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Effects of Allanblackia Floribunda Stem-Bark on Modulations of Calcium and Mitochondrial DNA in Crude Oil Stress

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Abstract

The effects of Allanblackia floribunda stem-bark on calcium (Ca^{2+}) and mitochondrial DNA (mt.DNA) concentrations in male Wistar rats exposed to crude oil were examined. The aqueous extract (AE) and ethanol extract (EE) were obtained by extracting the stem-bark of A. floribunda with water and ethanol (EE). Thirty male Wistar rats were distributed into five groups at random, with five rats belonging to each group over the fourteen-day trial. The results showed that when 5.0 (ml/kg bw) crude oil was orally administered to the rats, it resulted in a considerable rise ($p < 0.05$) in mt.DNA when equated to the values from the control group, but a significant decrease ($p < 0.05$) in Ca^{2+} concentration when compared to the control values. On the other hand, when the values are compared to the values of the control group, the group that received only A. floribunda stem-bark extracts showed no significant ($p > 0.05$) difference in Ca^{2+} and mtDNA. The comparison with the control values shows that the rats that received crude oil and A. floribunda extracts at the same time had a non-significant ($p > 0.05$) change in Ca^{2+} and mt.DNA concentrations. The considerable changes found in the crude oil-only group suggest that crude oil may have triggered unscheduled production of mt.DNA, resulting in regulation of mitochondrial Ca^{2+} sequestration and, as a result, decreased Ca^{2+} input into the cytoplasm. The non-significant ($p > 0.05$) changes seen could be attributable to A. floribunda's antioxidant properties. While crude oil alters Ca^{2+} and mt.DNA concentrations in the rat's liver, treatment with stem-bark extracts preserved Ca^{2+} and mt.DNA concentrations in the liver relative to control values. Because of the potential therapeutic and antioxidant role of A. floribunda in crude oil stress, the study implies that rats treated with crude oil and A. floribunda stem-bark extracts may be protected from cell damage.

1. Introduction

Petroleum (crude oil), is a natural resource as well as a pollutant otherwise referred to as environmental pollutant. It is comprised of different mixtures of hydrocarbons and other compounds [1, 2]. There is no doubt that crude oil and other associated petroleum activities have brought prosperity, better living conditions and general economic development to Nigeria [3], but it is apparent that these products are potential hazards to its biodiversity, environment, and human health. The use of petroleum and its products have increased several-fold in recent years due to the usefulness of their products and by-products when compared to other natural resources.

Crude oil has been shown to promote concentration-dependent inhibition of calcium influx and mitochondrial swelling [4]. In an earlier study, administration of crude oil at the dose of 5 ml/kg/day for 2 weeks resulted in significant increase in malondialdehyde (MDA) levels and altered antioxidant enzyme activities [5, 6, 7].

Crude oil exposure may be direct or indirect. The indirect methods of exposure may be by ingestion of food crops, water and organisms in water bodies such as fishes which has been exposed due to spill. Several studies have shown that exposure to crude oil may cause alterations in biochemical, physiological, hematologic and reproductive parameters [2, 5, 6, 7, 8, 9], enhanced by the production of free radicals/reactive oxygen species (ROS) leading to the induction of oxidative stress in plants and animal tissues stress [2, 5, 7, 9]. Oxidative stress has been shown to be one of the causes of several diseases, such as, degenerative diseases, cardiovascular disease, aging and cancer [6, 10].

The major producer of reactive oxygen species (ROS) in organisms is mitochondria and the close proximity of mitochondrial DNA (mt.DNA) to ROS, makes it prone to oxidative damage than nuclear DNA, suggesting that mt.DNA is more susceptible to oxidative damage [11, 12]. As the major producer and primary target of ROS, mitochondria are thought to play an important role in cell damage. The mitochondrial theory of degeneration, extended from the free radical theory, proposes that oxidative damage generated during oxidative phosphorylation of mitochondrial macromolecules such as mt.DNA, proteins, or lipids is responsible for degenerating illnesses [12]. As mt.DNA encodes essential components of oxidative phosphorylation and protein synthesis machinery, oxidative damage-induced mtDNA mutations that impair either the assembly or the function of the respiratory chain will in turn trigger further accumulation of ROS, which may result in a vicious cycle leading to energy depletion in the cell and ultimately cell death [12, 13, 14]. As mitochondria play a critical role in regulation of apoptosis, environment-related mitochondrial oxidative stress may contribute to apoptosis [12, 15].

