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Modulatory Effects of Almond (*Terminalia catappa*) Leaf Extract on Oxidative Stress in Wistar Rats Fed a Crude Oil-Contaminated Diet

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Abstract

This study evaluates the modulatory effects of *Terminalia catappa* (almond) leaf litter extract on oxidative stress markers in Wistar rats exposed to a crude oil-contaminated diet. Oxidative stress, a key driver of toxicity induced by environmental pollutants such as crude oil, was assessed to determine the protective potential of *T. catappa* extract. Thirty male Wistar rats were divided into ten experimental groups (n=3 per group): Group A (control) received a standard diet, while Groups B, C, and D were administered 100, 200, and 300 mg/kg *T. catappa* extract, respectively. Group E was treated with 200 mg/kg Vitamin C as a reference antioxidant. Group F was fed a crude oil-contaminated diet (4.0 mL/100 g feed), while Groups G, H, and I received 100, 200, and 300 mg/kg *T. catappa* extract alongside the contaminated diet. Group J was fed the crude oil-contaminated diet supplemented with 200 mg/kg Vitamin C. After a 14-day acclimatization period, the experiment lasted 28 days. Oxidative stress markers, including malondialdehyde (MDA) levels (a measure of lipid peroxidation) and Vitamin C concentrations, were assessed in the liver, kidney, heart, lungs, muscle, testis, and brain. Results showed that *T. catappa* extract significantly reduced MDA levels and enhanced Vitamin C concentrations in rats exposed to crude oil, comparable to Vitamin C treatment. These findings underscore the potential of *T. catappa* extract as a natural antioxidant in mitigating oxidative stress associated with environmental pollutants.

Keywords: *Terminalia catappa*, oxidative stress, crude oil contamination, antioxidant, malondialdehyde

Introduction

Petroleum, commonly referred to as crude oil, is a naturally occurring yellowish-black liquid found beneath the Earth's surface within geological formations. It originates from the remains of ancient marine organisms, such as algae and zooplankton, which have undergone transformation over millions of years under the influence of heat and pressure (Mohanta *et al*., 2024). Crude oil is a complex mixture of hydrocarbons, including alkanes, cycloalkanes, aromatic hydrocarbons, and compounds containing oxygen, nitrogen, and sulfur. The specific chemical composition of crude oil varies depending on its source, and its properties, such as viscosity and American Petroleum Institute (API) gravity, are influenced by the ratios of these components. Crude oil is classified into different grades based on density and sulfur content. Light, sweet crude oil, with a low sulfur content and high yield of petrol and diesel, is more desirable, whereas heavy, sour crude oil contains higher sulfur levels and requires extensive

processing (Achaw and Danso-Boateng, 2021).

Despite its industrial significance, crude oil poses severe environmental and health risks due to its toxic properties, which depend on the concentration. Exposure to crude oil has been linked to tissue and organ damage in living organisms, primarily through the induction of oxidative stress-a pathological process associated with cellular damage and various diseases (Paithankar *et al*., 2021). Alarmingly, crude oil is sometimes misused for medicinal purposes in regions such as the Niger Delta, where it is consumed for its purported laxative or antidotal properties. This practice has been shown to exacerbate oxidative damage by increasing free radical production, leading to the peroxidation of essential biomolecules like lipids, proteins, and DNA (Juan *et al*., 2021).

The toxic effects of crude oil on vital organs and systems have been extensively documented. Crude oil exposure impairs the functions of the kidney, liver, heart, lungs, testis, brain, and muscle, often through mechanisms involving oxidative stress, inflammation, and direct cellular toxicity. Studies have revealed significant tissue damage, biochemical dysfunction, and long-term health complications in both experimental animals and humans following exposure to hydrocarbons present in crude oil (Kuppusamy *et al*., 2020; Mallah *et al*., 2022).

In response to these health challenges, medicinal plants have gained attention for their potential to mitigate crude oil-induced toxicity. Medicinal plants, a cornerstone of African traditional medicine, are rich in bioactive compounds such as alkaloids, flavonoids, tannins, and essential oils, which possess antioxidant and anti-inflammatory properties (Gogoi *et al*., 2024). For instance, *Vernonia amygdalina* and *Moringa oleifera* have been shown to reduce oxidative stress and ameliorate organ damage in animals exposed to crude oil-contaminated diets (Achuba, 2018; Achuba *et al*., 2016).

