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Biochemical changes in rats exposed to crude oil and the antioxidant role of *Allanblackia floribunda* stem-bark

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ABSTRACT: The role of *Allanblackia floribunda* stem-bark on oxidative stress and biochemical changes induced by crude oil were investigated in rats. Thirty albino rats of both sexes, were randomly grouped into five with six rats in each group. A group which served as normal control had no treatment, a second group was orally administered crude oil at a dose of 5 ml/kg body weight, every other day for 28 days, a third group received 50 mg/kg *A. floribunda* stem-bark extracts, another two groups were orally administered crude oil at a dose of 5 ml/kg body weight and received *A. floribunda* stem-bark extracts (25 and 50 mg/kg) using oral dosing needle, every other day for 28 days. The results showed that administration of 5.0 (ml/kg bw), crude oil resulted in a significant increase in serum malondialdehyde (MDA) levels, and induced significant alterations in the activities of plasma superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) in the rats. A significant increase was also observed in the activities of alkaline phosphatases (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) and increase the level of uric acid and total protein. The biochemical changes in antioxidant enzymes and other marker enzymes may be due to oxidative stress and/or adaptive responses.
The rats that were simultaneously treated with crude oil and *A. floribunda* extracts however, maintained relatively no significant ($p>0.05$) biochemical changes in the serum and hepatic cells when compared with the control. The non-significant ($p>0.05$) changes recorded may be due to the antioxidant role of *A. floribunda*. The results shows that crude oil induces oxidative stress and biochemical changes in serum and liver in the rats. The crude oil treated rats needle-dosed with *A. floribunda* showed the probable therapeutic and antioxidative role of *A. floribunda* in crude oil oxidative stress.

Keywords: Antioxidant, Biochemical changes, Crude oil, *Allanblackia floribunda*, Oxidative stress

Introduction

Normal metabolic cells, tissues, and organs produce activated chemical species known as reactive oxygen species (ROS), an array of highly reactive molecules produced in enzymatic or non-enzymatic reactions through sequential reductions of oxygen (1). Reactive oxygen species (ROS) are responsible for toxic effects in the body through various tissue damages. The ability to maintain the production and degradation of generated ROS is contained in the cell. However, when excess generation or excess

degradation of ROS occurs, it may lead to abnormal oxidative damage (1, 2). Unfavourable conditions such as environmental/industrial toxicant-induced ROS could overwhelm the antioxidant defenses, leading to increased lipid peroxidation and oxidative damage (3). High concentration of ROS can result in non-controlled oxidation in cells, which is referred to as oxidative stress. Oxidative stress is defined as a serious imbalance between the production of ROS and antioxidant defense and this situation (ROS-attack) can cause damage to cellular macromolecules, including proteins (protein oxidation), membrane lipids (lipid peroxidation), carbohydrates and DNA (4, 5).

Crude oils contain hydrocarbons, some heavy metals and other chemicals (6, 7). It is the major form of income for the Nigerian economy and is produced in the Niger-Delta region where we have recurrent oil spills. These spills pollute the environment and pose treats to both aquatic and terrestrial organisms (5, 8). Heavy metals as well as crude oil poisoning has been reported to produce ROS and other free radicals which induce oxidative stress and peroxidation of lipids when plants and animals are exposed to them (1, 6, 7). Various researchers have reported increased incidence of organ damage in both chemical and environmental stresses (2, 9).

It has been estimated that about three million lives may be saved each year when plants in form of fruits, vegetables, spices and herbs are increased in diet. This they proposed will help overcome and/or ameliorate toxic effects of contaminants/pollutants (10).

Nigeria, like other Africa countries, is blessed with various arrays of medicinal plants. Majority of the people of Nigeria do not use these medicinal plants even though some people do rendering most of these plants under-utilized. Diverse plant species possesses nutritional and phytochemical compounds which are important in maintaining general well-being. They possess antioxidative potentials and are able to prevent or reduce damages such as lipid peroxidation, oxidative damage to membrane, etc (11).

