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Biochemical Modulation of Drug-Metabolizing Enzymes by Almond (*Terminalia catappa*) Leaf Litter Extract in Rats Exposed to Crude Oil-Induced Toxicity

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Abstract

This study investigated the impact of crude oil exposure on the activities of aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO) in rats, and assessed the modulatory effects of methanol extract from *Terminalia catappa* leaf litter (ALE) on these enzymes across vital organs. Sixty-four male Wistar albino rats (200.0 ± 1.5 g) were randomly assigned to eight groups (n = 8), receiving either a normal or crude oil-contaminated diet (4.0 mL/100 g feed), with or without ALE supplementation (100, 200, or 300 mg/kg body weight). After a 14-day acclimatization, rats were treated for 90 days. ALE increased oxidase activities in healthy rats, indicating enhanced detoxification. Crude oil exposure markedly reduced AO, SO, MAO, and XO activities, suggesting oxidative stress and mitochondrial dysfunction. Since these enzymes are in mitochondria or linked to mitochondrial redox metabolism, their reduced activities serve as indicative biomarkers of mitochondrial dysfunction. ALE co-treatment partially restored enzyme activities, though not fully, indicating modest protective effects. The results highlight the potential of *T. catappa* leaf extract in mitigating crude oil-induced oxidative damage.

Keywords: Crude oil exposure, Oxidase enzymes (AO, SO, MAO, XO), *Terminalia catappa* leaf extract, Oxidative stress, Detoxification

Introduction

Environmental pollution, especially from crude oil spills, has become a serious global health and ecological concern. Crude oil contains a complex mixture of hydrocarbons, heavy metals, and polycyclic aromatic hydrocarbons (PAHs), many of which are toxic, mutagenic, and carcinogenic (Patel *et al*., 2020). When ingested or absorbed into biological systems, crude oil disrupts metabolic and detoxification pathways, leading to oxidative stress and damage to critical organs such as the liver, kidney, heart, lungs, and skeletal muscle (Priyadarshanee *et al*., 2022; Ajarem *et al*., 2022).

One of the body's major defense mechanisms against xenobiotics and toxins is the activation of drug-metabolizing enzymes (DMEs), particularly the oxidase family of enzymes involved in Phase I metabolism. These include aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO) (Sayyed *et al*., 2022). AO is a molybdenum-containing enzyme that catalyzes the oxidation of aldehydes and nitrogenous heterocycles, playing a vital role in the metabolism of drugs and environmental toxins (Adamus *et al*., 2024). SO catalyzes the final step in sulfur amino acid catabolism, converting toxic sulfite to sulfate (Zhou *et al*., 2025). MAO, located on the outer mitochondrial membrane, metabolizes neurotransmitters such as dopamine and serotonin, influencing

neurological and metabolic balance (Tian *et al*., 2023). XO catalyzes the oxidation of hypoxanthine and xanthine to uric acid and is also involved in reactive oxygen species (ROS) generation (Han *et al*., 2025).

Exposure to crude oil can modulate the activity of these enzymes, either inhibiting or enhancing their function depending on the tissue, dose, and duration of exposure. For example, excessive oxidative stress can overwhelm antioxidant defense systems, leading to reduced enzymatic activities and exacerbation of tissue injury (Demirci-Çekiç *et al*., 2022). Altered DME activity contributes to impaired detoxification, accumulation of toxic intermediates, and increased susceptibility to metabolic diseases (Moghaddam and Hazlett, 2023).

As a result, there is growing interest in natural products with antioxidant properties that can restore or modulate enzyme function during oxidative damage. Among these, *Terminalia catappa* (commonly known as almond) has gained attention due to its pharmacological properties (Arunachalam *et al*., 2024). *T. catappa* is a tropical plant belonging to the Combretaceae family, traditionally used for the treatment of hepatitis, diarrhea, and inflammation (Arunachalam *et al*., 2024). Its leaves are rich in polyphenols, flavonoids, tannins, and other phytochemicals with well-documented antioxidant and hepatoprotective properties (Das *et al*., 2022).

Several studies have reported that green leaf extracts of *T. catappa* exhibit significant free radical scavenging activities and protect against chemical-induced organ damage (Shipa *et al*., 2022). Dada *et al*. (2021) demonstrated that *T. catappa* leaf litter extract provided hepatoprotective effects in rats exposed to carbon tetrachloride. In another study, Sarkar *et al*. (2007) isolated corilagin, a tannin from *T. catappa*, which significantly reduced liver injury induced by galactosamine (GalN) and lipopolysaccharide (LPS).

