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Protective influence of *Costus afer* Aqueous Extract In Rats Fed With Crude Oil Contaminated Diet as Measured by Employing Biochemical Indices

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Abstract
The use of medicinal plants in the treatment of harmful impacts of xenobiotics in animals is attracting an increasing attention in recent times. The aim of the current study is to assess the preventive potential of *Costus afer* aqueous leaves extract (CAAE) in treating metabolic aberrations imposed by crude oil contaminated diet in Wistar albino rats. Six groups of rats were treated as follows: A = Normal diet; B= Normal diet + 100 mg/kg body weight of CAAE; C =Normal diet + 200 mg/kg body weight of CAAE; D= Crude oil contaminated diet; E= crude oil contaminated diet + 100 mg/kg body weight of CAAE, F = crude oil contaminated diet + 200 mg/kg body weight of CAAE. After thirty days of exposure to the diet and administration of the corresponding plant extracts, the rats were sacrificed with chloroform and the required organs were excised. The hematological indices, as well as function indicators and levels of drug metabolizing enzymes in the liver and kidney, were investigated with standard protocols. The results indicated that the hematological parameters and kidney and liver function indices were altered in rats fed with crude oil contaminated diet. However, the values came close to those in control rats when *Costus afer* aqueous extracts were administered. Similarly, the activities of oxidase enzymes (aldehyde oxidase, monoamine oxidase, xanthine oxidase, and sulphite oxidase), following their inhibition by the ingestion of crude oil contaminated diet, equally restored close to control values upon treatment with *Costus afer* aqueous extract. This study, therefore, was able to establish an aqueous extract of *Costus afer* leave as an antidote for crude oil intoxication.

Keywords: Crude oil, *Costus afer*, hematological indices, kidney, Liver, Oxidase enzymes

Introduction

The importance of crude oil cannot be underestimated. This organic chemical is a major source of revenue for many countries and raw material for many industries [1-3]. All activities, such as production, distribution, and utilization of petroleum have been reported to expose denizens to toxicological health implications [4-9].The ability of crude oil to cause these aberrations is predicated on the induction of free radicals [10]. Free radicals have been implicated in the peroxidation of lipids in all forms of life [11, 12]. The major consequences

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of lipid peroxidation are the inactivation of antioxidant enzyme systems, genotoxicity, tissue and organ damages, and eventual death of all forms of life [12-17].

A good number of scientists who are in a bid to curtail the negative implications of ecotoxicological substances in animals have been testing a variety of plants and other organic products with antioxidant potentials [18-27]. It is on this basis that the protective potentials of many plants against crude oil toxicity are being examined [6, 8, 9].The emphasis of the current research is to determine the protective impact of *Costus afer* aqueous extract on crude oil-induced oxidative stress indicators in Wistar albino rats.

Materials and Methods

Materials

Bonny light crude oil was obtained from the Warri Refining and Petrochemical Company, Ekpan, Nigeria. *Costus afer* leaves were collected from the wild bush in the premises of the permanent site of the Delta State University, Abraka, Nigeria. Scientific identification was achieved at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria, by a specialized taxonomist who assigned the number of UBH-C287 to the leaf specimen. Experimental male Wistar albino rats were obtained from the animal house in the College of Medicine, Delta State University, Abraka, Nigeria. The feed used was produced by Rainbow Feed Limited, Lagos, Nigeria. Quality analytical grade reagents were used.

Methods

Preparation of plant extract and establishment of LD_{50}

The leaves were dried for 14days at laboratory conditions (28 ± 2ºC). The aqueous extract was prepared as described in Achuba [6] with slight modifications. The *Costus afer* leaves were washed, air-dried, and macerated with a warren blender into dry powder. This was followed by the dissolution of 100gmof the leaves in 400 ml of distilled water via sonication for 10 mins. The mixture was filtered using muslin cloth. The filtrate was concentrated with a rotary evaporator at 50 ^{o}C. The extract obtained was stored in air-tight containers for subsequent use. The adopted dose regimen and LD_{50}were earlier published by Ezejiofor *et al*. [28].