Allanblackia floribunda Oliv is one of a plant species of the nine species of the genus *Allanblackia* and belongs to the family, *Clusiaceae*. It is commonly known as tallow tree in English Language. It is found in a wide range of habitats and distributed in moist evergreen tropical rainforests across Africa. *A. floribunda* is a tree commonly seen in the Niger-Delta Region of Nigeria, especially in abandoned forests (acidic soils) with rainfall as high as 2400 mm. It is a seasonal fruit found during the rainy season in some countries but are available all seasons in Nigeria. It is an indigenous fruit tree that has been recently recognised as containing valuable edible oil, used for the manufacture of margarine, soap, chocolate, ointment and food products. As a medicine, the pounded bark, roots, leaves and/or seed oils is applied as herbal remedy in treatment of various illnesses (12, 13). The medicinal values of the plant lies in their phytochemicals, which includes polyphenols, flavonoids, saponins, tannins and glycosides, important for antioxidant, anti-inflammatory and analgesic activities (14, 15).

Some researchers reported the free-radical scavenging activity and anti-inflammatory property of *A. floribunda* (16). Ayoola *et al* (16) also reported that the leaves in *A. floribunda* have hypoglycemic activity in alloxan-induced diabetic rats. They inferred that the hypoglycemic activity may be due to the ability of *Allanblackia* antioxidant molecules such as *flavonoids, tannins* to reduce cell defense against oxidative stress through the free radical scavenging effect of the plant extracts.

Crude oil contamination has been on the increase in the past decade. The need to investigate the impacts of crude oil on living systems and their probable toxicity can therefore not be over emphasized. Also, since the growing focus to follow systematic research methodology to evaluate scientific basis for traditional herbal medicines claimed to possess antioxidant, anti-inflammatory and analgesic activities, is on the increase, this study is designed to evaluate the possible therapeutic and antioxidative effects of *A. floribunda* on crude oil oxidative stress in rats.

Materials and Methods

Study area

The experiment was carried out at the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria (Latitude 6° 23’ 44”; Longitude 5° 36’ 49”; Altitude 360 feet).

Collection of Crude oil, Plant Materials, Feeds and Animals

The crude oil was obtained from Warri Refinery and Petrochemical Company, Nigeria. Albino rats (Wistar strain) were obtained from the Department of Animal Science, Faculty of Agriculture, University of Benin, Edo State, Nigeria and rat pellets was purchased from vital feeds Nigeria. The fresh stem bark of *A. floribunda* was collected from a forest area in Edo State, Nigeria, by herbal practitioner. The plant was identified and authenticated by a botanist in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria and a voucher specimen deposited in the Herbarium of the Department for reference.

Preparation of Crude oil, Plant Materials and Animals

The stem bark was washed, dried at room temperature, macerated and sieved through a micro pore sieve. The macerated form of *A. floribunda* stem bark was soaked in ethanol for 72 hours with occasional stirring. The extracts were filtered using 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.

Thirty albino rats with a mean weight 170±5 g were maintained in the Laboratory Animal Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Benin. They were fed with standard diet and water ad libitum. They were acclimatize for two weeks. The study was approved by the ethics committee of the University of Benin, Nigeria. The rats were handled in accordance with the guidelines on the care and wellbeing of research animals.

Phytochemical Screening of the Ethanol Extract

The phytochemical screening of the *A. floribunda* stem bark extract was carried out using standard procedures (17).

Experimental Conditions

Albino Wistar rats were randomly divided into five groups of six rats each (n=6).
Group I served as Control and received distilled water ad libitum.
Group II received Crude oil (5ml/kg BW) by gavage every other day for 28 days of treatment.
Group III received *A. floribunda* extract (200mg/kg BW) by gavage for 28 days of treatment.
Group IV and Group VI received Crude oil (5 ml/kg BW) every other day with 200mg/kg *A. floribunda* and 400mg/kg *A. floribunda* extract combination respectively by gavage administration for 28 days.

Water was provided *ad libitium* to all groups. The experiment lasted for twenty eight (28) days and rats sacrificed on the twenty ninth (29^{th}) day. The blood and liver were recovered for analyses.

Collection of Blood and Liver Samples

After treatment, the rats were sacrificed by cervical decapitation on the 29^{th} day, 24 hours after the last treatment. The blood samples were collected into labeled sample tubes and allowed to clot for 30 min at room temperature. Serum was separated from the blood by centrifuging at 3000 rpm for 15 min. The supernatant (serum) was stored in sterile vial and kept in the freezer for biochemical analyses. The liver was excised and homogenised with 5 ml (50 mM, pH 7.4) phosphate buffer to give a 20% (w/v) liver homogenate. The homogenate was centrifuged at 3000 rpm for 20 min and the supernatant obtained for biochemical analyses.