Among medicinal plants, *Terminalia catappa*, commonly known as the Indian almond or tropical almond, has garnered scientific interest for its potent antioxidant properties. Native to tropical regions of Asia, Africa, and the Pacific, the tree is a rich source of polyphenols, flavonoids, and tannins, which are crucial for combating oxidative stress (Gupta *et al*., 2021). The leaves, fruits, and seeds of *Terminalia catappa* have demonstrated significant therapeutic potential in addressing oxidative damage and restoring cellular homeostasis. This study aims to evaluate the protective effects of *Terminalia catappa* leaf extract on lipid peroxidation and vitamin C levels in the kidney, heart, lungs, liver, testis, muscle, and brain of Wistar rats exposed to a crude oil-contaminated diet.

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. Thirty (30) healthy male Wistar albino rats weighing between 100 and 120 g were procured from the Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka. The rats were acclimatized for two weeks under standard laboratory conditions and fed growers mash (Top Feed, Premier Feed Mills Co. Ltd, Ibadan, Oyo State) with water provided *ad libitum.*

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 cheesecloth 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 thirty (30) male Wistar albino rats were used for the experiment. The rats were randomly assigned to ten (10) groups, each containing three (3) rats. Prior to the experiment, all animals were acclimatized under standard laboratory conditions for fourteen (14) days. The experimental exposure lasted for twenty-eight (28) days.

The rats were housed in polypropylene cages with paddy husk bedding to provide a comfortable, absorbent, and non-toxic environment that minimizes stress and prevents injuries**.** Paddy husk is commonly used as bedding because it helps control moisture, reduces ammonia buildup, and supports overall animal welfare. The rats were maintained on a diet of grower's mash and water ad libitum to ensure adequate nutrition and hydration throughout the study. The experimental groups were organized as follows:

Table 1: Experimental Grouping and Treatment Protocols

Group	Treatment
I	Control: Received a normal diet and water only.
II	Fed a normal diet and received 100 mg/kg of *T. catappa* leaf extract.
III	Fed a normal diet and received 200 mg/kg of *T. catappa* leaf extract.
IV	Fed a normal diet and received 300 mg/kg of *T. catappa* leaf extract.
V	Fed a normal diet and received 200 mg/kg of vitamin C (standard antioxidant) (Reason for this concentration?).
VI	Fed a crude oil-contaminated diet only (4.0 mL/100 g of diet).

VII	Fed a crude oil-contaminated diet and received 100 mg/kg of *T. catappa* leaf extract.
VIII	Fed a crude oil-contaminated diet and received 200 mg/kg of *T. catappa* leaf extract.
IX	Fed a crude oil-contaminated diet and received 300 mg/kg of *T. catappa* leaf extract.
X	Fed a crude oil-contaminated diet and received 200 mg/kg of vitamin C (standard antioxidant).

The crude oil-contaminated diet was prepared following the method described by Achuba (2019). Dosages of the *T. catappa* leaf extract were determined based on its established LD50 (Iheagwam *et al*., 2021).

The 200 mg/kg concentration of vitamin C was chosen as a standard antioxidant dose based on its proven efficacy in counteracting oxidative stress, its comparability to *Terminalia catappa* extract doses, its role in scavenging reactive oxygen species (ROS), and its established safety in toxicological studies.

Collection of Tissue Samples

At the end of the 28-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 (kidney, intestine, brain, heart, testes, and liver) 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

Catalase (CAT) activity was determined by Aebi (1974). Lipid peroxidation (LPI) was estimated by Nichius and Samuelsson (1968). Superoxide dismutase (SOD) activity was measured by Misra and Fridovich (1972). MnSOD and Cu/Zn SOD activities were determined as described by Crapo *et al*. (1978). Ascorbic acid was estimated by Omaye *et al*. (1979).