Experimental design

The rats were kept in rat cages and maintained on growers mash for two weeks to make them acclimatize to the new diet and laboratory conditions. The rats were treated according to the guidelines for the care of experimental animals published by the National Research Council (NRC) [29].The rats were divided into equal numbers that comprised ten rats in each group. The groups were treated as follows: A = Normal diet; B= Normal diet + 100 mg/kg body weight of CAAE; C =Normal diet+200 mg/kg body weight of CAAE; D= Crude oil tainted diet; E= crude oil tainted diet + 100 mg/kg body weight of CAAE, F = crude oil tainted diet + 200mg/kg body weight of CAAE. The extracts were administered by dissolving the appropriate dose in 1ml of distilled water and given by oral gavage. Each set of diet was prepared every day before administration and drinking water was allowed *adlibitum*.

Blood collection and tissue homogenization

Each group was exposed to the assigned regimen for thirty days and the rats were subjected to overnight fasting and sacrificed under chloroform tranquility. The blood samples were collected and divided into two sample containers, one for hematological analysis and the other was processed for sera collection. Thereafter, the liver and the kidney were also collected. One gram of each of the tissues (livers and kidneys) was separately weighed under iced conditions and homogenized with buffered saline (5 ml) in a mortar and pestle. Each homogenate was diluted with 5 ml of buffered saline (pH 7.4) and subjected to centrifugation at 2,500 rpm. The supernatants produced were collected in sample tubes and stored at 4 ^{o}C. All prepared homogenates and sera samples were used for the assays within forty eight hours [8].

Biochemical analysis

Anautomated hematology analyzer (Mindray Hematology analyzer, BC-2300) was employed to determine the hematological indices of the rats in each group. Randox kits were used to estimate serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities [30]. Total protein was measured as described by Tietz [31],whereas albumin was measured using the procedure described by Droumas *et al*.[32].Serum bilirubin was quantified following the method described by Cheerbrough [33].The monovalent ions of sodium and potassium were measured by using Sherwood scientific 410 flame photometer. Serum calcium was evaluated as specified in Cyaman assay kit, while chloride was determined by the colorimetric method. The bicarbonate ion was determined as described by Ochei and Kalhatkar [30]. Serum urea and creatinine were determined as described by Cheersbrough [33]. Aldehyde oxidase (AO) activities in the two organs were determined by the method of Omarov *et al*.[34]. Monoamine oxidase (MO) activity was estimated as described by Tabor I. [35]. Xanthine oxidase (XO) activity was analyzed according to the method of Dixon and Keilin [36]. Sulphite oxidase (SO) activity was evaluated as described by Macleod *et al*. [37].

Phytochemical Analyses of Costus afer leaf extract

The extract of *Costus afer* leaf was subjected to qualitative analyses for the presence of phytochemical constituents using methods documented by Sofowora [38] and Trease and Evans [39].The quantitative analyses were carried out as outlined by Harborne [40].

Statistical Analysis

Data were described as means ± SD and subjected to Analysis of variance (ANOVA). Significant differences between all treatment means were set at a probability level of $P < 0.05$.

Results and Discussion

The liver is an organ that is responsible for processing all foreign materials that enter the animal body either by accident or intentionally. By doing so, it inactivates toxic chemicals, which are then transferred to the kidneys by the blood stream to be filtered out of the general circulation [6]. Therefore, the complementary roles of the liver and the kidney in maintaining effective homeostasis cannot be ignored. To achieve this important function, the blood which is the conduit pipe must be in a healthy state. In this investigation, ingestion of crude contaminated diet caused a significant ($P<0.05$) decreases in various parameters of the hematological profile of the experimental rats (Table 1).