Biochemical Analyses

The serum sample prepared from the blood was used to determine alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA) and lactate dehydrogenase (LDH) activity. The liver function markers and LDH in the serum were measured using Randox test kit as specified by the manufacturers, Randox laboratory ltd (Antrim, UK, BT29 4QY). While the supernatant from liver homogenate was used to determine oxidative stress markers.

The total proteins was determined by the method described by (18) while superoxide dismutase (SOD) activity was assayed according to the method described by (19) and was expressed as units/mg tissue weight. One unit of enzyme was defined as the amount of the enzyme required for 50% inhibition of oxidation of epinephrine to adrenochrome in one minute. Catalase (CAT) specific activity was determined according to the method of (20) and was expressed as units/g wet tissue. Dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The amount of perchromic acid formed was taken as an activity unit. Peroxidase (POX) activity was determined using the method of (21) and was expressed as units/mg protein. The activity unit of the enzyme was defined as amount of purpurogallin formed in the oxidation of pyrogallol to purpurogallin by peroxidase at 20^0C. Malondialdehyde (MDA) levels was determined by reaction with thiobarbituric acid (TBA) and used as a lipid peroxidation marker (22). Uric acid was determined by enzymatic colorimetric method (23). All experiments were performed in five replicates.

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 represented mean ± standard error of mean (SEM) and Duncan's multiple comparisons test was used to determine significant differences between means. Instat-Graphpad software, San Diego, California, USA, was used for this analysis. A P value < 0.05 was considered statistically significant.

Results and Discussion

The results of the phytochemical constituents of the ethanol stem bark extracts of *A. floribunda* is shown in Table 1. The phytochemical screening of the stem bark extract of *A. floribunda* revealed the presence of tannins, flavonoids, saponins, glycosides, alkaloids, steroids and polyphenols (Table 1). This result is comparable to earlier reports of the phytochemistry of *A. floribunda* and other plant species by various researchers (9, 24, 25 & 26) who indicated the presences of various phytochemicals. However, we did not observe the presence of anthraquinone in our study. While Manikandan *et al* (27) reported that alkaloids and flavonoids protect cells by acting as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species, others reported that flavonoids possesses antioxidant, anti-inflammatory, anticancer and antimicrobial activity (9, 12, 24, 25, 26).

Tannins are reported to have some interactions with protein to give an effect which makes it important for management of inflamed or ulcerated cells. The phytochemical constituents present in the extract and the quantity of these constituents may be responsible for the antioxidant activities of the extracts and may also be responsible for their acclaimed medicinal uses.

The presence of these phytochemical constituents may have reduced the toxic effects of crude oil.

The presence of the phytochemical constituents in the extract of *A. floribunda* therefore, supports its use and pharmacological importance in the treatment of ailments.

Crude oil and its products have become an essential constituent of human life due to their industrial and domestic importance. However, clinical and experimental studies have shown that exposure to petroleum hydrocarbon and other constituents is a risk factor for oxidative stress and degenerative diseases (9, 28).

Table 1. Phytochemical constituents of the ethanol extracts of *A. floribunda*

Phytochemicals	Glycosides	Tannins	Saponins	Flavonoids	Alkaloids	Sterols	Polyphenols
Qualitative estimates	+++	+	++	+	++	++	++

(++) = Present in moderate concentrations; **(+)** = Present in low concentrations

Table 2 shows the effects of crude oil and *A. floribunda* on body weight in rats treated by using oral dosing needle with 5ml/kg crude oil and/or treatment with different doses of *A. floribunda.* While the rats in groups 2 significantly decreased in the percentage change in body weight when compared with control and the treatment groups, the rats in the other groups 4 (5ml/kg crude oil + 200mg/kg *A. floribunda*) and 5 (5ml/kg crude oil + 400mg/kg *A. floribunda*) recorded non-significant (P>0.05) increase in percentage change in body weight when compared with the control (groups 1). While groups 2 rats recorded 7% decrease in percentage change in body weight, groups 3, 4, and 5 recorded an increase of 25, 21 and 16% respectively when compared with control. Body weight loss is an indication of an abnormal condition and may arise from decreased food intake due to the immobility accompanying stress, or reduced absorption of food (29). The body weight were significantly increased in the rats given the extract and extract with 5ml/kg crude oil when compared to untreated crude oil rats. The extract may have inhibit the loss in body weight in rats by increasing food intake through attenuation of abnormal condition of oxidative stress or improving the absorption capacity of the intestine (29).