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

The moderate classification of MDA activity in the control group (Group I) was based on a comparative assessment of MDA levels across different organs, considering the physiological baseline of lipid peroxidation in normal conditions. The brain exhibited the

lowest MDA activity (24.36 µmol/ml), while the muscle showed the highest (44.34 µmol/ml), as presented in Tables 2a and b. In groups treated with *T. catappa* (100, 200, 300 µg), MDA activity decreased significantly compared to the control, indicating a reduction in oxidative stress. However, slight increases in MDA activity were observed in the muscle and brain, particularly in the group treated with 200 µg of *T. catappa*, where the MDA activity remained comparable to Group III. Vitamin C supplementation also led to a significant reduction in MDA activity, particularly in the muscle (42.06 µmol/ml) and lungs (37.92 µmol/ml), demonstrating its potent antioxidant effect across multiple tissues.

Table 2a: The Effect of MDA (Umol/ml) levels in the Organs of Rats fed with Crude Oil Contaminated Diet (Muscle, Testis, and Brain)

Groups	Muscle	Testis	Brain
I	44.34 ± 2.97^{a}	37.26 ± 2.97^{a}	24.36 ± 0.95^{a}
II (100 mg/kg)	44.04 ± 2.67^{a}	37.00 ± 1.72^{b}	23.56 ± 1.15^{b}
III (200 mg/kg)	42.12 ± 5.88^{b}	36.64 ± 4.38^{c}	21.58 ± 2.38^{b}
IV (300 mg/kg)	42.06 ± 1.52^{ac}	37.92 ± 1.23^{b}	23.57 ± 1.31^{b}
V (Vit C) (200 mg/kg)	$41.51 \pm 2.28^{a,b}$	$35.92 \pm 2.72^{a,b}$	$22.70 \pm 0.86^{b,b}$
VI (CO)	53.77 ± 1.51^{c}	42.45 ± 1.44^{c}	28.21 ± 0.90^{c}
VII (CO + 100)	46.92 ± 2.60^{a}	36.23 ± 4.83^{c}	26.14 ± 2.67^{c}
VIII (CO + 200)	46.43 ± 2.46^{a}	39.08 ± 1.63^{b}	26.76 ± 2.23^{c}
IX (CO + 300)	44.68 ± 3.26^{a}	39.73 ± 1.67^{b}	24.14 ± 2.40^{b}
X (CO + Vit C)	42.69 ± 1.15^{c}	38.41 ± 0.65^{a}	23.38 ± 1.54^{a}

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. CO refers to Crude oil, Vit C stands for Vitamin C, and 100, 200, 300 denote varying doses of *T. catappa* leaf extract.

Exposure to crude oil (Group VI) led to a significant increase in MDA activity across all organs compared to the control group (Group I) and other treatment groups, with the highest levels observed in the muscle (53.77 µmol/ml) and testis (42.45 µmol/ml), indicating substantial oxidative stress due to crude oil toxicity (Table 2a).

When rats were treated with *T. catappa* at doses of 100, 200, and 300 mg/kg alongside

crude oil exposure, MDA activity was significantly reduced across all groups (compared to Group VI). The most notable reductions were observed in the muscle and brain across the groups, particularly at 100 mg/kg and 200 mg/kg, which demonstrated strong protective effects against crude oil-induced oxidative damage.

Table 2b: The Effect of MDA (Umol/ml) levels in the Organs of Rats fed with Crude Oil Contaminated Diet (Liver, Kidney, Heart, and Lungs)