However, there remains a research gap concerning the potential biomedical application of *T. catappa* leaf litter—that is, the dried fallen leaves, which are often discarded as waste. Interestingly, during the natural decomposition of leaf litter, chemical changes may occur, possibly yielding novel secondary metabolites with enhanced biological activities (Malik *et al*., 2020). The use of leaf litter aligns with the principles of environmental sustainability, waste-to-wealth conversion, and low-cost natural therapy (Singh *et al*., 2024).

This study seeks to explore the protective role of methanol extract of *T. catappa* leaf litter on drug-metabolizing oxidase enzymes—AO, SO, MAO, and XO—in rats subjected to crude oil-induced toxicity. The organs of focus include the liver, kidney, heart, lungs, and muscle, which are integral to systemic detoxification, metabolism, and oxidative regulation. Does the methanol extract of *Terminalia catappa* leaf litter modulate the activities of drug-metabolizing oxidase enzymes (aldehyde oxidase, sulfite oxidase, monoamine oxidase, and xanthine oxidase) in the liver, kidney, heart, lungs, and muscle of rats exposed to crude oil-induced toxicity?

Previous studies carried out by the researcher have shown that crude oil exposure significantly altered the activities of key oxidase enzymes—aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO)—in various organs of rats, including the liver, kidney, heart, lungs, and skeletal muscle (O'Brien *et al*., 2005; Sadeghi *et al*., 2023). However, the effects of methanol extract of

almond leaf litter on these disruptions have not yet been reported.

Furthermore, oxidative stress, a hallmark of crude oil-induced toxicity, leads to increased ROS production and a subsequent decline in antioxidant enzyme activities, including those of AO and XO, which also participate in ROS generation (Madrigal-Santillán *et al*., 2022). Modulation of the balance between pro-oxidants and antioxidants by the extract of *Terminalia catappa* was observed to contribute to the restoration of redox homeostasis, reduction in lipid peroxidation levels, and preservation of oxidase enzyme integrity (Padmanaban *et al*., 2023).

The biochemical investigation of DMEs across various organs allows for an organ-specific assessment of toxicity and therapeutic response, which is critical since enzyme expression and sensitivity vary among tissues (Kulsharova and Kurmangaliyeva, 2021). The liver, being the principal site of detoxification, often shows the earliest and most pronounced enzymatic changes. The kidneys, due to their filtration role, also accumulate toxins, while the heart and lungs may exhibit systemic metabolic responses. Skeletal muscle, though less studied in this context, represents a key site for metabolic regulation during stress (Zumbaugh *et al*., 2022).

Beyond its effect on enzyme activity, the *Terminalia catappa* extract demonstrated potential to influence mitochondrial function, alter the expression of drug-metabolizing enzymes (DMEs), and modulate inflammatory cytokine responses; however, these underlying mechanisms require further investigation. The extract's ability to modulate MAO activity, for example, could have implications not just for detoxification but also for behavioral and neurochemical outcomes in toxicity-induced models (Kosmopoulou *et al*., 2024).

This study investigates the impact of crude oil exposure on the activities of key oxidase enzymes—aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO)—in rats, with a focus on vital organs including the liver, kidney, heart, lungs, and skeletal muscle. It further evaluates the modulatory effect of methanol extract from *Terminalia catappa* leaf litter on these enzymes. By examining the protective potential of this phytochemical-rich extract, the research highlights *T. catappa* leaf litter as a potential biochemical intervention against crude oil-induced toxicity. The findings contribute novel insight into the therapeutic utility of an underexplored botanical residue and its relevance in environmental toxicology and drug metabolism, supporting the broader application of phytotherapy in mitigating the effects of industrial pollutants.

Materials and Methods

Collection and Identification of Plant Materials

The leaves of *Terminalia catappa* were collected from Obiaruku, Delta State. The plant specimen was identified and authenticated at the Department of Botany, Delta State University, Abraka, and assigned a voucher number (DELSUH017). Crude oil was obtained from the Nigerian National Petroleum Corporation (NNPC) refinery in Warri, Delta State.

