Research Article

Effects of Siam Weed (*Chromolaena odorata*) Leaf Extract on Crude Oil-induced Toxicity

Fidelis Ifeakachuku Achuba*, Charles Offor

Abstract

The objective of this study was to investigate the effects of the different extracts (aqueous, ethanol and blended mixture) of *Chromolaena odorata* leaves on Wistar rats feed with crude petroleum (CP) tainted feed (4 ml/100 g diet).

Materials and methods: Twenty-five male Wistar rats weighing 140 -180 g were used for this study. The rats were kept one in a cage. Group A which was the control was allowed diet free of crude oil and were not treated with any of the extracts. Group B were exposed to a diet contaminated with crude oil that contained 4ml of crude oil per 100 gram of animal feed without treatment. Groups C-E were fed with diets contaminated with 4ml of crude oil per 100g of feed and treated with 500mg/Kg⁻¹ bodyweight of aqueous, ethanol and blended mixture of ethanol and aqueous extracts, respectively. The respective extracts and blended mixture were given daily to the rats via oral gavage in line with body weights. The study was for duration of twenty eight days after which the rats were sacrificed under sedation with chloroform. This was followed with collection of blood samples and organs of interest. All samples were stored at 4°C and used within forty eight hour for various biochemical analyses.

Results: There were significant decrease in haematological indices, liver proteins, oxidative enzymes, and antioxidant enzymes when compare with Group 1. Also, liver function maker enzymes, white blood cells (WBC), and malondialdehyde (MDA) were significantly increased by petroleum intoxication. Significant increase were observed in Hb, PCV, RBC, SO, MO, XO, SOD, CAT, albumin, total protein in the serum, liver and kidney; as against decreases in WBC, AST, ALT, ALP and MDA in rats fed crude petroleum (CP) tainted feed that was treated with *C. odorata* extract.

Conclusion: This study showed that administration of *C. odorata* leaves extracts to rats prevented health risk associated with crude petroleum toxicity.

Keywords

crude oil; siam weed; blood; liver; kidney; oxidative stress

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Problem statement and analysis of the latest research

Crude oil was discovered in Nigeria in 1956 and the production and uses have led to the release of various types of hydrocarbon related chemicals such as aliphatic and polycyclic aromatic hydrocarbons into the environment which constitutes danger to both aquatic and terrestrial animals [1, 2, 3].

The ingestion of crude oil or related chemi-

cals is one of the ways animals are exposed to petroleum hydrocarbon toxicity [4, 5]. The exposure to petroleum has been reported to induce oxidative stress due to free radicals production by the metabolism of petroleum hydrocarbons [6]. In addition, exposure to petroleum causes difficulty in breathing, nausea, headaches and other central nervous system abnormalities as well as haematological aberrations [7, 8].

These toxicities are attributed to the presence of complex aromatic and aliphatic hydrocarbons such as benzene, benzo [a] anthracene, benzo [b] pyrene, heptanes, hexane, isobutene and isopentane [4, 9, 10].

Many studies have been able to document the utilization of plants and its products in the management of petroleum induced toxicity. These studies include palm oil [11], *Gongronema latifolium* [12], *Ocimum gratissimum* [4]. *Chromolaena odorata* (commonly called Siam Weed) belongs to the family Asteraceae. It is very common in Nigeria [13]. Although, native to south and central America, it has spread throughout the tropics, Nigeria inclusive. Studies on the adverse effects of crude oil on exposed animals and the search for protective agent against its toxicity are on-going. However, no research has been carried out to investigate the ability of *C. Odorata* to mitigate the adverse effects of crude oil on exposed animals.

The objective of this research was carried out to determine the possible effect of Siam Weed (*C. odorata*) against crude petroleum-induced toxicity in experimental rats.

1. Materials and Methods

1.1 Animals

Twenty-five Wistar rats of albino strains were purchased from the animal house of the Delta State University College of Medicine, Abraka Nigeria. Animals were allowed to acclimatize to laboratory conditions and fed commercial growers mash and free access to water.

1.2 Chemicals/Reagents

All chemicals and reagents used were of analytical grade and were purchased from Merck, Germany, May and Baker Ltd, England and BDH chemicals Ltd, England.

1.3 Crude oil Sample

The test samples of crude oil were obtained from the Nigerian National Petroleum Cooperation (NNPC) refinery, Warri, Delta State.