Since several studies have shown that exposure to crude oil may induce toxic manifestations by enhancing the production of free radicals and DNA damage (oxidative stress), the administration of medicinal plants [6, 16], may protect against environment-induced oxidative stress and hepatotoxicity [6]. Also, the results of other researchers recorded the free-radical scavenging activity, anti-inflammatory and hypoglycemic properties of *A. floribunda* (9, 16) and attributed these activities to the ability of *Allanblackia* antioxidant molecules such as flavonoids and tannins, to reduce cell defense against oxidative stress through the free radical scavenging effect of the plant extracts. This is because plants have been reported to be antimicrobial, hepatoprotective, antihepatotoxic and/or antioxidants and as such have the ability to fight the toxicity induced on biological macromolecules [17].

The use of medicinal plants as a therapeutic and curative treatment for ailments and disorders has grown in popularity recently. This is owed to the existence of phytochemicals such as alkaloids, flavonoids, polyphenols, saponins, and other phytochemicals in plants. These phytochemicals have been found to have medicinal potential, implying that they can combat toxic lesions caused on biological macromolecules by pathogens, chemicals, and/or environmental stresses [2, 5, 7, 9].

Allanblackia floribunda (Oliver or tallow-tree), used in this study is a medicinal plant belonging to the family *Clusiaceae* or *Guttiferae*. It is identified as one out of nine species of the genus [18]. It can be found in Nigeria's rain forests, particularly in the south-eastern regions. Anticancer, antibacterial, anti-inflammatory, antioxidant, and antihelmintic activities have been discovered in *A. floribunda* [9, 10, 16, 19]. In this study, we evaluated the effects of the aqueous and ethanol extracts of the stem bark of *A. floribunda* on the modulation of the concentrations of cytoplasmic calcium (cyt.Ca^{2+}) and mitochondrial DNA in rats treated with crude oil.

2.0. Materials and Method

2.1. Study area

The experiment took place at the Biochemistry Department of 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, Plant Materials, Feeds and Animals

Nigeria's Warri Refinery and Petrochemical Company provided the crude oil. Rat pellets were purchased from Vital Feeds Nigeria and male Albino Wistar rats were procured from the Department of Animal Science, Faculty of Agriculture of the University of Benin, Edo State, Nigeria. A herbal practitioner obtained fresh stem bark of *A. floribunda* from a woodland location in Edo State, Nigeria. A botanist from the University of Benin's Department of Plant Biology and Biotechnology recognized and authenticated the plant, and a voucher specimen was put in the Department's Herbarium for future reference.

2.3. Preparation of Crude oil, Plant Materials and Animals

The stem bark was washed, air dried, macerated, and sieved using a micropore sieve. The macerated form of *A. floribunda* stem bark was weighed and divided into two groups. The extract was filtered after soaking one weighted portion in ethanol for 72 hours and the second weighed piece in distilled water for 48 hours with intermittent stirring. The aqueous extract (AE) and ethanol extract (EE) of *A. floribunda* were produced by drying the filtrate using a rotary evaporator at 40°C. The crude extracts were kept in the fridge until they were needed.

In the Laboratory Animal Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, forty Wistar rats (160–180 g) were kept. They were given a conventional feed and free access to water. They were given two weeks to acclimate. The rats were cared for in acquiescence with the guiding principle for research animal care and welfare.

2.4. Experimental Design

A total of forty (40) male Wistar rats with weight range of 160 to 180 g were placed into eight groups of five rats each. The rats were kept in steel cages and given time to adjust. To produce oxidative stress in rats, 5 ml/kg body weight crude oil was supplied through oral dosing needle (oral gavage).

The rats were divided into eight groups, each with five rats:

Group 1: untreated normal rats/Control
Group 2: normal rats were given oral Crude oil (5ml/kg crude oil)
Group 3: normal rats were given AE oral *A. floribunda*
Group 4: co-administered 5ml/kg crude oil + 25ml/kg AE *A. floribunda*
Group 5: co-administered 5ml/kg crude oil + 50ml/kg AE *A. floribunda*
Group 6: normal rats were given EE oral *A. floribunda*
Group 7: co-administered 5ml/kg crude oil + 25ml/kg EE *A. floribunda*
Group 8: co-administered 5ml/kg crude oil + 50ml/kg EE *A. floribunda*

Water was provided *ad libitium* to all groups. The experiment lasted for fourteen (14) days and rats sacrificed on the fifteenth (15^{th}) day. The livers were recovered for analyses.