Groups	Liver	Kidney	Heart	Lungs
I	42.08 ± 3.51[a]	40.19 ± 2.85a	43.63 ± 2.46a	29.64 ± 3.62a
II (100 mg/kg)	39.79 ± 3.46a	34.49 ± 3.38b	43.25 ± 2.10a	29.30 ± 1.94b
III (200 mg/kg)	38.19 ± 2.76ab	34.77 ± 2.86a	41.34 ± 5.40b	27.32 ± 2.61b
IV (300 mg/kg)	38.39 ± 2.03b	33.27 ± 3.68b	42.62 ± 4.72b	28.85 ± 1.72b
V (Vit C)	38.18 ± 2.09b	33.07 ± 3.46b	39.68 ± 2.52a	27.48 ± 1.53b
VI (CO)	47.59 ± 2.36b	45.79 ± 1.71c	48.48 ± 4.07b	34.50 ± 1.48b
VII (CO + 100)	43.36 ± 2.05a	41.11 ± 1.17c	48.28 ± 1.27c	31.36 ± 0.92c
VIII (CO + 200)	44.48 ± 3.01b	40.28 ± 2.46a	46.58 ± 2.79a	30.77 ± 1.23c
IX (CO + 300)	42.28 ± 4.06a	37.55 ± 2.55a	42.41 ± 5.20b	30.76 ± 2.50b
X (CO + Vit C)	40.97 ± 1.80c	36.72 ± 2.13a	43.62 ± 3.61a	29.84 ± 0.48c

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. CO refers to Crude oil, Vit C stands for Vitamin C, and 100, 200, 300 denote varying doses of *T. catappa* leaf extract.

Exposure to crude oil (Group VI) resulted in a significant elevation of malondialdehyde (MDA) levels across all examined organs compared to the control group (Group I), indicating heightened oxidative stress (Table 2b). Notably, the liver exhibited an MDA level of 47.59 ± 2.36 µmol/ml, and the heart showed 48.48 ± 4.07 µmol/ml, both surpassing the control group's values of 42.08 ± 3.51 µmol/ml and 43.63 ± 2.46 µmol/ml, respectively.

Administration of *Terminalia catappa* leaf extract at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg in conjunction with crude oil exposure (Groups VII, VIII, and IX) led to a reduction in MDA levels across all organs compared to Group VI. The 300 mg/kg dose (Group IX) was particularly effective in the kidney, reducing MDA levels to 37.55 ± 2.55 µmol/ml from 45.79 ± 1.71 µmol/ml in Group VI. However, this dose was less effective in the heart, achieving an MDA level of 42.41 ± 5.20 µmol/ml compared to 48.48 ± 4.07 µmol/ml in Group VI. Similarly, co-administration of vitamin C with crude oil (Group X) significantly reduced MDA levels

in all organs relative to Group VI, with the liver showing a decrease to 40.97 ± 1.80 µmol/ml and the lungs to 29.84 ± 0.48 µmol/ml, approaching control values.

In the control group (Group I), the liver exhibited the highest vitamin C levels (3.07 ± 0.59 µmol/ml). Administration of *Terminalia catappa* leaf extract at doses of 100, 200, and 300 mg/kg resulted in an increase in vitamin C levels in the liver, likely due to its antioxidant properties enhancing endogenous vitamin C retention or synthesis. These increases were significant when compared to the crude oil-only group (Group VI), which showed a marked reduction in vitamin C levels (1.88 ± 0.27 µmol/ml), indicating oxidative depletion (Table 3a). The highest dose of *T. catappa* (300 µg) resulted in a Vitamin C activity of 3.49 ± 0.45, which was slightly lower than that of the control group but still significantly higher than crude oil exposure alone. Vitamin C supplementation (Group V) also showed a significant improvement in Vitamin C activity (3.44 ± 0.39) in the liver.

Vitamin C levels in the kidney followed a similar trend to those in the liver. The control group (Group I) exhibited the highest levels (3.30 ± 0.64 µmol/ml) (Table 4), while the crude oil-only group (Group VI) showed the lowest levels (1.96 ± 0.17 µmol/ml), indicating significant depletion due to oxidative stress. Treatment with *T. catappa* (100, 200, and 300 mg/kg) resulted in a dose-dependent increase in vitamin C levels. However, since these groups were not directly supplemented with vitamin C, the observed increase suggests that *T. catappa* may enhance endogenous vitamin C retention or reduce its depletion by counteracting oxidative damage. Group III (200 µg dose) showed the highest increase (3.76 ± 0.47), significantly higher than the crude oil group (Group VI). Vitamin C supplementation in Group V (3.70 ± 0.42) also significantly improved Vitamin C activity in the kidney compared to crude oil exposure.

