Sahel Journal of Life Sciences FUDMA (SAJOLS)
June 2026 Vol. 4(2): 545-551
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2026-0402-57*

Research Article

Effect of *Citrullus colocynthis* Rind Ethanol Extract on Serum ALT, AST and ALP Activities in Rats Fed Crude-Oil-Contaminated Diet

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ABSTRACT

This study evaluated the effect of ethanol extract of *Citrullus colocynthis* rind on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities in rats fed a crude-oil-contaminated diet. Twenty-five male Wistar rats were randomly assigned to five groups of five rats each. Group 1 served as the positive control and received grower's mash, while Group 2 received a crude-oil-contaminated diet without treatment. Groups 3, 4 and 5 were fed a crude-oil-contaminated diet for 28 days. Following completion of the exposure period, the crude-oil-contaminated diet was discontinued and replaced with a normal diet, while the animals were administered 200, 400 and 600 mg/kg body weight of *C. colocynthis* rind ethanol extract, respectively, for an additional 28 days. Serum liver enzyme activities were determined using standard biochemical methods. Data were analysed at a significance level of $p < 0.05$. The untreated crude oil-exposed group showed marked elevations in ALT (137.72 U/L), AST (93.30 U/L) and ALP (385.41 U/L) compared with the control group, indicating alterations in hepatic enzyme activities. Administration of the extract produced progressive reductions in enzyme activities. The 600 mg/kg treatment produced the greatest attenuation, reducing ALT, AST and ALP activities to 107.82, 72.50 and 344.41 U/L, respectively. The findings indicated that ethanol extract of *C. colocynthis* rind attenuated crude-oil-associated elevations in serum enzyme activities, with the attenuating effect becoming more pronounced as the treatment dose increased. The study therefore indicated that *C. colocynthis* rind has the potential to attenuate crude-oil-induced increases in serum enzyme activities.

Keywords: Alanine aminotransferase; Alkaline phosphatase; Aspartate aminotransferase; *Citrullus colocynthis* rind; Crude oil

Citation: Onakurhefe, P. (2026). Effect of *Citrullus colocynthis* Rind Ethanol Extract on Serum ALT, AST and ALP Activities in Rats Fed Crude-Oil-Contaminated Diet. *Sahel Journal of Life Sciences FUDMA*, 4(2): 545-551. DOI: *https://doi.org/10.33003/sajols-2026-0402-57*

INTRODUCTION

Crude oil is a complex mixture of aliphatic hydrocarbons, cycloalkanes, polycyclic aromatic hydrocarbons (PAHs) and heavy metals like lead, chromium and nickel, which contribute to environmental pollution and health issues (Kanungo *et al*., 2024). In places such as the Niger Delta in Nigeria, which is a major petroleum-producing region, high levels of oil spillages are prevalent, causing persistent contamination of soil and water resulting in the exposure of people, plants and animals to toxic petroleum constituents (Ikhumetse *et al*., 2022). The toxicity associated with crude-oil exposure reflects the potential effects of this complex mixture rather than necessarily being attributable to any single petroleum constituent. Although individual components, such as PAHs and specific hydrocarbons, may exert distinct toxic effects, the toxicity examined in the present study reflects exposure to crude oil as a whole rather than to any single petroleum constituent.

The liver is the main organ involved in the metabolism of foreign chemicals and is therefore at high risk of crude oil toxicity (Almazroo *et al*., 2017). After exposure, crude oil components are metabolized by the cytochrome P450 enzyme system, which can generate reactive oxygen species (ROS), leading to lipid peroxidation, disruption of cell membranes, and the release of intracellular enzymes into the bloodstream (Aas *et al*., 2000). As a result, elevated serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), may occur (Kalas *et al.,* 2021) and these enzymes are widely recognized as indicators of hepatocellular injury and bile duct damage in rats exposed to crude oil. These enzymes therefore serve as useful biomarkers for assessing the extent of liver damage following crude oil exposure (Pathan *et al*., 2024).

Petroleum contamination is a large-scale environmental and public health issue in oil-producing communities, where people experience continuous exposure through contaminated soil,water and food, which has been associated with liver dysfunction and other non-communicable diseases. Although synthetic hepatoprotective agents are available, their cost, accessibility and potential side effects have driven the search for safer, more affordable and environmentally friendly alternatives (Gonfa *et al*., 2025). This has placed medicinal plants in the scientific spotlight because of their rich phytochemical composition and antioxidant properties.