1.4 Plant Collection and Extraction

Leaves of *Chromolaena odorata* were collected from a farmland at Abraka, Delta State, Nigeria. The leaves were washed with water to remove all unwanted materials and dirt. *C. odorata* leaves was identified at the Institute of Forestry Research of Ibadan, Nigeria, with the voucher number of FHI 109494 deposited at the Herbarium. Preparation of *C. Odorata* extracts was done using water and ethanol as solvent extractors. The plant leaves were air dried in open air at room temperature to obtain a constant weight for a period of two weeks after which it was pulverized to fine powder using electric blender. After blending, the aqueous extraction was carried out using the hot water extraction method previously described by George *et al.* [14] while ethanol extraction was carried out by the method described by Achuba [15] with little modification. In using this method, 50 g of the powdered *C. odorata* was soaked in 200ml of 95% (v/v) ethanol and allowed to stand for 48 hours. The extracted mixtures were filtered with a muslin cloth and the filtrate was concentrated using a rotary evaporator attached to a vacuum pump at 45°C. Further dryness was achieved using a water bath. The blended mixture was obtained by dissolving equal weight of both water extract and ethanol extract (1:1). From the dried sample extracts (crude extracts), 2.5 g of each sample, water extract and ethanol extract, was dissolved in 25 ml of clean water (tap water) which brings the concentration of each extract to 100mg/ml. The blended mixture was prepared by adding 1.25 g of each extract to give 2.5 g, which was dissolved in 25 ml of tap water to an equivalent concentration with the individual extracts.

1.5 Preliminary Qualitative and Quantitative Phytochemical Analysis

The quantitative and qualitative phytochemical analysis of plant extracts was carried out according to the methods below.

1.6 Qualitative Screening

This was carried out to identify possible phytochemicals in the various leaves extracts using standard

methods as described by Trease and Evans [16], Sofowora [17], Tiwari *et al.* [18]. The phytochemicals assayed for were alkaloids, flavonoids, glycosides, phenols and saponins.

1.7 Quantitative Screening

Alkaloid determination was carried out by the method of Obadoni and Ochuko [19], Flavonoid was determined by method of Harborne [20], Tannins determined by the method of Van-Burden and Robinson (1981) [21] and total phenol by method of Spanos and Wrolstad [22].

1.8 Experimental Design

After the acclimatization period, rats were distributed randomly into groups of 5 rats each. The rats were kept one in a cage. Group 1 normal control which was fed with normal rat diet and water only. Group 2 were exposed to diet contaminated (4ml of crude oil per 100 gram of animal feed) with crude oil without treatment. Groups 3-5 were fed contaminated diets (4ml of crude oil per 100g of feed) and treated with 500mg/Kg⁻¹ bodyweight of aqueous, ethanol and blended mixture of ethanol and aqueous extracts, respectively. The respective extracts and blended mixture were given daily to the rats through oral gavage in line with body weights. The study was for duration of twenty-eight days after which the rats were sacrificed under sedation with chloroform. This was followed with collection of blood samples and organs of interest. All samples were stored at 4°C and used within forty-eight hours.

1.9 Estimation of Liver Function Markers

Alkaline phosphatase (EC.3.1.3.1) activity assay was carried out using the method of Kaplan and Righetti [23], Aspartate aminotransferase (EC 2.6.1.1) activities were assayed by the method of Reitman and Frankel [24], Alanine aminotransferase (EC 2.6.1.2) activities were determined by the method of Reitman and Frankel [24], the method of Droumas *et al.* [25] was used in estimating albumin level while total protein was determined using the method of Tietz [26]

1.10 Estimation of Kidney Function Markers

Urea and creatinine levels were determined using the method of Henry [27].

1.11 Estimation of Haematological Parameters

The method described by Thrall and Weiser [28] was used in the determination of haematocrit. The cyanmethaemoglobin method described by of Tietz [26] was adopted for estimation of haemoglobin. White blood cell (WBC) count and red blood cell (RBC) count were determined as described by Thrall and Weiser [28].

1.12 Determination of Tissue Antioxidant Enzyme Analysis

The method of Buege and Aust [29] was used for the analysis of lipid peroxidation product (MDA), the method described by Misra and Fridovich [30] was used for the assay of superoxide dismutase activity, the method of Kaplan *et al.* [31] was adopted for the assay of catalase activity.

1.13 Determination of Tissue Oxidative Enzymes

The activity of the aldehyde oxidase (AO) was determined by the method of Omarov *et al.* [32]. The method used for the assay of monoamine oxidase (MO) activity was proposed by Tabor *et al.* [33]. The xanthine oxidase (XO) activity was determined by the method of Dixon and Keilin [34] while sulphite oxidase (SO) activity was determined by the reduction of ferricyanide [35].