Table 1-Effects of *Costus afer* leaves extract on hematological parameters in rats fed with crude oil contaminated feed**.**

Groups	Packed Cell Volume (%)	Hemoglobin (g/dl)	Red Blood Count ($x10^{12}$/L)	White Blood Count ($x10^{9}$/L)
Group A	41.30± 3.22^{a}	28.63± 5.43^{a}	38.72± 3.11^{a}	15.64± 1.66^{a}
Group B	43.22± 1.82^{b}	30.26± 2.86^{b}	39.60± 3.01^{a}	14.31± 3.12^{a}
Group C	43.41± 1.10^{b}	32.81± 4.34^{b}	39.44± 5.50^{a}	14.53± 2.30^{a}
Group D	30.20± 1.92^{c}	26.41± 4.25^{c}	31.20± 5.30^{b}	18.91± 2.80^{c}
Group E	38.43± 3.16^{d}	27.55± 6.46^{a}	36.61± 5.52^{c}	13.55± 2.22^{d}
Group F	39.56± 5.44^{d}	27.63± 3.54^{a}	37.01±4.32^{c}	13.93± 1.52^{d}

Values are represented in mean ± SD (n=5). Mean values with different superscript alphabet in the same column differ significantly at $p<0.05$.

This could culminate in the loss of functional integrity of the blood. This assertion is consistent with many previous reports in which exposure of animals to a variety of petroleum-related substances has been connected with derangements in blood composition and chemistry

[6, 41]. However, simultaneous administration of aqueous extracts of *Costus afer* offered some palliatives against crude oil-mediated abnormality in blood chemistry. The ability of the plant extract was highlighted by the enhancement of hematological parameters in rats fed diets without crude oil as well as in rats fed crude oil contaminated diet to near control values. The protective effects of this plant extract on hematological indices of rats exposed to metallic toxicant was reported previously [42]. Also,the efficacies of this plant in protecting against toxicant-related diseases are vast in the literature [43-47].

As previously asserted, the integrative function of the blood, liver, and kidney in the processing and elimination of foreign substances is clear. The involvement of the liver and kidney in the maintenance of the body‘s homeostasis has been a subject of simultaneous evaluation of the biochemical changes of the two organs in the midst of toxicological insults [8, 17]. The observed significant ((P<0.05) increases in the activities of aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase(ALP),in addition to thedecrease in protein and albumin levels in rats fed with crude oil contaminated diet as compared to the control signifies induced liver architectural derangements (Table 2).

Table 2-Effects of *Costus afer*leavesextract on serum AST, ALT and ALP activities and albumin and total bilirubin levels in rats fed with crude oil contaminated feed.

Groups	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)	Serum Total Protein (µmol/l)	Serum Albumin (µmol/l)	Serum Total bilirubin (µmol/l)
Group A	29.40±1.93^{a}	38.50±2.77^{a}	122.00± 5.00^{a}	32.22±1.59^{a}	17.33±3.66^{a}	38.76± 4.65^{a}
Group B	27.50±5.23^{b}	36.63±2.55^{b}	120.83±11.70^{a}	33.83±3.55^{a}	18.72±2.56^{a}	37.87± 3.87^{a}
Group C	26.66±2.32^{b}	36.30±4.93^{b}	120.50± 5.88^{a}	33.94±4.63^{a}	18.97±3.75^{a}	37.66± 2.64^{a}
Group D	40.20±5.54^{c}	45.80±5.44^{d}	131.55± 5.66^{c}	40.53±4.55^{b}	14.81±4.54^{b}	43.21±5.54^{c}
Group E	33.54±4.40^{d}	40.10±2.98^{a}	126.55±10.44^{d}	35.61±2.54^{c}	15.65±1.51^{b}	40.44± 4.44^{a}
Group F	32.41±3.55^{d}	41.09±3.33^{a}	126.05±11.53^{d}	35.03± 3.74^{a}	15.78± 3.22^{b}	39.11± 4.41^{a}

Values are represented in mean ± SD (n=5). Mean values with different superscript alphabet in the same column differ significantly at p<0.05.