Citrullus colocynthis is a traditional medicinal plant used for the management of various ailments. While previous studies have focused mainly on the seeds and pulp, recent research has reported that the fruit rind contains phenolics, flavonoids and cucurbitacins, which are associated with antioxidant and anti-inflammatory activities (Onakurhefe *et al*., 2026b). Ethanol is an effective solvent for extracting a wide range of polar bioactive compounds while maintaining their stability (Lee *et al*., 2024).

Despite the reported pharmacological and biological activities of *C. colocynthis*, comparatively limited attention has been given to the rind, particularly in relation to crude-oil-associated biochemical alterations. Thus, there is insufficient information on the potential of *C. colocynthis* rind extract to mitigate changes in serum ALT, AST and ALP activities following crude-oil exposure. The rind was selected in the present study because it is an underutilized fruit component that is commonly discarded as agricultural waste, despite containing phytochemicals with potentially beneficial biological activities. Investigating this plant part therefore addresses an underexplored research area while also providing an opportunity to utilize a neglected agricultural resource. This knowledge gap provided the rationale for evaluating the effect of ethanol extract of *C. colocynthis* rind in crude-oil-exposed rats.

It was hypothesized that oral administration of ethanol extract of *C. colocynthis* rind would dose-dependently attenuate crude-oil-associated increases in serum ALT, AST and ALP activities in rats.

MATERIALS AND METHODS

Chemical and reagents

L-alanine, 2,4-dinitrophenylhydrazine (DNPH), L-Aspartate, p-nitrophenyl phosphate, distilled water, sodium hydroxide, p-nitrophenol, and ethanol were products of British Drug House, Poole, England. A RII-HB rotary evaporator manufactured by BüchiLabortechnik AG, Switzerland (CH-9230), was used for solvent evaporation. A Superfit digital water bath, manufactured by Superfit Continental (P) Ltd., Mumbai, India, was used. An AP223CN weighing balance manufactured by Ohaus Corporation, Pine Brook, NJ, USA, was used for weighing. Centrifugation was performed using a centrifuge manufactured by Search Tech Instrument. A Techmel&Techmel S23A spectrophotometer (USA) was used for absorbance measurements. The crude oil was obtained from the Warri Refining and Petrochemical Company, Warri, Delta State, Nigeria

Collection of Plant Material and Identification

Citrullus colocynthis rinds were collected with permission from a farmer's residence in Idumuesah community, Ika North East Local Government Area, Delta State, Nigeria, during the harvest season in August 2025. The plant was identified based on a previously authenticated voucher specimen (UBH-C641), which was authenticated in 2024 by Prof. H. A. Akinnibosun and deposited at the Herbarium Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Extract Preparation

The rinds of *C. colocynthis* were rinsed with distilled water to remove debris. They were air-dried to a constant weight, then pulverized into fine particles using an electric blender. One hundred grams (100g) of the powdered melon rinds were macerated in 400mL of 95% v/v ethanol for 24 hours. The mixture was filtered using clean muslin cloth. The filtrate was concentrated using a rotary evaporator at 45°C and

then transferred to a water bath maintained at 45°C, where it was evaporated to dryness.

The extraction yield was calculated based on the weight of the dried extract relative to the initial weight of the powdered plant material and was 43.24%.

The obtained extract was stored in a specimen container in a refrigerator at 4°C until further analysis.

Preparation of Stock Solution and Doses

One gram (1 g) of ethanol extract from the dried sample was dissolved in 9 mL of 5% aqueous Tween 80 yielding a final concentration of 0.11 g/mL. For the administration of 200, 400 and 600 mg/kg body weight of the extract, the required doses were calculated as follows:

$$V(ml) = \frac{D(g/Kg) \times W(Kg)}{C\ (g/mL)}$$

Where;

D= Dose used (g/Kg body weight)

W= Body Weight (Kg) of rat

C= Concentration of extract (g/mL)

V= Volume of extract (mL)

(An example of the dose calculation is provided in the Supplementary Material)

Experimental Animals

Twenty-five mature male albino Wistar rats (100.59-152.78 g) were purchased from the animal house of Department of Anatomy, Delta State University, Abraka. The experimental rats were kept in plastic cages. Acclimatization was allowed for two weeks and the rats were fed on grower's mash. All animals completed the experimental protocol; no deaths or exclusions occurred and data from all 25 animals were included in the statistical analysis.