2.5. Isolation and Quantification of Mitochondrial DNA (mt.DNA)

The livers were standardized to 10% (w/v) in an ice-cold 0.05M potassium phosphate buffer of pH 7.4 which contains 0.2mM EGTA, and mitochondria were isolated using conventional distinction centrifugation in a buffer of 210 mM mannitol, 70 mM sucrose, 5 mM HEPES (pH 7.4), and 1 mM EGTA. The mitochondrial fraction's DNA was isolated using the phenol–chloroform extraction procedure and quantified using Oruambo's diphenylamine method [19]. Using Bovine Serum Albumin as a standard, the protein content was calculated using the Folin-Ciocalteu technique [20].

2.6. Extraction and Quantification of Cytoplasmic (Extra-mitochondrial) Calcium (Ca^{2+})

The cytoplasmic fraction is represented by the supernatant from the mitochondrial fraction, which was collected and utilized to measure Ca^{2+} concentration. The calcium concentration was evaluated using Hildebrand and Reilley's EDTA-titrimetric technique [21].

2.7. Statistical Analysis

To assess for differences in groups, all data was statistically appraised using one-way analysis of variance (ANOVA). All of the results were expressed as mean standard error of the mean (SEM), and Duncan's multiple comparisons investigation was employed to evaluate whether there existed any significant differences amongst the means. This study was conducted using Instat-Graphpad software from San Diego, California, USA. Statistical significance was defined as a p value of less than 0.05.

3.0 Results

Tables 1 and 2 indicate the effects of Allanblackia floribunda stem-bark on the content of liver mitochondrial DNA in Wister rats treated with crude oil. After 14 days of therapy, the group 2 rats had a substantial increase ($p < 0.05$) in mt.DNA concentration as compared to the untreated rats/control (group 1). The other groups (groups 3–5), which received aqueous stem bark extracts and were co-administered, saw increases in mt.DNA concentrations, although the differences were not significant (groups 3–5). (Table 1). The ethanol extracts showed similar tendencies, with minor changes in the percentage of significant ($p < 0.05$) and non-significant ($p > 0.05$) differences in the various groups (Table 2).

Table 1: Effects of aqueous stem-bark extracts of *Allanblackia floribunda* stem-bark on liver mitochondrial DNA concentration in Wister rats treated with crude oil

Group/Assay (mg/ml)	mt.DNA concentration	mt.DNA difference over control	% Change (%Δmt.DNA) over control
Group 1	0.62 ± 0.17	-	-
Group 2	2.10 ± 0.09*	1.48	239
Group 3	0.68 ± 0.07	0.06	10
Group 4	0.64 ± 0.19	0.02	03
Group 5	0.75 ± 0.09	0.13	21

Results are expressed as means ± SEM of four (4) replicates. %Δ, percentage change; mt. DNA, mitochondria DNA is expressed as mg/ml while increase/change in mt. DNA over control is expressed as %. Means carrying different notations are statistically different at $p < 0.05$.

Table 2: Effects of ethanol stem-bark extracts of *Allanblackia floribunda* on liver mitochondrial DNA concentration in Wister rats treated with crude oil.

Group/Assay (mg/ml)	mt.DNA concentration	mt.DNA difference over control	% Change (%Δmt.DNA) over control
Group 1	0.62 ± 0.17	-	-
Group 2	2.10 ± 0.09*	1.48	239
Group 6	0.75 ± 0.16	0.13	21
Group 7	0.69 ± 0.10	0.07	11
Group 8	0.84 ± 0.01	0.22	35

Results are expressed as means ± SEM of four (4) replicates. %Δ, percentage change; mt. DNA, mitochondria DNA is expressed as mg/ml while increase/change in mt. DNA over control is expressed as %. Means carrying different notations are statistically different at $p < 0.05$.