The control group (Group I) had the lowest oxidative stress, as indicated by the highest vitamin C levels in the heart (3.81 ± 0.73) (Table 3a). Exposure to crude oil (Group VI) significantly depleted vitamin C levels (2.34 ± 0.33), suggesting increased oxidative stress. Treatment with *T. catappa* (100, 200, and 300 mg/kg) and vitamin C supplementation (Group V) led to a significant restoration of vitamin C levels, with the 200 mg/kg *T. catappa* group (Group III) showing the highest recovery (4.34 ± 0.55). This suggests that *T. catappa* may enhance antioxidant defense mechanisms and counteract crude oil-induced oxidative damage.

Table 3a: The Effect of *T. catappa* on Vitamin C Activity in the Liver, Kidney, and Heart of Rats Fed with Crude Oil Contaminated Diet

GROUPS	LIVER	KIDNEY	HEART
I	3.07 ± 0.59^{a}	3.30 ± 0.64^{a}	3.81 ± 0.73^{b}

II 100	3.55 ± 0.31^{b}	3.65 ± 0.42^{b}	3.02 ± 2.40^{a}
III 200	3.50 ± 0.44^{b}	3.76 ± 0.47^{b}	4.34 ± 0.55^{b}
IV 300	3.49 ± 0.45^{b}	3.57 ± 0.53^{b}	4.12 ± 0.61^{b}
V Vit. C	3.44 ± 0.39^{b}	3.70 ± 0.42^{b}	4.27 ± 0.48^{b}
VI CO	1.88 ± 0.27^{c}	1.96 ± 0.17^{c}	2.34 ± 0.33^{c}
VII CO + 100	1.99 ± 0.19^{c}	2.14 ± 0.20^{c}	2.50 ± 0.28^{c}
VIII CO + 200	2.19 ± 0.17^{c}	2.36 ± 0.19^{b}	2.72 ± 0.22^{a}
IX CO + 300	2.27 ± 0.11^{c}	2.44 ± 0.12^{b}	2.82 ± 0.14^{a}
X CO + Vit C	2.48 ± 0.16^{b}	2.67 ± 0.17^{b}	2.91 ± 0.12^{a}

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. CO refers to Crude oil, Vit C stands for Vitamin C, and 100, 200, 300 denote varying doses of *T. catappa* leaf extract.

In the lungs, the control group (Group I) exhibited moderate vitamin C levels (2.84 ± 0.55) (Table 3b), indicating a balanced oxidative status. Exposure to crude oil (Group VI) significantly depleted vitamin C levels (1.74 ± 0.25), suggesting increased oxidative stress. Treatment with *T. catappa* at doses of 100, 200, and 300 mg/kg significantly restored vitamin C levels, likely due to its antioxidant properties reducing oxidative damage and preserving endogenous vitamin C. Among the treatment groups, the 100 mg/kg dose (Group VII) showed the greatest improvement (3.13 ± 0.36). This suggests that *T. catappa* may effectively counteract crude oil-induced oxidative stress, potentially offering protection comparable to or even better than vitamin C supplementation. Vitamin C supplementation (Group V) significantly increased vitamin C levels in the lungs (3.18 ± 0.36), similar to the effect observed with the 100 mg/kg dose of *T. catappa* (Group VII). This suggests that *T. catappa* may exert antioxidant effects comparable to vitamin C by preserving endogenous vitamin C levels.

In the muscle, the control group (Group I) exhibited the highest vitamin C activity (3.49 ± 0.67) (Table 3b). Crude oil exposure (Group VI) significantly reduced vitamin C activity (2.00 ± 0.08), indicating increased oxidative stress. Treatment with *T. catappa* at doses of 100, 200, and 300 µg significantly improved vitamin C activity, likely by reducing oxidative damage and preserving endogenous vitamin C levels. The 100 µg dose (Group VII) showed the most pronounced effect, with a significant increase (3.85 ± 0.44), reaching levels comparable to the control. This suggests that *T. catappa* may enhance antioxidant defenses in a manner similar to vitamin C. Vitamin C supplementation (Group V) significantly improved muscle vitamin C activity (3.91 ±

0.44), the highest observed across all groups. This suggests that exogenous vitamin C effectively counteracts oxidative stress and enhances antioxidant defense mechanisms in muscle tissues.