Preparation of Plant Extract

The leaves of *T. catappa* were thoroughly washed with distilled water to remove dirt and debris, air-dried for two weeks until a constant weight was achieved, and then ground into coarse powder using a manual grinder. A total of 100 g of the powdered leaves was subjected to extraction with 400

mL of ethanol (95% v/v) via cold maceration for 24 hours. The extract was initially filtered through cheese cloth and then filtered again using Whatman No. 1 filter paper (to obtain crude filtrate). The filtrate was concentrated at 50°C using a rotary evaporator for 2 hours and further evaporated to dryness on a water bath maintained at 50°C, yielding a dark brown residue. The dried extract was stored at 4°C in a refrigerator until further use (Arabshahi *et al*., 2007).

Experimental Design

A total of sixty-four (64) healthy male Wistar albino rats, weighing between 200.0 ± 1.5 g, were obtained from the Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka. The animals were acclimatized for fourteen (14) days under standard laboratory conditions prior to the commencement of the experiment. These conditions included a 12-hour light/dark cycle, an ambient temperature of 22 ± 2°C, and relative humidity maintained at 50–60%. During this period, the rats were provided with growers' mash (Top Feed, Premier Feed Mills Co. Ltd, Ibadan, Oyo State) and clean drinking water ad libitum.

The animals were randomly divided into eight (8) groups, with eight (8) rats per group. Each group was housed in well-ventilated polypropylene cages lined with clean paddy husk bedding to provide a non-toxic, absorbent, and stress-minimizing environment. Throughout the 90-day experimental period, the rats were maintained on a consistent diet of growers' mash and water *ad libitum* to ensure optimal nutritional and hydration status.

The experimental groups were organized as follows:

Groups	Treatment
I	Control: Rats maintained on normal diet.
II	Fed a normal diet and received ALE 100 mg/kg. bwt.
III	Fed a normal diet and received ALE 200 mg/kg. bwt.
IV	Fed a normal diet and received ALE 300 mg/kg. bwt.
V	Fed a crude oil-contaminated diet only (4.0 mL/100 g of diet).
VI	Fed a crude oil-contaminated diet and received ALE 100 mg/kg. bwt.
VII	Fed a crude oil-contaminated diet and received ALE 200 mg/kg. bwt.
VIII	Fed a crude oil-contaminated diet and received ALE 300 mg/kg. bwt.

The crude oil-contaminated diet was prepared following the method described by Achuba (2018), the crude oil-contaminated diet was prepared by incorporating 4.0 mL of crude oil per 100 g of feed, a dosage shown to be tolerable over a 30-day period. Dosages of the *Terminalia catappa* leaf litter methanol extract (ALE) used in this study were determined based on its previously established median lethal dose (LD_{50}), reported as 2500 mg/kg body weight (Iheagwam *et al*., 2021).

Collection of Tissue Samples

At the end of the 90-day experimental period, the rats were fasted for 12 hours and then euthanized via cervical decapitation. Each rat was placed in a supine position, and a laparotomy was performed to expose the internal organs. Tissue samples (liver, kidney, heart, lungs and muscle) were excised, rinsed with cold saline, and homogenized in a mortar and pestle under cold conditions using 0.1 M phosphate buffer (pH 7.4). The homogenates were transferred into plain tubes and centrifuged at 3000 g. The resulting supernatants were collected for various biochemical analyses. All tissue

samples and homogenates were stored at 4°C in a refrigerator until further use.

Biochemical assay

Drug metabolizing enzyme activities were analyzed with standard protocols, aldehyde oxidase (AO) (Omarov *et al* 1998), Sulphite oxidase (SO) (Macleod *et al*., 1961), monoamine oxidase (MO), xanthine oxidase (XO) (McEwen, 1971).

Ethical approval

The care and use of the animals and the experimental protocol were in accordance with the Research and Ethics Committee (REC), Faculty of Science, Delta State University, Abraka. Ethical Clearance was applied for and obtained before the commencement of the study (REC/FOS/2025/23).

Statistical analysis

All data obtained were subjected to statistical analysis. Values were reported as Mean and Standard deviation (Mean ± S.D) while one-way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$). Turkey post hoc tool was used as basis for comparison for level of significance.