Preparation of crude-oil-contaminated feed: Crude-oil-contaminated feed was prepared freshly each day by thoroughly mixing 4 mL of crude oil with 100 g of growers' mash feed to ensure uniform distribution of the crude oil throughout the feed (Achuba *et al*., 2016). The contaminated feed was prepared immediately before administration to the experimental animals. The actual amount of feed consumed by individual animals was not measured. The detailed feed composition is provided in the Supplementary Material

Ethical Considerations

At the time the study was conducted, a formal institutional ethics review committee was not yet in place. Therefore, ethical approval could not be obtained. However, the animals were handled according to the care and use of laboratory animals (8th ed.) of National Research Council (NRC, 2011).

Experimental Design

The rats were fed Crude Oil Contaminated Feed (COCF) for four weeks and treated with *C. colocynthis* rinds ethanol extract for another four weeks before the determination of various biochemical indices.

Treatment of Animals

Twenty-five albino rats were assigned randomly into five (5) groups with five in each group. Rats in Group 1 served as positive control and were fed with grower's mash only. Rats in Group 2 served as negative control and were fed COCF (4 mL per 100 g of feed). Rats in Group 3 were fed with grower's mash contaminated with crude oil treated with 200 mg/kg body weight (b. wt) of ethanol extract of *C. colocynthis* rinds. Rats in Group 4 were fed with COCF treated with 400 mg/kg. b. wt of ethanol extract of *C. colocynthis* rinds. Rats in Group 5 were fed with COCF treated with 600 mg/kg. b. wt of ethanol extract of *C. colocynthis* rinds. The rats in each group were given free access to drinking water (Ragolis water)

Collection of Blood Samples

The rats were allowed to fast overnight after the last day of the experiment. This was to allow for stabilization of the rats (Nowland *et al*. 2011). The rats were humanely euthanized by cervical dislocation performed by trained personnel in accordance with accepted laboratory animal welfare guidelines. Following euthanasia, 5 mL sterile syringe with needle were used for collection of blood from the vena cava into properly labelled plain sample bottles for biochemical tests.

Assay for Alkaline phosphatase (ALP) (EC.3.1.3.1)

Alkaline phosphatase activity was assayed for using the method of Kaplan and Righetti (1955).

Procedure

For the assay, 0.5 mL of alkaline phosphatase substrate were added in labelled tubes (sample and blank) and equilibrated to 37℃ for three minutes. Subsequently, 0.05 mL of serum was added to tubes and incubated at 37℃ for ten minutes. Thereafter, 2.5 mL ALP colour developer was added at time interval. The absorbance was measured spectrophotometrically at 580 nm at 1-min intervals for 3 min against a reagent blank containing distilled water.

Calculation

$$ALP = \frac{Absorbance\ of\ Assay\ (sample) \times Value\ of\ Standard}{Absorbance\ of\ Standard}$$

Value of Standard= 50U/L

Determination of Aspartate Aminotransferase (AST, EC 2.6.1.1)

Aspartate Aminotransferase (AST) activity was determined by Reitman and Frankel (1957) method.

Procedure

Into test tubes labelled sample and blank, 250 μL of reagent 1 were dispensed. Then, 50 μL of samples was added and mixed. 250 μL of reagent 2 was added to tubes after incubation at room temperature for 60 minutes and exactly 2.5 mL of NaOH reagent was added. The absorbance was recorded against reagent blank at 540nm after ten minutes.

Assay of Alanine Aminotransferase (ALT, EC 2.6.1.2)

Alanine aminotransferase activity was assayed by the method of Reitman and Frankel (1957).

Procedure

Into sample and blank test tube were dispensed 250 μL of reagent 1. Into sample test tubes, 50 μL of sample was added and 50 μL of distilled water into blank, then incubated for 60 minutes at 37 ℃. 250 μL of reagent 2 was added and then incubated at room temperature for 20 minutes. Into each test tubes, 2.5 mL of working NaOH reagent was added, mixed well and allowed to stand for 10 minutes. Absorbance was read against reagent blank at a wavelength of 540nm.

Data Analysis

Data were analyzed using SPSS version 21.0. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to assess differences among the experimental groups, followed by the least significant difference (LSD) post hoc test for multiple comparisons. Differences between means were considered statistically significant at $p < 0.05$.

RESULTS

The effect of *C. colocynthis* rind ethanol extract on serum liver function biomarkers of Wistar rats fed crude oil-contaminated feed is presented in Table 1. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were evaluated to determine the extent of alterations in hepatic enzyme activitiesand the attenuating potential of the extract.