Since crude oil induces lipid peroxidation as a result of elevated production of reactive oxygen species (ROS) (30, 31), the observed decrease (P<0.05) in body weight in group 2 (crude oil treated rats) may be due to crude oil toxicity. Crude oil may have impaired normal growth and development through a variety of mechanisms. This research is in agreement with other researchers who reported weight loss in animals exposed to crude oil and other contaminants (9, 30 & 31). The non-significant alteration in body weight with the combined treatment may be due to the antioxidant protective role of *A. floribunda*.

Table 2: Effects of ethanol extracts of *A. floribunda* stem bark and crude oil on the body weight in albino Wistar rats

Group/Assay (g)	Initial Body Weight	Final Body Weight	% Change in Body Weight (%ΔBWt.)
Group 1: Control	165.52±2.17	205.40 ± 3.89[a]	24
Group 2: Co only	174.50±3.38	162.89.± 4.20[b]	-7
Group 3: E only (200mg/kg)	166.20±5.00	207.46 ± 5.06[a]	25
Group 4: Treated(200mg/kg)	163.44±5.30	198.16 ± 3.17[a]	21
Group 5: Treated(400mg/kg)	172.46±3.90	200.52 ± 5.07[a]	16

Results are expressed as means ± SEM of five (5) replicates. Body weight is expressed as g while change in body weight is expressed as %.

The results of total protein concentration and the serum marker enzymes are shown in Table 3. The concentration of total protein in group 2 decreased significantly (p<0.05) when compared with control and the other groups (p<0.05). The result also recorded increased activity of ALP, AST, ALT and LDH in crude oil exposed rats when compared with control and other groups (Table 3).

The administration of crude oil to the rats recorded significant increase in marker enzymes (ALT, AST, ALP and LDH) in the serum after 28 days of alternate treatment with dosing needle. Administration of the *A. floribunda* stem bark extract of two different doses, provided significant antioxidative protection,

resulting in decreased elevated serum activities of marker enzymes (Table 3 and Figure 1) when compared to crude oil untreated rats. The toxicity induced by crude oil in the liver tissues was assessed by measuring the antioxidant defense enzymes like SOD, POX, CAT and MDA levels (Table 4). Table 4 recorded reduction in the activities of SOD and POX but increase in CAT and MDA when compared with the control group. Treatment of the rats with *A. floribunda* stem bark extract to group 4 and group 5 reduced the toxicity of crude oil.

Table 3: Effects of crude oil and *A. floribunda* extracts on marker enzymes and total proteins in albino rats

Group /Assay	Group 1 Control	Group 2 Co only	Group 3 E only	Group 4 Co+E200mg/kg	Group 5 Co+E400mg/kg
Protein	54.6±2.9[a]	46.0+1.6[b]	57.6+2.0[a]	54.7+2.3[a]	58.6+2.4[a]
AST	221.5±10.82	242.14±8.10	220.5±8.5	222.18±8.09	220.46±10
ALT	46.37±1.52	63.16±6.7	46.17±3.12	49.33±5.23	45.83±1.28
ALP	260.56±19.1[a]	332.4±15[b]	249.11±11.81[a]	272.14±19.7[a]	301.9±12.44[a]
LDH	143.64±6.5[a]	172.65±9.5[b]	143.77±6.7[a]	144.78±6.8[a]	142. 94±7.6[a]

Results are expressed as means ± SEM of determinations from five samples. ALT = Alanine aminotransferase, AST=Aspartate aminotransferase, ALT=alkaline phosphatase and LDH = Lactate Dehydrogenase, were expressed as U/L. Means carrying different notations are statistically different at $p< 0.05$.

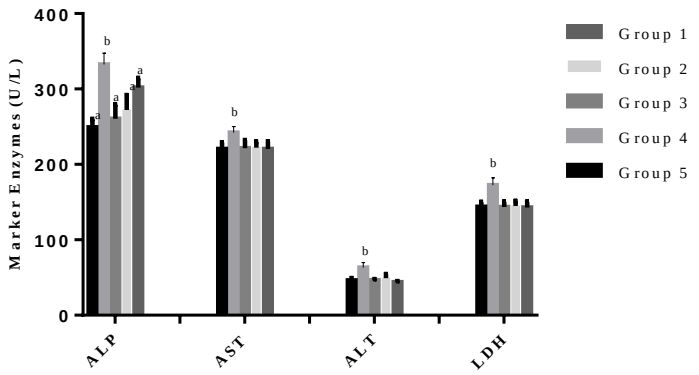

Fig. 1: Effects of crude oil and *A. floribunda* extracts on liver marker enzymes in rats.Each bar represents the means ± SEM from five samples. ALT = Alanine aminotransferase, AST=Aspartate aminotransferase, ALT=alkaline phosphatase and LDH = Lactate Dehydrogenase, were expressed as U/L. Bars carrying different notations are statistically different at p< 0.05 using Instat Graphpad.