Results and Discussion

The activities of key drug-metabolizing enzymes—aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO)—in the liver of rats were significantly affected by crude oil exposure and supplementation with varying doses of methanol extract of *Terminalia catappa* leaf litter (ALE). Administration of ALE to rats on a normal diet resulted in increased activities of these enzymes, suggesting enhanced detoxification capacity and improved oxidative stress response. Notably, AO activity increased to 94.4 ± 4.4 units/g tissue in ALE-treated rats (Group IV) compared to 79.6 ± 2.1 units/g tissue in the control group (Group I), supporting the antioxidant potential of the extract (Rodríguez-Yoldi, 2021).

In contrast, crude oil exposure significantly reduced the activities of AO (74.0 ± 6.6 units/g tissue), SO (511.3 ± 29.7 units/g tissue), MAO (145.5 ± 6.5 units/g tissue), and XO (51.7 ± 8.0 units/g tissue), indicating impaired liver enzyme function. This reduction reflects oxidative damage and disruption of hepatic metabolism caused by toxic constituents of crude oil, compromising the liver's ability to detoxify xenobiotics. These findings align with previous reports linking environmental pollutants to liver dysfunction and oxidative stress (Priyadarshanee *et al*., 2022; Ajeel *et al*., 2021).

Table 1. Effect of methanol extract of almond (*Terminalia catappa*) leaf litter on oxidase enzyme activities in liver of rats fed crude oil treated diet

Groups	AO (Units/g tissue)	SO (Units/g tissue)	MAO (Units/g tissue)	XO (Units/g tissue)
I	79.6 ± 2.1^{b}	600.1 ± 48.2^{b}	149.4 ± 5.8^{c}	65.2 ± 3.5^{b}
II	89.8 ± 2.8^{a}	632.2 ± 28.9^{a}	172.4 ± 5.9^{a}	67.1 ± 2.9^{b}

Groups	AO (Units/g tissue)	SO (Units/g tissue)	MAO (Units/g tissue)	XO (Units/g tissue)
III	84.1 ± 6.5^{ab}	637.3 ± 22.1^{a}	169.5 ± 2.0^{a}	72.0 ± 4.4^{ab}
IV	94.4 ± 4.4^{a}	635.3 ± 43.3^{a}	167.2 ± 5.4^{a}	74.6 ± 3.2^{a}
V	74.0 ± 6.6^{c}	511.3 ± 29.7^{c}	145.5 ± 6.5^{c}	51.7 ± 8.0^{c}
VI	78.7 ± 2.0^{b}	611.8 ± 78.2^{b}	165.0 ± 6.2^{ab}	62.0 ± 4.8^{b}
VII	78.0 ± 6.9^{b}	569.1 ± 94.4^{bc}	160.7 ± 3.6^{b}	76.6 ± 8.4^{a}
VIII	87.8 ± 2.2^{a}	594.1 ± 25.0^{b}	162.8 ± 8.4^{b}	63.6 ± 5.6^{b}

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. Aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO) and xanthine oxidase (XO)

ALE supplementation partially restored oxidase activities in crude oil-treated rats. For AO, ALE increased activity to 87.8 ± 2.2 units'/g tissue (Group VIII), and for SO, it partially restored activity to 611.8 ± 78.2 units'/g tissue (Group VI), suggesting ALE's potential to counteract toxic disruptions in sulfur metabolism (McLoone *et al*., 2021). The partial recovery of MAO (160.7 ± 3.6 units'/g tissue, Group VII) and XO (76.6 ± 8.4 units'/g tissue, Group VII) further suggests ALE's neuroprotective and antioxidant roles (Lobine *et al*., 2021; Akbari *et al*., 2022). ALE enhances oxidase enzyme activities in rats on a normal diet and restores these activities in crude oil-treated rats, indicating its protective and restorative effects against oxidative stress and environmental toxins (Akbarpour *et al*., 2020; Mao *et al*., 2021). This effect is likely due to the rich presence of bioactive phytochemicals in *Terminalia catappa* leaf litter extract, such as ellagitannins, flavonoids, and phenolic compounds, which exhibit strong antioxidant properties. These compounds can scavenge reactive oxygen species (ROS), reduce lipid peroxidation, and stabilize cellular membranes, thereby preserving the structural integrity and functionality of drug-metabolizing enzymes. Additionally, ALE may upregulate the expression of genes involved in antioxidant defense and detoxification pathways, further contributing to the normalization of enzyme activities disrupted by crude oil toxicity (Kim *et al*., 2023; Arunachalam *et al*., 2024).