Tables 3 and 4 show the effects of Allanblackia floribunda stem-bark on extra-mitochondrial calcium concentration in the liver of Wister rats fed with crude oil. Table 3 shows a substantial decrease in extra-mitochondrial Ca^{2+} concentration ($p < 0.05$) when compared to control values. When compared to the control group, the percentage drop for group 2 rats was more than 90%, and when compared to the other groups, it was more than 50%.
The results recorded for the ethanol extract reflected relatively the same trend observed in the aqueous extract with lower percentages recorded for other groups with the group 8 rats recording the least percentage Ca^{2+} concentration of less than 5.

Table 3: Effects of aqueous stem-bark extracts of *Allanblackia floribunda* on extra-mitochondrial calcium concentration in the liver of Wister rats treated with crude oil

Group/Assay (mg/ml)	Cytoplamic Ca^{2+} Concentration	Ca^{2+} difference over control	% Change over control
Group 1	1.65 ± 0.15	-	
Group 2	0.10 ± 0.04*	1.55	94
Group 3	1.43 ± 0.09	0.22	13
Group 4	1.39 ± 0.11	0.26	16
Group 5	1.51 ± 0.10	0.14	8

Results are expressed as means ± SEM of four (4) replicates. %Δ, percentage change; Ca^{2+} is expressed as mg/ml while decrease/change in Ca^{2+} over control is expressed as %. Means carrying different notations are statistically different at $p < 0.05$.

Table 4: Effects of ethanol stem-bark extracts of *Allanblackia floribunda* on extra-mitochondrial calcium concentration in in the liver of Wister rats treated with crude oil

Group/Assay (mg/ml)	Cytoplamic Ca^{2+} Concentration	Ca^{2+} difference over control	% Change over control
Group 1	1.65 ± 0.15	-	
Group 2	0.10 ± 0.04*	1,55	94
Group 6	1.57 ± 0.04	0.08	5
Group 7	1.52 ± 0.05	0.13	8
Group 8	1.62 ± 0.13	0.03	2

Results are expressed as means ± SEM of four (4) replicates. %Δ, percentage change; Ca^{2+} is expressed as mg/ml while decrease/change in Ca^{2+} over control is expressed as %. Means carrying different notations are statistically different at $p < 0.05$.

3.1. Discussion

The results from this study shows that 5ml/kg body weight of crude oil orally given to male Wistar rats, modulated over 100 percent significant increases in liver mt.DNA concentrations and over 90 percent significant reductions in cyt.Ca^{2+} when compared to the control groups. Oruambo and Jones, [4], found that adult male guinea pigs subjected to Nigerian 'Bonny' Light Crude Oil (BLCO) by intraperitoneal injection had similar results. They found dose-dependent rises in mtDNA and decreases in cyt.Ca^{2+} concentrations in their report. Despite the fact that mitochondrial DNA reproduces self-sufficiently of nuclear DNA, it codes for proteins involved in mitochondrial and cellular metabolism. As a result, they hypothesized that the rise in BLCO-treated guinea pigs was due to metabolites that triggered unscheduled mtDNA synthesis.
The findings of the study revealed that crude oil has the power to significantly alter the concentrations of mt.DNA and cytoplasmic calcium in the livers of Wistar rats. This finding is consistent with prior findings of crude oil's potential to alter biochemical parameters in organisms' systems [2, 4]. Significant increases in mt.DNA levels in the liver of group 2 rats could indicate liver injury caused by a lack of hepatocyte membrane

integrity [9, 16]. The rise in mt.DNA concentration could be attributed to liver damage resulting from liver inflammation and disturbance caused by crude oil stress [5, 9].

Administration of *A. floribunda*, stem-bark extract inhibited the rise of mt.DNA caused by crude oil in this investigation. These results suggest that the aqueous and ethanol extracts of *A. floribunda* may have an antioxidant and antiinflammatory effect which varied with each extraction solvent. This may be because permeabilization of the mitochondrial membranes causes the release of cytochrome c, which triggers death via caspase-cascade pathways [23]. It is possible that the extracts (aqueous and ethanol) preserved the cell membranes or countered the negative effects of crude oil. It is also possible that phytochemicals in the plant extracts, such as tannins and alkaloids, may have suppressed cytochrome c or boosted the gene responsible for liver cell regeneration, or scavenged free radicals, to avoid cell death [23]. Other research has indicated that combining toxicants and therapeutic plants may help to prevent or delay the onset of different illnesses and disorders [5, 9, 20].