1.14 Histopathology Analysis

The liver and kidney histology was done following the method of Al-Attar *et al.* [36].

1.15 Statistical Analysis

All the results were expressed as means $\pm$ SD and mean bars. The data were analyzed using Analysis of Variance (ANOVA). Significant difference between the control and treatment means were determined at 5% ($P < 0.05$) confidence level using least significant difference (LSD)

Figure 1. Effect of *C. odorata* leaves extracts on weight changes in rats fed with crude oil contaminated feed. Bars represent mean values from five rats in each group. Bars of same groups with different superscript letter differ significantly at $p < 0.05$.

2. Results and Discussion

Biochemical consequences of consuming hydrocarbon tainted diet have been voraciously reported [11, 37, 38, 39, 40]. Moreover, one major health implication of exposure to crude petroleum is decrease in body weight [4]. Loss in body weight is a sensitive indicator of a toxic impact. Apparently, the animals consumed less of the food due to poor palatability of feed because of the crude oil. This is in line with the observation made by Ekwere *et al.* [41] which stated that body weights of exposed organisms declined in a predictable way with respect to the concentration of pollutants and duration of exposure in their tissue. However, rats fed with rat chow contaminated with crude oil but treated with aqueous, ethanol and blended mixture extract of *C. odorata* leaves had the loss of body weight ameliorated (Figures 1, 2). The blended mixture extract was more effective when compared with the aqueous and ethanol extracts.

Besides the toxicant-stimulated decrease in weight gain, hydrocarbon-induced alterations in blood chemistry are widely reported in literature [42, 43, 44]. This may account for the obvious reduction recorded

for RBC, PCV and Hb which suggest crude oil-induced anaemia (Table 1).

Many researchers in this field have reported that crude oil or its various constituents have demonstrated anaemic potency in experimental animals by reduction in two or all of RBC, PCV and Hb [43]. In fact, study has reported that benzene, one important constituent of crude oil, can suppress erythropoietic activity by activating the production of prostaglandins (PGE2) [45]. Crude oil can cause a lot of damage to the red cell membrane via generation of reactive oxygen species (ROS) and lipid peroxidation thereby resulting in the susceptibility of the cell to lysis and oxidation of Hb to reduce its concentration [6, 38, 46]. The increase in WBC counts due to the intake of crude oil mixed feed is in line with the normal physiologic response following perception of a foreign attack by the body defence mechanisms [47]. From the results of this study, it is evident that ingestion of crude oil orally, results in toxicity targeted at the haematopoietic system. However, administration of *C. odorata* leaves extracts to rats consuming crude oil contaminated feed significantly reversed the situation by reducing the fragility, WBC counts as well as raising the

Figure 2. Effect of *C. odorata* leaves extracts on feed intakes in rats fed with crude oil contaminated feed. Bars represent mean values from five rats in each group. Bars of same groups with different superscript letter differ significantly at $p < 0.05$.

Table 1. Effect of *C. odorata* leaves extracts on haematological parameters in rats fed with crude oil contaminated feed.

Groups	Packed Cell Volume (%)	Haemoglobin (g/dl)	Red Blood Count ($\times 10^{12}/L$)	White Blood Count ($\times 10^9/L$)
Group 1	40.00 $\pm$ 4.42 ^a	29.70 $\pm$ 7.11 ^a	38.16 $\pm$ 5.06 ^a	5.26 $\pm$ 2.22 ^a
Group 2	18.00 $\pm$ 4.72 ^b	8.34 $\pm$ 1.56 ^b	20.37 $\pm$ 2.95 ^b	18.21 $\pm$ 5.11 ^b
Group 3	20.00 $\pm$ 5.10 ^b	12.38 $\pm$ 6.04 ^b	25.53 $\pm$ 6.75 ^b	14.50 $\pm$ 3.40 ^b
Group 4	30.00 $\pm$ 9.82 ^c	18.88 $\pm$ 6.20 ^{b,c}	31.19 $\pm$ 4.38 ^{b,c}	10.53 $\pm$ 3.75 ^{b,c}
Group 5	28.00 $\pm$ 6.96 ^{b,c}	26.31 $\pm$ 5.49 ^{a,c}	36.36 $\pm$ 4.35 ^a	6.85 $\pm$ 2.04 ^{a,c}

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

erythrocyte parameters.