Table 3b: The Effect of *T. catappa* on Vitamin C Activity in the Lungs, Muscle, Testis, and Brain of Rats Fed with Crude Oil Contaminated Diet

GROUPS	LUNGS	MUSCLE	TESTIS	BRAIN
I	2.84 ± 0.55a	3.49 ± 0.67a	3.07 ± 0.59a	3.63 ± 0.70a
II 100	3.13 ± 0.36b	3.85 ± 0.44a	3.39 ± 0.39b	4.01 ± 0.46b
III 200	2.21 ± 1.48a	3.97 ± 0.50a	3.50 ± 0.44b	4.13 ± 0.52b
IV 300	3.07 ± 0.45b	3.77 ± 0.56a	3.32 ± 0.49b	3.94 ± 0.56b
V Vit. C	3.18 ± 0.36b	3.91 ± 0.44a	3.44 ± 0.39b	4.07 ± 0.46b
VI CO	1.74 ± 0.25c	2.00 ± 0.08b	1.88 ± 0.27c	2.22 ± 0.32c
VII CO + 100	1.84 ± 0.17c	2.26 ± 0.21b	1.99 ± 0.19c	2.35 ± 0.22c
VIII CO + 200	2.03 ± 0.16c	2.49 ± 0.20b	2.19 ± 0.17c	2.59 ± 0.21c
IX CO + 300	2.10 ± 0.10b	2.58 ± 0.12b	2.27 ± 0.11b	2.69 ± 0.13b
X CO + Vit C	2.29 ± 0.15a	2.82 ± 0.18a	2.48 ± 0.16b	2.93 ± 0.19b

The result is expressed as mean ± standard deviation. Values with different superscripts are significantly different at $p<0.05$. CO refers to Crude oil, Vit C stands for Vitamin C, and 100, 200, 300 denote varying doses of *T. catappa* leaf extract.

In the testis, the control group (Group I) had the highest Vitamin C activity (3.07 ± 0.59) (Table 5). The crude oil group (Group VI) exhibited significantly lower Vitamin C activity (1.88 ± 0.27). *T. catappa* treatment at doses of 100, 200, and 300 µg significantly increased Vitamin C activity, with the 200 µg dose (Group III) showing the greatest improvement (3.50 ± 0.44), significantly higher than the crude oil-only group. Vitamin C supplementation (Group V) also improved Vitamin C activity in the testis (3.44 ± 0.39).

In the brain, the control group (Group I) displayed the highest Vitamin C activity (3.63 ± 0.70) (Table 5). Crude oil exposure (Group VI) resulted in a significant decrease in Vitamin C activity (2.22 ± 0.32). Treatment with *T. catappa* at doses of 100, 200, and 300 µg significantly improved Vitamin C activity, with the 100 µg dose (Group VII) showing the greatest improvement (4.01 ± 0.46), comparable to Vitamin C supplementation (Group V: 4.07 ± 0.46). The 200 µg dose (Group III) also enhanced Vitamin C activity (4.13 ± 0.52), which was higher than the crude oil-only group.

Discussion

The present study investigated the potential of *T. catappa* and Vitamin C as antioxidants to mitigate oxidative stress induced by crude oil exposure in rats. The evaluation of MDA activity across various organs revealed significant findings that underline the protective effects of these antioxidants.

In the control group (Group I), baseline MDA activity was observed, with the brain exhibiting the lowest MDA levels (24.36 µmol/ml) and the muscle showing the highest levels (44.34 µmol/ml). These results are in line with previous studies that have shown the varying baseline MDA activity across different tissues, depending on the metabolic demands and the oxidative stress levels in those tissues(Menzel *et al*., 2021). Upon exposure to crude oil (Group VI), all organs exhibited significantly increased MDA activity, especially the muscle (53.77 µmol/ml) and lungs (42.45 µmol/ml), indicating substantial oxidative stress induced by the crude oil. This increase in MDA levels aligns with findings from other studies that have demonstrated elevated oxidative stress markers, such as MDA, in tissues of rats exposed to environmental pollutants like crude oil (Attia *et al*., 2024). These findings confirm that crude oil exposure exacerbates oxidative damage, likely due to the presence of reactive oxygen species (ROS) and other free radicals.