The increase recorded in serum ALT, AST, ALP and LDH may be an indication of liver injury resulting from a loss of hepatocytes membrane integrity (26). This increase in serum marker parameters may be due to the liver damage resulting from the liver inflammation and disruption induced by crude oil toxicity (9). In this study, the plant extract prevented crude oil-induced increase of ALT, AST, ALP and LDH. It is possible, that the extract protects cell membranes or counteracts the deleterious effects of crude oil. These results could suggest the presence of some compounds (tannins, alkaloids) which may inhibit the cytochrome P_{450} responsible for metabolism or boost the gene responsible for the regeneration of liver cells, or scavenged free radicals. The administration of the extract inhibited the increase in serum markers at both doses though non-significantly when compared with the control values. These results suggest that the plant extract could protect the liver against inflammation and disorders.

Crude oil, *A. floribunda* stem bark extract and a combination of both, recorded significant ($p<0.05$) decrease and non-significant increases in total protein respectively. This could be due to the oxidation of proteins by crude oil-induced free radicals. The oxidation of these proteins could cause protein denaturation

thus reduced their liver and blood concentrations. The treatment with the extract maintained total protein to within the normal control values. This may be due to its antioxidant character.

Table 4 recorded the effects of crude oil and *A. floribunda* on oxidative stress parameters in rats treated by using oral dosing needle with 5ml/kg crude oil and/or treatment with different doses of *A. floribunda* stem bark extract for 28 days. Significant variations were observed in oxidative stress parameters [superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), peroxidase (POX)] and Uric acid (UA) (Table 4). 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 CAT, MDA and UA, the groups treated with *A. floribunda* stem bark extract relatively maintained a non-significant alteration from the control values in a dose dependent manner.

Table 4: Effects of crude oil and *A. floribunda* extracts on oxidative stress markers in albino rats

Group /Assay	Group 1 Control	Group 2 Co only	Group 3 E only	Group 4 Co+E25mg/kg	Group 5 Co+E50mg/kg
POX	3.50±0.35[a]	1.38±0.33[b]	2.86±0.15[a]	2. 94±0.45[a]	3. 01±0.25[a]
Catalase	3.54 ±0.47[a]	5.84±0.38[b]	3.23±0.21[a]	3.32±0.37[a]	4.15± 0.36[a]
SOD	4.94±0.25[a]	2.75±1.09[b]	4.04±0.50[a]	4.38 ±0.58[a]	4.60±0.86[a]
MDA	8.02±1.05[a]	15.82±1.25[b]	7. 94±1.02[a]	7.76±1.04[a]	7.89±0.58[a]
UA	3.61±0.27[a]	4.98±0.83[b]	3.86±0.47[a]	3.94±0.36[a]	4.01±0.84[a]

Results are expressed as means ± SEM of determinations from five samples. Catalase activity is expressed as unit/g wet tissue. 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.

Antioxidant defense systems under physiological condition may induce a slight oxidative stress as a compensatory response against ROS and thus protect the organisms from oxidative damage. The activity of antioxidant may be increased or inhibited under chemical stress depending on intensity and duration of the applied stress as well as susceptibility of the exposed species (32).

Catalase (CAT), SOD and POX are important 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 antioxidative functions which may have resulted in induced formation of pro-oxidants and relative decrease in antioxidants status of cells, while the increase in CAT may be a physiological adaptation for the elimination of generated ROS. Other researchers also reported decrease in antioxidant enzymes (1, 5, 32 & 33). This result is in agreement with the report of other researchers who used other types of treatments (34, 35). 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 (36) or decrease due to overwhelming effect of the pollutants (37), we may say that the decrease in the activity of SOD is due to the overwhelming effect 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 is suggestive of antioxidative, and hepato-protective ability of the stem bark extract (38**)**.