Apart from the noxiousness of hydrocarbon on blood chemistry, it exhibits same effect on major organs of the animal. An example of such organ is the liver. The liver is the organ responsible for the metabolism and detoxification of chemical compounds. In rats fed crude oil contaminated feed only, there were markedly increases in serum and liver AST, ALT and ALP activities (Tables 2 and 3).

In a related development, Asomugha *et al.* [48] found that the activities of liver function enzymes

correlate with the synthesis of the enzymes in the liver and are important indicators of liver tissue derangement [49]. The authors further stated that the activities of these enzymes are a measure of liver integrity [49]. The level of serum and liver protein and albumin shows a significant decrease ($p < 0.05$) in group 2 (Table 4).

This study also investigates alterations in albumin and total protein representing one of the metabolic functions of the liver. Therefore, since all proteins are synthesized in the liver, a decrease

Table 2. Effect of *C. odorata* leaves extracts on serum AST, ALT and ALP activities in rats fed with crude oil contaminated feed.

Groups	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)
Group 1	28.20±12.81 ^a	40.00±3.80 ^a	120.57±6.99 ^a
Group 2	69.90±8.23 ^b	82.10±2.75 ^b	190.70±10.42 ^b
Group 3	60.10±25.40 ^c	71.30±15.90 ^c	172.27±5.79 ^c
Group 4	44.19±10.35 ^d	60.10±22.66 ^d	153.43±6.42 ^d
Group 5	32.20±11.93 ^a	54.30±21.78 ^a	140.46±11.14 ^e

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 3. Effect of *C. odorata* leaves extracts on liver AST, ALT and ALP activities in rats fed with crude oil contaminated feed.

Groups	Liver AST (U/L)	Liver ALT (U/L)	Liver ALP (U/L)
Group 1	30.30 ± 7.99 ^a	48.10 ± 4.67 ^a	141.33 ± 13.62 ^a
Group 2	100.20 ± 13.97 ^b	92.20 ± 5.27 ^b	220.24 ± 19.06 ^b
Group 3	81.10 ± 16.27 ^c	81.00 ± 14.52 ^c	201.43 ± 22.60 ^c
Group 4	70.20 ± 30.84 ^d	67.00 ± 10.77 ^d	185.20 ± 18.97 ^d
Group 5	51.10 ± 9.92 ^e	55.10 ± 12.48 ^a	165.33 ± 14.86 ^e

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 4. Effect of *C. odorata* leaves extracts on serum total protein and albumin levels in rats fed with crude oil contaminated feed.

Groups	Serum Total Protein	Liver Total Protein	Serum Albumin	Liver Albumin
Group 1	32.22 ± 1.59 ^a	37.04 ± 3.14 ^a	15.19 ± 4.48 ^a	20.53 ± 3.86 ^a
Group 2	12.50 ± 1.45 ^b	16.36 ± 4.51 ^b	5.48 ± 1.97 ^b	8.67 ± 2.10 ^b
Group 3	16.32 ± 1.62 ^b	18.42 ± 2.19 ^b	7.26 ± 2.04 ^b	10.69 ± 3.75 ^b
Group 4	17.32 ± 2.84 ^{b,c}	25.22 ± 3.97 ^c	9.45 ± 2.12 ^{b,c}	13.36 ± 5.89 ^{b,c}
Group 5	20.38 ± 5.28 ^{b,c}	34.43 ± 2.40 ^a	12.59 ± 1.73 ^{a,c}	18.71 ± 2.49 ^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.

in total protein portends failure in hepatic metabolic function. The crude oil-caused decrease in protein levels is similar to toxicant-mediated decrease in serum albumin and total protein [50] also demonstrated that rats administered with the aqueous, ethanol and blended mixtures of *C. odorata* leaves extracts, respectively in addition to consuming crude oil contaminated feed, revealed substantial reduction in the

levels of AST, ALT and ALP when compared with group 2 (rats fed crude oil contaminated feed only). In addition, the level of serum protein and albumin increases significantly (p < 0.05) when compared with the rats fed crude oil contaminated feed only. The significant change in the levels of AST, ALT, ALP, protein and albumin in the rats fed with crude oil contaminated feed and administered with the

aqueous, ethanol and blended mixture of *C. odorata* leaves extract point towards an improvement in the function of the hepatic cells. This effect was more reliable with the blended mixture of *C. odorata* leaves extract. Thus *C. odorata* leaves extracts possess hepatoprotective effect (Figure 3).