In this study, the administration of crude oil-contaminated diet markedly influenced liver enzyme activities in the tested rats. Group 2, which received the contaminated feed without treatment, recorded the highest serum ALT (137.72 U/L), AST (93.30 U/L), and ALP (385.41 U/L) activities, suggesting hepatic dysfuntion. The elevation of these enzymes may result from damage to hepatocyte membranes, leading to the leakage of intracellular enzymes into the bloodstream.

Treatment with 200 mg/kg body weight of *C. Colocynthis* rind ethanol extract (Group 3) reduced all three liver enzymes activities compared with the untreated group. Serum ALT decreased to 127.90 U/L, AST to 78.30 U/L and ALP to 353.62 U/L. This reduction may be associated with the bioactive phytochemicals present in the plant, which may contribute to the preservation of hepatocellular integrity. Younis *et al.* (2022) reported that *C. colocynthis* possesses hepatoprotective activity, which may be mediated through its antioxidant properties and stabilization of hepatocyte membranes. Although the enzyme activities remained higher than those of the normal control, the observed reductions may indicate an improvement in hepatic status following treatment.

In Group 4, which received the 400 mg/kg body weight dose, greater improvement was observed compared with Group 3. Serum ALT decreased to 119.50 U/L and AST to 73.36 U/L, approaching the values recorded for the normal control. Although serum ALP remained relatively elevated at 359.32 U/L, the marked attenuation of ALT and AST activities suggests a reduction in crude-oil-associated hepatocellular dysfunction. The greater response observed in Group 4 may be associated with the higher dose of the extract, which could provide a greater amount of antioxidant and other bioactive phytochemicals capable of scavenging reactive oxygen species generated by crude oil exposure. This interpretation is consistent with Marcel *et al.* (2025), who reported that antioxidant phytochemicals can counteract oxidative stress induced by environmental pollutants.

Group 5, which received the 600 mg/kg body weight dose produced the most pronounced attenuation of ALT, AST and ALP activities among the treatment groups. Serum ALT decreased to 107.82 U/L, AST to 72.50 U/L and ALP to 344.41 U/L, with the values approaching those of the normal control. The progressive reduction in liver enzyme activities with increasing extract concentration suggests a dose-related response to the ethanol extract. This observation may be attributed to the various phytochemicals reported in *C. colocynthis*, including flavonoids, phenolic compounds, tannins, alkaloids and saponins. These compounds possess antioxidant and anti-inflammatory properties (Onakurhefe *et al.,* 2026b) and may contribute to the reduction of oxidative stress and lipid peroxidation, thereby supporting the maintenance of hepatocellular

integrity. The findings are consistent with Ali *et al*. (2019), who reported that higher doses of hepatoprotective plant extracts can produce greater improvements in liver biochemical markers. The findings indicate that ***C. colocynthis*** rind ethanol extract attenuated crude-oil-associated alterations in liver enzyme activities, with the 600 mg/kg body weight dose producing the most pronounced improvement, followed by the 400 and 200 mg/kg body weight doses**.** The progressive reduction in ALT, AST and ALP activities across the treatment groups suggests a dose-related response to the extract.

Table 1: Serum Transaminases and Alkaline Phosphatase Activities of rat fed crude oil contaminated feed treated with *C. colocynthis* rind ethanol extract

Experimental Group	Serum ALT (U/L)	Serum AST(U/L)	Serum ALP(U/L)
Group 1	92.78 ± 6.97^{a}	66.46 ± 6.03^{a}	303.69 ± 4.81^{a}
Group 2	137.72 ± 9.32^{b}	93.30 ± 5.00^{b}	385.41 ± 12.78^{b}
Group 3	127.90 ± 5.98^{c}	78.30 ± 5.84^{c}	353.62 ± 17.42^{c}
Group 4	119.50 ± 5.50^{d}	73.36 ± 8.87^{d}	359.32 ± 7.24^{d}
Group 5	107.82 ± 5.55^{e}	72.50 ± 6.32^{d}	344.41 ± 14.10^{e}

Values are represented in mean ± SD. n = 5. Mean values with different superscript on the same column differ significantly at ($p < 0.05$). ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline Phosphatase), Group 1: Positive control fed with growers mash only, Group 2: Negative control; fed crude oil-contaminated feed, Group 3: treated with 200 mg/kg body weight of ethanol extract, Group 4: Treated with 400 mg/kg body weight of the extract, Group 5: Treated with 600 mg/kg body weight of the extract

