O., Fayemi, D.K., & Osagie, O.A. (2021b). Biochemical changes in rats exposed to crude oil and the antioxidant role of Allanblackia floribunda stem-bark. Biokhimia, 33(1): 67–76. http://www.ojs.klobexjournals.com/index.php/bkr/article/viewFile/1357/1346

Otunrinin, S.O., & Uguru, M.O. (2006). Acute toxicity studies, anti-inflammatory and analgesic activities of the methanolic extract of the stem bark of Enantia chlorantha and Nauclea latifolia. J. Pharm. Bioresources., 3 (2): 111-115. DOI: 10.4314/jpb. v3i2.32105

Oyedeji, T.A., Akobi, C.I. Onireti, D.O. & Babalola, O. (2006). Acute toxicity studies, anti-inflammatory and analgesic activities of the methanolic extract of the stem bark of Enantia chlorantha and Nauclea latifolia. J. Pharm. Bioresources., 3 (2): 111-115. DOI: 10.4314/jpb. v3i2.32105

Ray, P.D., Huang, B.W., & Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24, 981–990. DOI: 10.1016/j.cellsig.2012.01.008

Sies, H., & Jones, D.P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat. Rev. Mol. Cell Biol. 21, 363–383. DOI: 10.1038/s41580-020-0230-3

Sinha KA. (1971). Colorimetric assay of catalase. Anal. Biochem.47 : 389 – 394. DOI: 10.1010/1.cellsiq.2012.01.008

Slater, T.I. (1984). Overview of methods used for detecting lipid peroxidation. Methods Enzymol. 105: 283-93. DOI: 10.1016/s0076-6879(84)50536-9

Ujowundu, C.O, Kalu, F.N., Nwaoguikpe, R.N., Okechukwu, R.I., & Ihejirika, C.E. (2012). The antioxidants potentials of Gongronema latifoliumon diesel petroleum induced hepatotoxicity. Journal of Applied Pharmaceutical Science, 2(1): 90-94.
https://www.japsonline.com/admin/php/uploads/349_pdf.pdf

Ujowundu, C.O, Nwaoguikpe, R.N, Okwu, G.N., & Ene, A.C. (2014). Crude oil induced oxidative changes and chemoprotective roles of Ocimum gratissimum and Gongronema latifolium formulated diet. J. Adv. Biol., 5(2): 645-650.
https://doi.org/10.24297/jab.v5i2.3771

Ujowundu, C.O, Ogbede, J.U, Igwe, K.O., & Nwaoguikpe, R.N. (2016). Modulation of biochemical stress initiated by toxicants in diet prepared with fish smoked with polyethylene (plastic) materials as fuel source. Afr. J. Biotech. 15(30): 1628-1640. DOI: 10.5897/AJB2015.15119

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M.,Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44–84. DOI: 10.1016/j.biocel.2006.07.001

Vasanth, S., Arul, G., Vijayakumar, T.S., Karthikeyeni, S., Manimegalai, M., ...Subramanian, P. (2012). Assessment of anthracene on hepatic and antioxidant enzyme activities in Labeorohita (Hamilton, (1822). Int. J. Pharm. Life Sci. 3(5):1696-1704.