Liver damage such as necrosis and hepatic degeneration were confirmed through histopathological examinations in rats fed with crude oil contaminated feed only. However, rats given *C. odorata* leaves extracts and fed with crude oil contaminated feed showed less necrosis and hepatic degeneration. These showed that the *C. odorata* leaves extracts may help in the prevention of liver tissue damage. Similar protections by plant extracts were documented [44, 51, 52]

Serum and kidney concentrations of urea and creatinine are indices of renal function. Consequent rise in urea and creatinine serum levels are pointers to impaired renal function [53]. This study revealed that exposure of rats to petroleum hydrocarbon contaminated feed caused a significant increase in serum and kidney urea and creatinine concentrations (Table 5).

This is in accord with previous study conducted by Adebayo *et al.* [54] and Achuba [55], that reported hydrocarbon-induced kidney dysfunction. This observation suggests impaired kidney damage and agrees with previous publication [56]. Rats fed with chow contaminated with crude oil and treated with 500mg/Kg⁻¹ of ethanol and blended mixture leaf extracts of *C. odorata* respectively had significant ($p < 0.05$) decreases in urea and creatinine levels in the serum and kidney when compared with the group 2. Urea and creatinine levels in the serum and kidney of rats fed blended mixture of *C. odorata* leaves extracts did not exhibit significant difference ($p > 0.05$) when compare with the control. This may indicates the presence of antioxidant phytochemicals such as phenol, flavonoids and alkaloids in the extracts and conforms to an earlier study that was reported by Abo *et al.* [57], which stated the possible anti-diarrhoeal potential of ethanol leaf extract of *C. odorata* in castor oil-induced rats. The results of histology of rat kidney tissues also showed necrotic tubular cell, glomeruli

and Bowman capsule atrophy (Figure 4).

Rats fed growers mash contaminated with crude oil and administered with 500mg/Kg⁻¹ of ethanol and aqueous extract of *C. odorata* leaves, respectively showed reduced necrotic tubular cell and glomeruli atrophy. The preventive potentials of *C. odorata* leaves extracts blended mixture was better as an indication of no atrophy of glomerulus.

The main mediator in cellular damage is generation of excess free radical. This free radical extracts electrons from biomembrane, leading to the formation of lipid peroxidation products. One of these products is malondialdehyde (MDA). The level MDA in the liver and kidney of rats administered with *C. odorata* leaves extracts and fed with crude oil contaminated feed are shown in Figure 55. There were significant ($p < 0.05$) increases in MDA levels in the liver and kidney tissues of group 2 when compare with group 1. Increase in MDA concentration in tissues is an indicator of oxidative stress condition and is usually accompanied by alteration in antioxidant enzyme activities [58]. Crude oil toxicity leading to generation of free radicals could cause lipid peroxidation [59]. Therefore, the significant increase in MDA concentration in the rats fed crude oil contaminated feed only (Figure 5) is an indication of membrane damage resulting from crude oil toxicity.

This is in line with earlier studies that reported increase in MDA contents of rat fed crude oil contaminated feed [59]. The reduced MDA levels observed in the rats fed with crude oil contaminated feed and administered with *C. odorata* leaves extracts is an indication of the antioxidative effect of the *C. odorata* leaves extracts. *C. odorata* leaves extracts may reduce the spontaneous production of oxygen radicals through blocking of early events in the membrane inflammation cascade, thus preventing tissue damage caused by lipid peroxidation and membrane inflammation. *C. odorata* leaves extracts have secondary metabolites including phenols and flavonoids that have the ability to direct enzymatic breakdown of free radicals through endogenous antioxidants thereby inhibiting the actions of the inflammatory cells.

The activities of these enzymatic antioxidants

(a) Group 1 (Control): Microscopic representation of rat liver showing normal hepatic cell (HC) and central vein (CV). (H&E stain, x200)

(b) Group 2 (Rats fed growers mash contaminated with crude oil (4 ml/100 g feed): Microscopic representation of rat liver showing degeneration of hepatic cell (HC), necrotic cell (NC) and inflammation (IF). (H&E stain, x200)

(c) Group 3 (Rat fed growers mash contaminated with crude oil (4 ml/100 g feed plus 500mg/Kg⁻¹ of aqueous extract of *C. odorata* leaves): Microscopic representation of rat liver showing mild necrosis (N) and portal vein (PV). (H&E stain, x200)

(d) Group 4 (Rat fed growers mash contaminated with crude oil (4 ml/100g plus 500mg/Kg⁻¹ of ethanol extract of *C. odorata* leaves): Microscopic representation of rat liver showing mild inflammation (IF) and degeneration of hepatic cell (HC), no necrosis. (H&E stain, x200)