When rats were treated with *T. catappa* at doses of 100, 200, and 300 µg, a significant reduction in MDA activity was observed in comparison to the control group. These results suggest that *T. catappa* exhibits a strong antioxidant effect, reducing lipid peroxidation and oxidative stress. The reduction was most noticeable in the muscle and brain tissues, with some doses showing MDA levels comparable to those of the control group. However, a slight increase in MDA activity was noted in the muscle and brain when treated with 200 µg of *T. catappa*, which may reflect varying responses based on tissue-specific antioxidant defense mechanisms. The findings are consistent with previous studies where *T. catappa* was shown to possess antioxidant properties, alleviating oxidative damage induced by environmental toxins like pollutants and heavy metals (Ajibade *et al*., 2022; Adejumo *et al*., 2023).

Vitamin C supplementation also demonstrated significant reductions in MDA levels, particularly in the muscle (42.06 µmol/ml) and lungs (37.92 µmol/ml). This is in agreement with studies that have demonstrated the potent antioxidant activity of Vitamin C in protecting against oxidative stress induced by various environmental toxins, including crude oil (Unsal *et al*., 2020). Vitamin C, a water-soluble antioxidant, plays a crucial role in neutralizing ROS and preventing oxidative damage to cellular structures, which was evident in the significant reduction of MDA levels observed in our study.

In the crude oil-exposed group (Group VI), MDA activity was significantly elevated across all organs, which is indicative of oxidative stress. The protective effect of *T. catappa* and Vitamin C was evident as both antioxidants led to a marked reduction in MDA activity in all organs, with the muscle and brain showing the most pronounced reductions. The results suggest that *T. catappa* and Vitamin C can effectively

counteract the oxidative damage induced by crude oil exposure, supporting their potential as protective agents against environmental pollutants.

In comparison with other studies, the reduction in MDA levels observed in this study aligns with the results from (Bin Jardan *et al*., 2020), who reported significant improvements in oxidative stress markers in rats treated with plant extracts and antioxidants after exposure to pollutants. Furthermore, (Vicidomini *et al*., 2024) reported that antioxidants like Vitamin C and plant-based extracts could mitigate oxidative damage in the kidneys and liver following exposure to industrial pollutants. Similarly, (Vicidomini *et al*., 2024) found that *T. catappa* was effective in reducing lipid peroxidation markers, including MDA, in rats exposed to environmental toxins such as mycotoxins, drugs & pharmaceuticals, and food contaminants. These findings corroborate our study's results, confirming the antioxidant potential of *T. catappa* in mitigating oxidative damage.

In addition to MDA levels, Vitamin C activity was measured in various organs. The control group had the highest Vitamin C activity in the liver (3.07 ± 0.59), but this significantly decreased in the crude oil-exposed group (1.88 ± 0.27). Treatment with *T. catappa* resulted in a dose-dependent increase in Vitamin C activity, particularly at the 300 µg dose, which showed values close to the control group. This result aligns with other studies, such as (Zuñiga-Miranda *et al*., 2024), where antioxidants were found to increase Vitamin C levels in various tissues following exposure to environmental pollutants. The combination of *T. catappa* and Vitamin C significantly improved Vitamin C activity in the kidney and heart, with the 200 µg dose of *T. catappa* showing the highest increases, consistent with findings from (Atanu *et al*., 2021), who observed similar improvements in Vitamin C levels following antioxidant treatment.

Conclusion

This study provides compelling evidence that *T. catappa* and Vitamin C possess significant antioxidant properties that can mitigate oxidative stress induced by crude oil exposure. The reduction in MDA levels and the enhancement of Vitamin C activity in various organs highlight their potential as effective strategies for combating oxidative damage caused by environmental pollutants. These findings are consistent with previous studies that have demonstrated the protective effects of antioxidants in alleviating oxidative stress caused by pollutants and toxins. Future studies should explore the synergistic effects of *T. catappa* and Vitamin C in different animal models and their potential use in protecting human health from environmental oxidative stressors.

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