The decrease observed in cytoplasmic Ca^{2+} concentration may be related to crude oil-induced oxidative stress, which may have adverse effects on adenosine triphosphate (ATP) synthesis. This is because low blood glucose concentration coupled with reduced ATP levels, causes the liver to release glucose molecules into the blood for onward transport to other tissues, such as, skeletal muscle or brain. When this occurs, Ca^{2+} is regarded as a second messenger. Calcium as a secondary messenger to hormonal bio-signal triggers a cascade reaction in the liver which leads to the activation of glycogen phosphorylase, which breaks down liver glycogen to release glucose which is transported to skeletal muscle for glycolysis and ATP synthesis [4].

The endoplasmic reticulum (ER) and mitochondria sequester Ca^{2+} and keeps intracellular Ca^{2+} very low by the action of Ca^{2+} pumps found in the ER, mitochondria and plasma membranes. The process may cause either an influx of extracellular Ca^{2+} into the liver cell through the plasma membrane or release of sequestered Ca^{2+} from the ER or mitochondria. When this happens, raising the intracellular Ca^{2+} concentration would triggers the sequence of cascade reactions for the breakdown of glycogen and release of glucose. It therefore means that there may be a Ca^{2+}-concentration gradient which acts to trigger the calcium pumps for Ca^{2+} influx or release.

Several studies reported a crude oil dependent inhibition of calcium influx into the mitochondria for sequestration, and the production of swelling of the mitochondria *in vitro.* They suggested in their results that increased permeability of the mitochondrial and microsomal membranes to calcium may be a contributing factor in the inhibition of calcium uptake by crude oil. Their results agreed with our results where both aqueous and ethanol extracts of *A. floribunda* stem-bark caused over 90% reductions in cyt.Ca^{2+} when compared to the control groups.

It is possible that Ca^{2+} influx or release from the ER or mitochondrial may have been modulated. We suggested that crude oil may have damaged the ER, mitochondrial or plasma membrane so that normal level of intracellular (cytoplasmic) Ca^{2+} may leach-out to increase extracellular (cytosolic) Ca^{2+} while reducing cyt. Ca^{2+}. Also, the crude oil may have altered the Ca^{2+}- concentration gradient across the plasma or mitochondrial membrane so that the calcium pumps are inhibited leading to disorganization of the permeability of the plasma or mitochondrial membrane, thus inhibiting Ca^{2+} uptake. This may result in liver cell alteration of the bio-signal cascade in which Ca^{2+} serves as second messenger thus inhibiting ATP-synthesis and probable cell and tissue damage. This may also have toxic effects for organs like skeletal muscle and brain that rely on the liver for glucose [4, 24]. Administration of *A. floribunda* extracts significantly increased cyt. Ca^{2+} to levels comparable to control values in the rats. The results suggest that the aqueous and ethanol extracts may have an antioxidant effects which may have cushioned the modulated extracellular calcium sequestration induced by crude oil. The treatment of the crude oil-induced oxidative stress rats with the plant extracts significantly increased the concentration of cyt. Ca^{2+} and reduced that of mt.DNA, suggesting that *A. floribunda* stem-bark extract may act by directly trapping free radicals and/or reducing their production, thus limiting subsequent induction of unscheduled mt.DNA synthesis, and modulation of ER/mitochondrial Ca^{2+} sequestration or Ca^{2+} concentration gradient, that may have led to the decrease of Ca^{2+} influx into the cytosol. These results are in conformity with various research works [5, 9, 16, 17, 20] which showed that plant extracts have ameliorative effects on animals treated with chemical or pathologic or environmental stressors. The study indicates that *A. floribunda* stem-bark extracts may protect the liver from cell damage and diseases.

4.0. Conclusion

The results suggest that crude oil may be hepatotoxic because it modulated cyt. Ca^{2+} and mt. DNA concentrations. However, the stem- bark extracts on administration was able to regulate the effects in the rat liver. *A. floribunda* stem-bark extracts in water and ethanol include putative phytochemicals that regulate cyt. Ca^{2+} and mitochondrial DNA concentrations in rat liver to various degree. This action may be related to the antioxidative and ameliorating effects of the extracts which is associated with improvement of the cyt. Ca^{2+} and mt. DNA concentrations in the rat liver. More research is needed to determine the processes by which the extracts prevented crude oil stress.

Conflict of interest

The authors declare no conflict of interest.

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