Table 2 illustrates the impact of *T. catappa* leaf litter methanol extract on renal oxidase enzymes—aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO)—in rats fed crude oil-contaminated diets. These enzymes are crucial for oxidative metabolism, detoxification, and renal function. Crude oil exposure (Group V) significantly suppressed all four oxidases compared to the control (Group I), indicating oxidative stress and mitochondrial dysfunction, likely due to the toxic effects of hydrocarbons and heavy metals (Sun *et al*., 2022; Reddam *et al*., 2022). This downregulation suggests compromised renal detoxification and redox homeostasis.

Table 2. Effect of methanol extract of almond (*Terminalia catappa*) leaf litter on oxidase enzyme activities in kidney of rats fed crude oil treated diet

Groups	AO (Units/g tissue)	SO (Units/g tissue)	MAO (Units/g tissue)	XO (Units/g tissue)
I	60.7 ± 1.9^{b}	556.1 ± 46.0^{b}	125.7 ± 3.9^{c}	48.0 ± 2.1^{b}
II	70.1 ± 3.6^{a}	595.3 ± 28.0^{a}	153.8 ± 4.6^{a}	54.9 ± 4.4^{b}
III	73.5 ± 3.6^{a}	606.5 ± 19.6^{a}	155.7 ± 6.4^{a}	59.0 ± 2.9^{a}
IV	75.8 ± 2.2^{a}	603.4 ± 43.6^{a}	158.0 ± 5.8^{a}	61.6 ± 2.1^{a}
V	50.6 ± 2.0^{c}	508.4 ± 8.5^{c}	117.1 ± 6.4^{c}	36.6 ± 1.8^{c}
VI	66.9 ± 2.7^{b}	512.4 ± 11.4^{c}	124.5 ± 3.5^{c}	38.3 ± 1.7^{c}
VII	71.4 ± 2.5^{b}	517.0 ± 6.3^{c}	124.5 ± 3.7^{c}	40.9 ± 4.1^{c}
VIII	59.7 ± 4.0^{c}	511.4 ± 0.7^{c}	127.4 ± 2.0^{c}	40.0 ± 4.3^{c}

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. Aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO) and xanthine oxidase (XO).

Extract treatment (Groups II–IV, VI–VII) led to a dose-dependent restoration of oxidase enzyme activities in the kidney, with Group IV exhibiting the most pronounced effect. However, Group VIII did not follow this trend, showing a decline in enzyme activities, suggesting that beyond a certain concentration or treatment condition, the restorative effect of the extract may diminish. This suggests that the extract not only reverses crude oil-induced inhibition but may enhance oxidase expression, possibly via gene upregulation or enzyme synthesis (Ben Hsounna *et al*., 2022). The extract's renoprotective effects are likely due to its flavonoids, phenolics, and tannins, which are known for antioxidant, metal-chelating, and enzyme-inducing properties (Xu *et al*., 2021). The restoration of monoamine oxidase (MAO) activity indicates potential neuro-renal protective modulation, while the increase in xanthine oxidase (XO) activity suggests enhanced purine metabolism. However, caution is warranted, as overexpression of XO can lead to excessive generation of reactive oxygen species (ROS), potentially exacerbating oxidative stress if not counterbalanced by sufficient antioxidant defenses (Liu *et al*., 2021). Therefore, maintaining a balance between XO activity and antioxidant capacity is critical to avoid unintended oxidative damage. *T. catappa* extract shows strong potential to mitigate crude oil-induced renal oxidative dysfunction through modulation of oxidase enzymes and redox pathways.

Table 3. Effect of methanol extract of almond (*Terminalia catappa*) leaf litter on oxidase enzyme activities in heart of rats fed crude oil treated diet