This observation agrees with previous studies on organ damage stimulated by crude petroleum and petroleum-related chemicals [8,48,49]. The elevated levels of serum marker enzymes, in addition to the decrease in serum albumin, are indicative of damage to the structural integrity of the liver[6, 17, 50, 51]. The decrease in the activities of marker enzymes in rats fed with crude oil contaminated diet and administered with *Costus afer* aqueous extract demonstrate the protective influence of the extract on the liver cells (Table 4).This agrees with previous studies on the hepatoprotective effect of *Costus afer* on chemically-induced hepatic damage [52, 53]. The reduction of the activities of the hepatic maker enzymes by *Costus afer* in spite of the intake of crude oil contaminated diet is related to the stabilizing effect of the plant on the cell membrane. This is attributed to the antioxidant-rich potentials of the plant that prevents oxidative damage of the cell membrane and the consequent enzyme leakages. *Costus afer* is reported by many authors to exhibit remarkable antioxidant properties [54, 55].This efficacy is predicated on the presence of antioxidant vitamins and antioxidant micronutrients [56, 57].

Similarly, kidney derangement was indicated by the significant (P<0.05) aberrations in serum kidney dysfunction indicators in rats fed with crude oil contaminated diet compared to the control (Table 3).

Table 3- Effects of *Costus afer* leaves extract on serum kidney function indices in rats fed with crude oil contaminated feed.

Groups	Chloride (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Bicarbonate (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Group A	98.50±12.88^{a}	113.70± 13.50^{a}	3.92± 0.30^{a}	27.54± 3.52^{a}	5.51± 0.48^{a}	64.53± 5.13^{a}
Group B	97.30±10.53^{a}	112.60±13.61^{a}	4.22± 0.11^{a}	25.77± 5.00^{b}	3.99± 0.76^{b}	62.40± 6.50^{a}
Group C	96.50±12.48^{a}	109.20±12.82^{a}	4.04± 0.52^{a}	26.60± 3.13^{c}	3.74± 0.55^{b}	62.00± 5.83^{a}
Group D	101.55±13.60^{b}	129.71±13.66^{b}	5.30± 0.62^{b}	32.92± 4.12^{d}	5.98± 1.11^{c}	71.71± 2.55^{b}
Group E	99.50±14.81^{a}	112.10±14.73^{a}	4.88±0.67^{c}	28.53± 5.30^{a}	4.73± 0.55^{d}	66. 53±3.56^{a}
Group F	99.90±13.88^{a}	111.50±13.55^{a}	4.64± 0.31^{c}	28.65± 4.01^{a}	4.55±0.50^{d}	65.35±6.11^{a}

Values are represented in mean ± SD (n=5). Mean values with different superscript alphabet in the same column differ significantly at p<0.05.

Thus, these effects indicate compromised kidney function by intake of crude oil [8, 13,24]. Nonetheless, administration of the aqueous extract of *Costus afer* relieved the derangement of kidney dysfunctions imposed by crude oil contaminated diet. This treatment significantly (p<0.05) improved the values of these kidney function indicators in a close resemblance with the values observed in the control rats. In fact, this investigation agrees with earlier studies on the nephroprotective credentials of *Costus afer* [45, 46, 58]. As stated above in relation to the liver, the protective capabilities of the plant extract are centered on the antioxidant potentials occasioned by the enormous presence of antioxidant-rich substances [54-56]. The efficacy of this plant extract against crude toxicity is beyond doubt, since one major principle in the induction of toxicity by crude oil is the stimulation of free radical generation [10]. This explains the application of a variety of substances with antioxidant capabilities against crude oil linked free radical generation which leads to organ and tissue damages [6, 12, 16, 24, 26].
This investigation recorded a significant (p<0.05) alteration in the activities of xenobiotic metabolizing enzymes in the liver and kidney by the crude oil contaminated diet (Tables 4 and 5).

Table 4-Effects of *Costus afer* leaves extract on serum aldehyde oxidase, monoamine oxidase, xanthine oxidase and sulphite oxidase activities in the liver of rats fed with crude oil contaminated feed.