DISCUSSION

The activities of serum ALT, AST and ALP differed significantly among the experimental groups ($p < 0.05$). The crude-oil-exposed group differed significantly from the control group for all three hepatic enzymes, indicating that crude-oil exposure produced marked alterations in hepatic biochemical function. Such alterations may reflect disruption of hepatocellular integrity and impairment of normal hepatic function. The hepatotoxic effects associated with crude-oil contamination have been documented in the literature, with petroleum hydrocarbons recognised as important environmental contaminants capable of inducing adverse biological effects (Ikhumetse *et al.,* 2022).

Administration of *C. colocynthis* rind ethanol extract significantly modified the activities of ALT, AST and ALP compared with the crude-oil-exposed group. For ALP and ALT, the different superscripts among the extract-treated groups indicate significant differences between the treatment levels. For AST, the first extract-treated group differed significantly from the subsequent treatment groups, whereas the latter two groups did not differ significantly from each other, as indicated by their shared superscript. These findings indicate that administration of the extract significantly ameliorated the alterations in hepatic enzyme activities associated with crude-oil exposure, although the degree of response varied among the enzymes.

The present findings are consistent with previous reports of the hepatoprotective and antioxidant properties of medicinal plants. Ali *et al*. (2019) documented the hepatoprotective activity of medicinal plants and highlighted the importance of plant-derived bioactive constituents in protecting against hepatic injury. Similarly, Vakiloddin *et al*. (2015) reported significant hepatoprotective and antioxidant activities of *C. colocynthis* fruit extract. Since the rind constitutes part of the fruit, this finding provides supportive evidence for the hepatoprotective potential of the rind. More specifically, Onakurhefe (2026a), through GC–MS profiling of aqueous, ethanolic, acetone and petroleum ether extracts of the rind, identified thirty-six (36) phytocomponents in the ethanolic extract, while Onakurhefe *et al.* (2026b) reported *in vitro* antioxidant efficacy of *C. colocynthis* rind. The bioactive constituents identified by GC–MS may contribute to mitigating oxidative disturbances and preserving cellular integrity, thereby supporting the observed amelioration of crude-oil-associated alterations in hepatic enzyme activities. Collectively, the findings suggest that the antioxidant and phytochemical properties of *C. colocynthis* rind may underlie its observed hepatoprotective effect in the present crude-oil-associated hepatic model.

Generally, the significant differences observed among the experimental groups indicate that crude-oil exposure adversely altered serum hepatic enzyme activities, whereas subsequent administration of *C. colocynthis* rind ethanol extract significantly attenuated these alterations. However, the statistical pattern was not identical for all three enzymes,

particularly for AST at the higher treatment levels, suggesting that the response to the extract may vary according to the biomarker and treatment dose.

The present study has some limitations that should be considered when interpreting the findings. First, the assessment was primarily based on serum biochemical markers, including ALT, AST and ALP, and therefore did not provide a comprehensive evaluation of tissue-level alterations. Histopathological examination of the liver was not performed; consequently, the extent to which the observed biochemical changes corresponded to structural alterations in hepatic tissue could not be established. In addition, other relevant indicators of hepatic injury, oxidative stress, and inflammation were not assessed. The study also involved a relatively small number of animals per experimental group, which may limit the statistical power and generalizability of the findings. Furthermore, only three doses of the ethanol extract were investigated and the study duration was limited to the experimental period; therefore, the long-term effects and the optimal therapeutic dose could not be established. Finally, the study evaluated the ethanol extract as a whole and did not isolate or characterize individual bioactive constituents responsible for the observed effects. Further studies incorporating histopathological assessment, a broader panel of biochemical and molecular biomarkers, larger sample sizes, and characterization of individual active constituents are warranted to confirm and extend these findings.

CONCLUSION

In the findings of this study, ethanol extract of *C. colocynthis* rind attenuated the elevations in serum ALT, AST and ALP associated with crude-oil-contaminated feed, with the 600 mg/kg dose showing the greatest numerical improvement among the tested doses. However, the biomarkers did not consistently return to control values. Further studies incorporating liver histopathology, oxidative-stress and inflammatory biomarkers, phytochemical characterization, larger sample sizes and appropriate positive controls are required to confirm hepatoprotective efficacy and clarify the underlying mechanisms.

Conflicts of Interest

The author declare no conflict of interest.

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