Groups	AO (Units/g tissue)	SO (Units/g tissue)	MAO (Units/g tissue)	XO (Units/g tissue)
I	80.1 ± 2.4^{b}	641.6 ± 55.9^{b}	153.8 ± 5.7^{c}	47.4 ± 2.1^{b}
II	90.2 ± 3.1^{a}	677.5 ± 24.6^{a}	174.0 ± 3.8^{a}	55.0 ± 4.4^{b}
III	84.1 ± 7.5^{b}	696.9 ± 26.5^{a}	170.9 ± 6.7^{a}	58.8 ± 3.8^{a}
IV	93.7 ± 3.3^{a}	689.2 ± 49.2^{a}	167.5 ± 5.3^{a}	61.4 ± 2.7^{a}
V	61.2 ± 4.4^{c}	694.5 ± 51.3^{a}	146.2 ± 2.5^{c}	34.2 ± 1.8^{c}
VI	70.6 ± 1.7^{b}	764.9 ± 26.9^{a}	150.2 ± 4.9^{c}	37.8 ± 0.8^{c}
VII	73.9 ± 3.7^{b}	777.1 ± 12.6^{a}	156.8 ± 2.7^{c}	40.9 ± 4.1^{c}
VIII	71.2 ± 2.3^{b}	778.5 ± 26.4^{a}	156.5 ± 6.1^{c}	40.3 ± 4.1^{c}

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. Aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO) and xanthine oxidase (XO)

Table 3 presents the activities of key cardiac (heart) oxidases—aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO)—in rats subjected to crude oil toxicity and treated with *Terminalia catappa* leaf litter extract. Rats exposed to crude oil alone (Group V) showed significant reductions in AO (61.2 ± 4.4) and XO (34.2 ± 1.8) compared to controls (Group I: AO, 80.1 ± 2.4; XO, 47.4 ± 2.1), suggesting mitochondrial dysfunction and impaired redox metabolism (Colon Hidalgo *et al*., 2022; Amorim *et al*., 2022). MAO activity also declined, while SO remained elevated, possibly due to compensatory enzymatic responses.

Pre-treatment with *T. catappa* extract (Groups II–IV) significantly restored AO, MAO, and XO activities, with Group IV showing the highest values (AO: 93.7 ± 3.3; MAO: 167.5 ± 5.3; XO: 61.4 ± 2.7), surpassing even the control. This suggests bioactive constituents in the extract—likely flavonoids and polyphenols—enhance cardiac enzymatic defense mechanisms (Khan *et al*., 2021; Mihailović *et al*., 2021). In post-treatment groups (VI–VIII), modest improvements in AO and MAO were observed compared to the crude oil-only group, though less pronounced than in pre-treated rats. Interestingly, SO activity increased markedly in these groups (e.g., Group VIII: 778.5 ± 26.4), potentially reflecting activation of sulfite detoxification pathways during recovery. The extract demonstrated both preventive and therapeutic potential, with pre-treatment offering superior protection against crude oil-induced cardiac oxidase suppression. The life implication of pre-treatment is that it may serve as a proactive protective strategy to fortify the heart's enzymatic antioxidant defenses before exposure to environmental toxins, thereby minimizing tissue damage,

preserving cardiac function, and reducing the risk of chronic cardiovascular complications associated with oxidative stress and toxic insult. This highlights the potential for *T. catappa* extract to be used as a preventive dietary supplement or phytotherapeutic agent in populations at risk of pollutant exposure (Ajibade *et al*., 2022).

Table 4. Effect of methanol extract of almond (*Terminalia catappa*) leaf litter on oxidase enzyme activities in lungs of rats fed crude oil treated diet

Groups	AO (Units/g tissue)	SO (Units/g tissue)	MAO (Units/g tissue)	XO (Units/g tissue)
I	68.3 ± 3.2^{b}	581.0 ± 44.9^{b}	139.4 ± 5.3^{c}	52.7 ± 5.2^{b}
II	77.4 ± 3.3^{a}	615.8 ± 39.8^{a}	161.2 ± 4.7^{a}	56.1 ± 5.2^{b}
III	79.4 ± 3.9^{a}	629.4 ± 17.6^{a}	157.4 ± 4.1^{a}	59.9 ± 3.0^{a}
IV	83.7 ± 2.8^{a}	612.9 ± 76.1^{b}	157.1 ± 5.8^{a}	61.9 ± 4.8^{a}
V	60.2 ± 3.7^{c}	444.5 ± 4.9^{c}	128.7 ± 3.9^{c}	40.9 ± 2.0^{c}
VI	63.5 ± 4.6^{b}	446.3 ± 14.7^{c}	147.4 ± 7.9^{c}	43.3 ± 2.7^{c}
VII	62.7 ± 3.5^{c}	450.2 ± 11.3^{c}	150.8 ± 4.1^{c}	51.3 ± 2.4^{b}
VIII	67.0 ± 2.2^{b}	467.6 ± 17.7^{c}	147.3 ± 3.7^{c}	43.7 ± 1.9^{c}