(e) Group 5: (Rats were fed growers mash contaminated with crude oil (4 ml/100 g plus 500mg/Kg⁻¹ of blended mixture (aqueous and ethanol extract) of *C. odorata* leaves): Microscopic representation of rat liver showing mild inflammation, no necrosis. Normal hepatic cell (HC) when compare with group 1. (H&E stain, x200)

Figure 3. Effect of *C. odorata* leaves extracts on liver micrograph of rats fed with crude oil contaminated feed.

in the serum, liver, and kidney tissues of group 2 decrease significantly ($p < 0.05$) when compared with the control (Tables 6 and 7). Oxidative stress

occurs when free radicals overwhelm antioxidant capacity of the cell and culminates in macromolecular damages, and hence, structure and functional

Table 5. Effect of *C. odorata* leaves extracts on urea and creatinine levels in the serum and kidney of rats fed with crude oil contaminated feed.

Groups	Serum Urea (mg/dl)	Kidney Urea (mg/dl)	Serum Creatinine (mg/dl)	Kidney Creatinine (mg/dl)
Group 1	5.37 ± 3.03 ^a	8.42 ± 2.97 ^a	2.10 ± 0.89 ^a	7.54 ± 2.09 ^a
Group 2	12.32 ± 3.78 ^b	18.39 ± 6.42 ^b	9.40 ± 5.06 ^b	15.43 ± 3.85 ^b
Group 3	10.40 ± 3.60 ^b	14.60 ± 2.98 ^b	7.28 ± 3.18 ^b	13.62 ± 2.34 ^b
Group 4	8.37 ± 3.03 ^{a,b}	12.40 ± 0.67 ^{b,c}	5.47 ± 1.69 ^b	10.60 ± 1.58 ^{a, b}
Group 5	6.39 ± 2.40 ^a	9.45 ± 1.96 ^a	4.81 ± 2.31 ^a	8.42 ± 2.76 ^a

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

integrity of the cell [60, 61].

Observations from this study revealed that exposure to crude oil contaminated feed led to oxidative damage of the liver and kidney. This was evident by the reduction of superoxide dismutase (SOD) and catalase (CAT) activities. This agrees with a previous study of Alisi *et al.* [53] that reported the prooxidant effect of hydrocarbons. The observed oxidative stress on the liver and kidney could be associated with the hydrocarbon-induced hepatic and renal dysfunction seen in this study. The results of this study suggest that oxidative stress is a primary mode of action of crude oil-induced hepatic and renal dysfunction. Hepatotoxicity and nephrotoxicity could also be explained by the impaired antioxidant enzyme activities in the liver and kidney of the rats. Indeed, the antioxidant enzymes SOD and CAT limit the effects of oxidant molecules in tissues and act in the defense against oxidative cell injury by means of their being free radical scavengers [58]. These enzymes work together to eliminate active oxygen species. In this respect, SOD accelerates the dismutation of hydrogen peroxide (H_2O_2), also termed as a primary defense, as it prevents further generation of free radicals whereas, CAT helps in the removal of H_2O_2 formed during the reaction catalyzed by SOD [58]. Biochemical alterations accompanied by crude oil toxicity were alleviated following the administration of *C. odorata* leaves extracts. This could be attributed to the antioxidant capacity of *C. odorata* leaves extracts.

Monoamine oxidase (MO) is an important en-

zyme in the mitochondria outer membrane that is involved in the oxidation amines. MO inhibition is accompanied by marked changes in the sensitivity of the organism to some dietary constituents (e.g. tyramine, tryptophan and other amines and amine precursors) as well as many drugs (e.g. sympathomimetics, opiates, reserpine and caffeine). Xanthine oxidases (XO) play a vital role in purine catabolism, in which it catalyzes the oxidation of hypoxanthine to xanthine, then to uric acid. Aldehyde oxidase (AO) and XO have been implicated in oxidative injury to tissues [62]. Sulphite oxidase (SO), another molybdoprotein, is involved in the oxidation of endogenous sulphite arising from the degradation of sulphur amino acids [63]. There were significant ($p < 0.05$) decrease in the activities of these oxidative enzymes (MO, SO, AO and XO) in the liver and kidney tissues of rats fed crude oil contaminated feed only, when compare with the control (Tables 8 and 9).