The increase observed in the levels of malondialdehyde (MDA) [lipid peroxidation product] confirms the induction of oxidative stress in the rats exposed to crude oil only (32, 33).

The significant increase recorded in uric acid level of group 2 rats compared to the control and the other groups treated with the plant extracts, shows that crude oil have toxic effect on uric acid level in blood. The reason may be over production by liver, poor elimination by the kidneys or as non-enzymatic antioxidant defense against excess ROS produced as a result of oxidative stress (8, 39). *A. floribunda* recorded good results in combating crude oil-induced toxicity in albino rats by reducing lipid peroxidation and normalize the levels of uric acid in the serum of rats treated with the extracts. Other researchers also

recorded increased uric acid levels during intoxication and ameliorative effects of other plant species (8, 39)

When hepatocytes membranes are allowed to be damaged by ROS, as observed by significant variation in the oxidative stress parameters in this study, a variety of liver transaminases are released into the blood from the cytosol (11). This indicates hepatocytes damage even without evident hepatic impairment. The elevated levels of AST and ALT are indicative of cellular leakage and loss of functional integrity of cellular membrane and liver. Increased activity of AST has been reported in CCl_4-intoxicated experimental animals (40). This increase may be due to abnormal dynamic properties of cellular membranes following exposure to hydrocarbon fractions present in crude oil. Metabolisms of aliphatic and aromatic hydrocarbons which are major constituents of crude oil as well as other environmental contaminants have resulted in changes in cell membrane due to ROS (32, 40).

Uboh *et al* (40) also reported that serum ALT activity increase as a result of liver injury in patients developing severe hepatotoxicity. The ALP activities on the other hand are related to functioning of hepatocytes, its increase in serum is due to increased synthesis in the presence of increased biliary pressure (1). This could be attributed to damage to the structural integrity of the liver and possible necrotic lesions in the hepatocytes (1). ALT might have leaked from damaged cells, due to increased permeability of the hepatocellular membrane, or due to necrosis, indicating organ dysfunction (40). However, the close to normal control levels of the marker enzymes in the treated groups affirm the antioxidant protective role of the stem bark extracts on the hepatocytes. This is in agreement with the reports of Ujowundu *et al* (33) who recorded the hepato-protective and antioxidant potentials of *O. gratissimum* and *G. latifolium* respectively against petroleum-based products-induced hepatotoxicity in albino rats. The antioxidant role of *A. floribunda* stem bark extract (26) may have prevented the induction of oxidative stress in the liver (1).

The significantly higher ($p < 0.05$) alkaline phosphatase (ALP) activity in the rats exposed to crude oil when compared with control may imply damage in the liver cells, since the activity of this enzyme in the serum is reported to be increased in liver damage (32, 40). Alkaline phosphatase is involved in the transport of metabolites across the cell membranes, protein synthesis and synthesis of certain enzymes, secretory activities and glycogen metabolism. The increase in ALP activity may not be unconnected with a disturbance in the transport of metabolites or alteration in the synthesis of certain enzymes as in other hepatotoxic conditions (32, 40).

The significant decrease in total protein in crude oil group may be attributed to decrease in synthetic function of liver due to crude oil exposure. 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 George *et al* (41), which recorded hepato-protective potential of *O. gratissimum* and *G. latifolium* against ethanol-induced and CCl_4-induced hepatoxicity in albino rats respectively (11, 33).

Conclusion

Administration of *A. floribunda* to rats exposed to crude oil can prevent severe alterations of serum marker enzymes and oxidative status. This study has demonstrated that treatment with *A. floribunda* stem bark extract significantly attenuated the alterations induced by crude oil. Crude oil significantly increased the levels of some serum marker enzymes (AST, ALP, ALT and LDH), and oxidative stress markers (CAT, SOD, POX, UA) and increased the level of total protein. In the case of using the extract alone, there was little alteration in the activity of these parameters. On the other hand, co-administration of crude oil with *A. floribunda* stem bark extract-treated rats improved most of the biochemical parameters to within normal levels. This may be due to the antioxidant properties of the stem bark. We can deduce that crude oil-induced lipid peroxidation and oxidative stress observed in the albino rats, and treatment with *A. floribunda* stem bark extract resulted in significant ameliorating effect. The antioxidant properties of the plant species support the bioactive roles of its protective effects on crude oil toxicity. Further study should be undertaken to strengthen the evidence for dietary antioxidants as modulators of the adverse effects caused by increased chemical or environmental contamination.

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