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. Aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO) and xanthine oxidase (XO)

The activities of aldehyde oxidase (AO), sulphite oxidase (SO), monoamine oxidase (MAO), and xanthine oxidase (XO) were significantly altered in the lungs of rats exposed to crude oil, with or without treatment with *Terminalia catappa* leaf extract (Table 4). Crude oil exposure (Group II) significantly increased AO, SO, MAO, and XO activities compared to the control (Group I), reflecting oxidative stress and altered enzymatic function.

Co-treatment (the rats received *Terminalia catappa* leaf extract simultaneously with the crude oil-contaminated diet) with the methanol extract (Groups III and IV) maintained or slightly elevated oxidase activities, suggesting the extract did not completely prevent crude oil-induced enzyme induction. However, post-treatment (the rats were first exposed to the crude oil-contaminated di*et al*one (to induce toxicity), and afterward, they were treated with the *Terminalia catappa* leaf extract) with the extract (Groups V–VIII) led to a marked reduction in all oxidase activities, with Group V showing the most significant decreases. This indicates a therapeutic effect of the extract, likely due to its phytoconstituents such as flavonoids and phenolic compounds with known antioxidant properties (Mutha *et al*., 2021; Nwozo *et al*., 2023).

The reduction in xanthine oxidase (XO) and monoamine oxidase (MAO) activities observed in the post-treatment groups is

particularly notable, as both enzymes are key sources of reactive oxygen species (ROS) in biological systems. XO catalyzes the oxidation of hypoxanthine to xanthine and subsequently to uric acid, a process that generates superoxide anions—a potent contributor to oxidative damage (Bortolotti *et al*., 2021). MAO, on the other hand, is involved in the oxidative deamination of monoamine neurotransmitters, producing hydrogen peroxide as a by-product, thereby contributing to neuroinflammation and oxidative stress (Ostadkarampour and Putnins, 2021; Banerjee *et al*., 2024). The observed decline in these enzyme activities suggests that *Terminalia catappa* extract may play a role in mitigating ROS production and dampening neuroinflammatory pathways triggered by crude oil exposure.

This aligns with the findings of Oyeyemi *et al*. (2024), who reported that exposure to crude oil-contaminated diet significantly elevated oxidative stress markers in rats, including enhanced lipid peroxidation and reduced antioxidant enzyme activities. In contrast, treatment with red palm oil ameliorated these effects by restoring antioxidant balance. Similarly, in the present study, post-treatment with *T. catappa* extract not only normalized oxidase activities but also likely curtailed the cascade of oxidative and inflammatory damage induced by crude oil toxicity. These findings reinforce the extract's therapeutic potential as a post-exposure intervention, consistent with previous reports on the antioxidative and neuroprotective properties of phytochemicals and polyphenol-rich plant extracts (Seferli *et al*., 2024; Saint Martin *et al*., 2024).

Table 5. Effect of methanol extract of almond (*Terminalia catappa*) leaf litter on oxidase enzyme activities in muscle of rats fed crude oil treated diet

Groups	AO (Units/g tissue)	SO (Units/g tissue)	MAO (Units/g tissue)	XO (Units/g tissue)
I	93.6 ± 2.6^{b}	777.7 ± 60.2^{b}	155.8 ± 6.3^{c}	61.5 ± 3.2^{b}
II	103.8 ± 2.1^{a}	821.4 ± 38.4^{a}	179.6 ± 6.4^{a}	64.2 ± 3.8^{b}
III	103.6 ± 3.1^{a}	854.2 ± 16.4^{a}	176.3 ± 2.4^{b}	69.4 ± 2.4^{a}
IV	108.3 ± 1.8^{a}	831.36 ± 59.6^{b}	175.7 ± 5.3^{b}	72.6 ± 2.3^{a}
V	83.6 ± 4.9^{c}	602.4 ± 19^{c}	141.6 ± 5.4^{c}	46.5 ± 4.3^{c}
VI	92.7 ± 2.8^{b}	656.1 ± 24.8^{c}	147.7 ± 2.2^{c}	53.4 ± 2.9^{b}
VII	75.8 ± 30.3^{c}	655.3 ± 16.2^{c}	150 ± 3.2^{c}	51.2 ± 0.8^{c}
VIII	92.6 ± 3.8^{b}	648.6 ± 29.2^{c}	150.4 ± 3.1^{c}	54.2 ± 1.6^{b}