MO, SO, AO and XO play important roles in the metabolism of many exogenous and endogenous compounds. They exhibit oxidative activity towards various heterocyclic compounds [64]. The decrease observed in the activities of these oxidative enzymes would affect their contribution towards the radical detoxification of crude oil toxicity. The radical scavenging activity and the reducing ability of the *C. odorata* leaves extracts can be attributed to the presence of redox active substances such as phenol, flavonoids, alkaloids and tannin. This may be due to the presence of more of the bioactive constituents in

(a) Group 1 (Control): Microscopic representation of rat kidney showing normal proximal tubules (PT), glomeruli (G) and Bowman capsule (BC). (H&E stain, x200)

(b) Group 2 (Rats fed growers mash contaminated with crude oil (4 ml/100 g feed): Microscopic representation of rat kidney showing necrotic tubular cell (NT) and glomeruli atrophy (GA), renal abscess (RA). (H&E stain, x200)

(c) Group 3 (Rat fed growers mash contaminated with crude oil (4 ml/100 g feed plus 500mg/Kg⁻¹ of aqueous extract of *C. odorata* leaves): Microscopic representation of rat kidney showing reduced necrotic tubular cell (NT) and mild glomeruli atrophy (GA), reduced renal abscess (RA). (H&E stain, x200)

(d) Group 4 (Rat fed growers mash contaminated with crude oil (4 ml/100 g feed plus 500mg/Kg⁻¹ of ethanol extract of *C. odorata* leaves): Microscopic representation of rat kidney showing reduced necrotic tubular (NT) cell and glomeruli atrophy (GA), slight renal abscess (RA). (H&E stain, x200)

(e) Group 5: (Rats were fed growers mash contaminated with crude oil (4 ml/100 g plus 500mg/Kg⁻¹ of blended mixture (aqueous and ethanol extract) of *C. odorata* leaves): Microscopic representation of rat kidney showing normal proximal tubular (PT) and no atrophy of glomerulus (G). (H&E stain, x200)

Figure 4. Effect of *C. odorata* extracts on kidney micrograph of rats fed with crude oil contaminated feed.

the blended mixture when compared with the aqueous and ethanol leaf extracts of *C. odorata*. The

phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, steroids,

Figure 5. Effect of *C. odorata* leaves extracts on lipid peroxidation in rats fed with crude oil contaminated feed. Bars represent mean values from five rats in each group. Bars of same parameters with different superscript letter differ significantly at $p < 0.05$.

Table 6. Effect of *C. odorata* leaves extracts on superoxide dismutase activity in serum, liver and kidney in rats fed with crude oil contaminated feed.

Groups	Serum superoxide dismutase (units/ml)	Liver superoxide dismutase (units/g wet tissue)	Kidney superoxide dismutase (units/g wet tissue)
Group 1	25.52 ± 3.04^a	24.54 ± 6.99^a	22.67 ± 2.61^a
Group 2	11.36 ± 4.06^b	13.20 ± 3.76^b	12.48 ± 3.98^b
Group 3	14.20 ± 3.19^b	19.51 ± 2.69^b	14.34 ± 3.08^b
Group 4	16.28 ± 2.87^b	$21.29 \pm 5.31^{a,b}$	$18.200 \pm 5.62^{a,b}$
Group 5	$19.57 \pm 5.32^{a,b}$	23.39 ± 3.84^a	20.47 ± 3.15^a

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

saponins, cardiac glycosides, alkaloids, and cardiac glycosides (Tables 10 and 11). These compounds have been documented to possess medicinal properties and health-promoting effects [65]. The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. [66] Saponins have hypotensive and cardiodepressant properties [67]. Glycosides are natural phytochemical that is used in the treatment of cardiovascular

diseases. Therefore the presence of these bioactive substances may be responsible for the medicinal and antioxidant property of this leaf [68, 69].

3. Conclusions

On the basis of the findings from this work and correlation with other works, it is evident that hydrocarbons found in crude oil contaminated feed influenced alteration of biochemical parameters of the experimental animals. The observed biochemical

Table 7. Effect of *C. odorata* leaves extracts on catalase activity in serum, liver and kidney in rats fed with crude oil contaminated feed.