Groups	Aldehyde oxidase (units/g wet tissue)	Monoamine oxidase (units/g wet tissue)	Xanthine oxidase (units/g wet tissue)	Sulphite oxidase (units/g wet tissue)
Group A	43.55± 3.77^{a}	48.66± 4.51^{a}	46.91± 4.36^{a}	250.88± 10.00^{a}
Group B	44.62±2.53^{a}	50.32±3.52^{b}	47.87±4.30^{a}	254.91± 13.56^{a}
Group C	44.75± 2.44^{a}	49.60± 3.35^{a}	47.93±3.92^{a}	253.97±11.45^{a}
Group D	38.55±3.56^{b}	40.31± 2.94^{c}	34.92 ±3.37^{b}	220.98± 18.67^{b}
Group E	41.61± 3.66^{c}	47.05± 5.00^{a}	43.75± 5.10^{c}	241.78 ± 17.66^{c}
Group F	42.53± 4.44^{c}	46.06± 4.71^{a}	44.65± 4.09^{c}	242. 15±14.55^{c}

Values are represented in mean ± SD. n=5. Mean values with different superscript alphabet in the same column differ significantly at p<0.05.

Table 5- Effects of *Costus afer* leaves extracts on aldehyde oxidase, monoamine oxidase, xanthine oxidase, and sulphite oxidase activities in kidney of rats fed with crude oil contaminated feed.

Groups	Aldehyde oxidase (units/g wet tissue)	Monoamine oxidase (units/g wet tissue)	Xanthine oxidase (units/g wet tissue)	Sulphite oxidase (units/g wet tissue)
Group A	33.53± 2.75[a]	44.66± 2.65[a]	36.35± 3.66[a]	256.92 ±18.83 [a]
Group B	37. 65 ± 3.65 [b]	45.88±5.56[a]	37.88±4.54[b]	260.87 ±17.33 [b]
Group C	37.41± 3.50 [b]	46.04±3.45[a]	38.65±5.35[b]	261.66 ± 17.84 [c]
Group D	28.77± 3.77 [c]	36.99± 3.77[b]	32.45±3.46[a,b]	227.85±15.81 [d]
Group E	31.66± 4.01[d]	41.55±3.87[c]	35.77±3.71[a]	245.77 ± 19.46 [e]
Group F	30.55± 2.66 [d]	42.75±4.55[c]	35.88 ± 5.68	247.66±18.67

Values are represented in mean ± SD. n=5. Mean values with different superscript alphabet in the same column differ significantly at $p< 0.05$.

These enzymes, i.e. aldehyde oxidase, monoamine oxidase, xanthine oxidase, and sulphite oxidase, act simultaneously to inactivate and solubilize harmful substances for eventual elimination from the general circulation [59]. These actions complement those of the antioxidant enzymes in quenching of free radical production. Therefore, the enhancement of the enzyme activities in non-crude oil intoxicated rats portents proper positioning of the rats by the plant extract to handle oxidative insults. The efficacies of pretreatment of animals with antioxidant-enriched substances are vastly stated in the literature [21, 60, 61, 62, 63]. Equally, the plant extract restored the activities of these enzymes near to control values in rats fed with crude oil contaminated diet. It is, thus, suggested that this plant is an efficient antidote for crude oil toxicity. This influence is certainly attributed to its rich phytochemical and ethnopharmocological properties. Tables 6 and 7 elucidate the chemical composition of the tested leaves extract.

Table 6-Qualitative composition of aqueous extract of *Costus afer* leaves.

Phytochemicals	Presence
Saponins	+
Proteins	+
Terpenes	+
Alkaloids	+
Steroids	-
Carbohydrates	+
Thiols	+
Phenol	+
Phlobatannin	-
Flavonoids	+
Tannins	+
Cardiac glycosides	-

Key: + present, - Absent

Table 7-Quantitative composition of aqueous extract of *Costus afer* leaves.

Phytochemical	Quantity (mg/100g)
Alkaloids	4.5
Flavonoids	28.5
Saponins	3.5
Tannins	3.6
Phenols	3.5

Previous studies implicated the inherent potential of numerous plant extracts in eliminating crude oil initiated clinical aberrations [64-67].This justifies the efficacy of traditional herbal preparations in the management of an array of human diseases, as reported in the literature [68].

This study therefore was able to establish the aqueous extract of *Costus afer* leave as an antidote for crude oil intoxication. This conclusion comes against the backdrop of the restoration of the alterations in hematological indices, liver and kidney functional indicators, and oxidative enzymes caused by the consumption of crude oil contaminated diet to values near to those recorded in control rats.

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