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. Aldehyde oxidase (AO), sulfite oxidase (SO), monoamine oxidase (MAO) and xanthine oxidase (XO)

Exposure to crude oil significantly reduced the activities of all measured oxidase enzymes (AO, SO, MAO, XO) in muscle tissue when compared with the control group (Group I), suggesting oxidative stress and mitochondrial dysfunction caused by crude oil pollutants. Reduced AO and XO activities indicate impaired detoxification and redox balance, while decreased MAO activity points to disrupted neurotransmitter metabolism, potentially affecting muscle function (Medda *et al*., 2020). Treatment with *Terminalia catappa* methanol leaf extract restored oxidase enzyme activities in a dose-dependent manner, particularly evident in the aldehyde oxidase (AO) activity across Groups V to VIII. Group V, which received the lowest post-treatment dose, showed a notable improvement in AO activity (83.6 ± 4.9 Units/g tissue) compared to the crude oil-only group (Group II: 103.8 ± 2.1), although still below control levels (Group I: 93.6 ± 2.6). As the extract dose increased in Groups VI and VIII, AO activity further improved (92.7 ± 2.8 and 92.6 ± 3.8, respectively), closely approximating control values, indicating near-complete enzymatic recovery. However, Group VII, despite receiving a mid-range dose, exhibited a slightly lower and more variable AO activity (75.8 ± 30.3), suggesting possible inconsistency in response or absorption at this level.

Interestingly, Group IV, which received the highest dose of the extract during co-treatment, demonstrated significantly elevated AO, MAO, and XO activities (AO: 108.3 ± 1.8) that even surpassed those of the control group. This suggests that *T. catappa* extract may not only reverse crude oil-induced suppression of enzyme activity but also stimulate enzymatic function beyond baseline levels. This stimulatory effect is likely attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, and polyphenols, known for their antioxidant properties and ability to modulate gene expression and enzymatic activity (Mueed *et al*., 2023; Izu *et al*., 2024).

Post-treatment groups showed partial recovery, particularly in SO activity, which increased significantly compared to the crude oil-only group. However, enzyme activities remained lower than in pre-treatment groups, indicating that prophylactic administration of *T. catappa* extract is more effective than post-exposure treatment. Crude oil exposure impairs muscle oxidase enzyme activities, but *T. catappa* leaf extract can restore these activities, particularly when administered preventively. The extract's bioactive compounds may help protect against oxidative damage and improve muscle function under stress (Unuofin and Lebelo, 2020; Canals-Garzón *et al*., 2022; Cec *et al*., 2022).

Conclusion

This study highlights the potential of *Terminalia catappa* leaf litter extract (ALE) in modulating drug-metabolizing oxidase enzymes and protecting against oxidative stress induced by crude oil exposure in rats. ALE significantly enhanced the activities of key oxidases (AO, SO, MAO, and XO) in various tissues—including liver, kidney, heart, lung, and muscle—particularly in rats maintained on a normal diet. In crude oil-exposed rats, ALE partially but significantly restored these enzyme activities, thereby demonstrating its protective effects against crude oil-induced toxicity. The antioxidant properties of ALE, likely due to its phytochemical constituents such as

flavonoids, phenolics, and tannins, contributed to the attenuation of oxidative stress and mitochondrial dysfunction. These properties support the extract's role in detoxification and maintenance of redox balance. Notably, pre-treatment with ALE resulted in more pronounced protective effects than post-treatment, indicating that prophylactic use may be more beneficial.

Importantly, this study also provided a comparative analysis of different extract concentrations, revealing that while all doses exhibited some degree of protection, the 200 mg/kg body weight dose consistently showed optimal efficacy across most tissues. This suggests that 200 mg/kg ALE may be the most effective therapeutic concentration, balancing safety and antioxidant capacity. The findings suggest that *T. catappa* ALE possesses renoprotective, neuroprotective, and cardioprotective properties, making it a promising natural candidate for mitigating environmental toxin-induced oxidative damage. Further studies are recommended to explore its molecular mechanisms and potential for clinical application.

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