Groups	Serum catalase (units/ml)	Liver catalase (units/g wet tissue)	Kidney catalase (units/g wet tissue)
Group 1	50.66 ± 6.69 ^a	60.41 ± 6.34 ^a	54.55 ± 3.50 ^a
Group 2	28.20 ± 5.69 ^b	29.36 ± 19.72 ^b	30.43 ± 8.63 ^b
Group 3	37.24 ± 12.66 ^c	40.62 ± 4.46 ^c	35.41 ± 3.15 ^b
Group 4	46.05 ± 4.87 ^a	46.36 ± 5.94 ^d	41.54 ± 4.98 ^{b,c}
Group 5	48.48 ± 9.50 ^a	52.12 ± 4.49 ^d	50.49 ± 15.23 ^{a,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 8. Effect of *C. odorata* leaves extracts on aldehyde oxidase, monoamine oxidase, xanthine oxidase and sulphide oxidase activities in liver of rats fed with crude oil contaminated feed.

Groups	Liver aldehyde oxidase (units/g wet tissue)	Liver monoamine oxidase (units/g wet tissue)	Liver xanthine oxidase (units/g wet tissue)	Liver sulphide oxidase (units/g wet tissue)
Group 1	41.31 ± 5.51 ^a	50.30 ± 14.96 ^a	48.42 ± 1.51 ^a	254.34 ± 9.64 ^a
Group 2	20.40 ± 7.44 ^b	30.41 ± 7.72 ^b	23.51 ± 3.40 ^b	190.48 ± 15.79 ^b
Group 3	24.40 ± 9.04 ^b	35.36 ± 4.65 ^b	32.45 ± 4.29 ^c	210.45 ± 8.08 ^c
Group 4	32.78 ± 5.32 ^{a,d}	40.44 ± 3.09 ^c	36.49 ± 4.23 ^c	227.48 ± 24.40 ^d
Group 5	38.41 ± 8.78 ^a	47.87 ± 11.03 ^a	45.69 ± 9.17 ^a	245.29 ± 31.89 ^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.

Table 9. Effect of *C. odorata* leaves extracts on aldehyde oxidase, monoamine oxidase, xanthine oxidase and sulphide oxidase activities in kidney of rats fed with crude oil contaminated feed.

Groups	Kidney aldehyde oxidase (units/g wet tissue)	Kidney monoamine oxidase (units/g wet tissue)	Kidney xanthine oxidase (units/g wet tissue)	Kidney sulphide oxidase (units/g wet tissue)
Group 1	30.29 ± 3.80 ^a	44.42 ± 5.80 ^a	35.25 ± 4.03 ^a	250.24 ± 38.41 ^a
Group 2	17.00 ± 6.26 ^b	25.37 ± 4.50 ^b	21.72 ± 5.09 ^b	186.40 ± 9.10 ^b
Group 3	21.49 ± 7.56 ^b	30.28 ± 2.30 ^b	28.51 ± 6.53 ^b	201.22 ± 7.11 ^c
Group 4	26.26 ± 13.07 ^{a,b}	36.28 ± 5.08 ^{a,b}	32.26 ± 1.66 ^{a,b}	222.31 ± 10.78 ^d
Group 5	31.35 ± 17.81 ^a	40.42 ± 2.72 ^a	35.02 ± 11.67 ^a	240.31 ± 31.32 ^e

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

findings are also pointers to the toxicity of crude oil. The possible mechanism of crude oil toxicity was found to be elaborated in the liver and kidney via

decrease in oxidant/antioxidant enzymes concentrations, increase in the lipid peroxidation products, and amino transaminases activities. The presence

Table 10. Qualitative phytochemical analysis of *C. odorata* leaves extracts.

Phyto-chemicals	Water extract	Ethanol extract	Blended mixture of extracts
Alkaloids	++	++	++
Saponins	+	++	++
Tannin	+	+	+
Cardiac glycoside	+	+	+
Phenols	+	+	+
Flavonoids	++	++	++

+ = moderately present, ++ = highly present

Table 11. Quantitative phytochemical analysis of *C. odorata* leaves crude sample.

Phytochemical	Mean $\pm$ SD (mg/dl)
Alkaloids	3.99 $\pm$ 0.51
Saponins	3.61 $\pm$ 0.14
Tannins	0.95 $\pm$ 0.34
Cadiac glycoside	0.22 $\pm$ 0.09
Phenols	0.63 $\pm$ 0.19
Flavonoids	2.29 $\pm$ 0.18

of phytoconstituents in *C. odorata* leaves extracts (aqueous, ethanol and blended mixture) make the plant useful for treating different ailments and have a potential of providing useful drugs of human use in the treatment of crude oil toxicity. However, the results of this study will be more reliable with the blended mixture leaf extract *C. odorata* when compare with the aqueous and ethanol extract.

Conflict of Interest

The authors stated no conflict of interest.

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