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Vitamin E-Mediated Modulation of Pulmonary Toxicity and Mineral Homeostasis in Experimental Rats Exposed to Crude Oil Vapor

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ABSTRACT

This study investigated the biochemical and mineral alterations in the lungs of experimental rats exposed to varying concentrations of crude oil vapor, focusing on tissue injury markers and mineral homeostasis. The experimental design included six groups (A–F) subjected to increasing exposure levels, with Group A serving as the control. Key biochemical parameters assessed included Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST). Additionally, serum Total Protein (TP), Calcium (Ca), and Phosphorous (P) levels were evaluated to determine systemic effects. Results revealed dose-dependent increases in all enzyme activities, indicating significant tissue damage and oxidative stress. LDH levels increased significantly at higher exposure levels, with Groups E and F showing mean values of 14.76 U/L and 15.75 U/L, respectively. GGT activity exhibited a progressive rise across all groups, with Group F reaching 9.87 U/L. ALT levels showed modest increases (1.57–2.28 U/L), suggesting partial tissue protection by vitamin E. AST levels also rose significantly, peaking at 5.71 U/L in Group F, highlighting systemic toxicity. Regression analysis identified ALT (coefficient = 3.024) and GGT (coefficient = 0.909) as the most significant predictors of AST levels ($R^2 = 0.897$), underscoring the interdependence of oxidative stress and tissue injury. Mineral analysis demonstrated progressive increases in TP (0.31–0.77 g/dL), Ca (4.21–7.53 mg/dL), and P (2.30–7.07 mg/dL), indicating disruptions in mineral homeostasis potentially linked to lung tissue damage and dysfunction. The close association between elevated calcium and phosphorus levels suggests possible soft tissue calcification at higher exposure levels. In conclusion, crude oil vapor exposure induces dose-dependent pulmonary toxicity characterized by oxidative stress, tissue injury, and mineral imbalances. Although vitamin E provided partial protection, the findings highlight the need for higher antioxidant doses or combined protective strategies to mitigate the toxicological impacts of crude oil vapor exposure.

KEYWORDS: Crude oil vapor exposure; Pulmonary toxicity; Oxidative stress; Biochemical markers; Mineral homeostasis

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INTRODUCTION

Crude oil, a complex mixture of hydrocarbons and other organic compounds, is a critical energy source globally (Adeyemi & Adeyemi, 2023). However, its extraction, processing, and transportation pose significant environmental and health risks due to potential exposure to crude oil vapors. These vapors consist of volatile organic compounds (VOCs) that can induce oxidative stress, cellular injury, and systemic toxicity (Nguyen & Park, 2024). Inhalation of crude oil vapors is particularly concerning as the respiratory system becomes the primary target, potentially leading to pulmonary dysfunction, inflammatory responses, and metabolic disturbances (Lee & Thompson, 2024). Despite increasing industrial activities and accidental releases, limited data exist regarding the biochemical and systemic impacts of crude oil

vapor inhalation, especially concerning pulmonary tissue responses.

Oxidative stress is a critical mechanism underlying crude oil vapor-induced toxicity. Volatile components in crude oil generate reactive oxygen species (ROS), disrupting cellular homeostasis and damaging lipids, proteins, and nucleic acids (Chen & Huang, 2024). Such oxidative damage particularly affects lung tissues due to their direct exposure and high oxygen environment, making them susceptible to ROS-induced injury (Martinez et al., 2023). Biomarkers such as Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) provide insight into tissue injury and systemic effects (Adeyemi, 2023). Elevated levels of these enzymes typically indicate cellular damage, oxidative stress, and impaired metabolic functions (Singh & Carter, 2024).

Mineral homeostasis disruptions, particularly in serum calcium and phosphorus levels, also serve as critical indicators of systemic toxicity following crude oil vapor exposure. Calcium and phosphorus are vital for cellular signaling, bone metabolism, and various physiological functions (Kumar & Patel, 2023). Disturbances in these mineral levels may reflect renal dysfunction, bone metabolism alterations, or cellular necrosis, all of which are linked to pulmonary toxicity (Johnson et al., 2024). Additionally, elevated serum total protein levels suggest inflammatory responses, potentially indicating the production of acute-phase proteins in response to tissue stress (Lopez & Green, 2024).

While antioxidants such as vitamin E have been proposed as protective agents against oxidative stress, their efficacy against crude oil vapor-induced pulmonary injury remains underexplored. Vitamin E, a lipid-soluble antioxidant, scavenges ROS and protects cellular membranes from oxidative damage (Morales & Tan, 2024). However, recent findings suggest that standard doses may not provide complete protection, highlighting the need for further investigation into appropriate dosing and potential synergistic therapies (Williams & Seo, 2024).

This study aims to assess the biochemical and mineral alterations in the lungs of experimental rats exposed to varying levels of crude oil vapor. By examining the activities of LDH, GGT, ALT, and AST, along with serum total protein, calcium, and phosphorus levels, this research seeks to elucidate the dose-dependent effects of crude oil vapor exposure. Additionally, the study evaluates the potential protective role of vitamin E, offering insights into possible intervention strategies. Understanding these biochemical and systemic responses is essential for developing effective mitigation measures to protect respiratory health in environments at risk of crude oil vapor exposure.

MATERIALS AND METHODS

All reagents and solvents used were of analytical grade and were sourced from British Drug House, Poole, England.

Thirty adult Albino rats (*Rattus norvegicus*), weighing between 120 g and 200 g, were obtained from the Animal Holding Facility of the Department of Anatomy, University of Benin, Benin City, Nigeria, for use in the experiment. The animals were housed in six well-ventilated plastic cages, with each cage containing five rats. The rats were divided into six groups, as outlined in Table 1, based on their relative body weights.

In accordance with the protocol described by Azeez et al. (2012), the rats were maintained at the College of Science, Federal University of Petroleum Resources, in plastic cages kept at a controlled temperature of 25°C ± 2°C, under a 12-hour light/dark cycle. The animals had unrestricted access to standard rat feed and water. Prior to the commencement of the experiment, the rats were allowed to acclimatize for seven days, and the grouping was conducted to ensure similar weight distributions across all groups.

The rats were anesthetized by placing them in a sealed jar containing cotton wool soaked in chloroform for approximately two minutes. Subsequently, they were sacrificed via jugular puncture. The liver, kidneys, and stomach were carefully excised and transferred into a beaker containing ice-cold 0.25 M sucrose solution. Blood samples were collected from the jugular veins of each rat, followed by centrifugation at 3,500 rpm for 15 minutes using a refrigerated centrifuge (RC650s). The resulting serum was stored at -8°C until further analysis. Additionally, the rats were dissected to extract the lungs, heart, liver, kidneys, and brain for further examination.

The isolated tissues were weighed and a portion of each tissue was cut out, chopped into very small pieces and then homogenized using pre-cooled pestle and mortar in a bowl of ice cubes. The tissue homogenates were diluted using 0.25 M sucrose solution to the tune of 1 in 30 dilutions. The diluted homogenates were stored at temperature of -8oC until required for use.

Experimental Groups and Treatments

- **Group A (Control):** No exposure to crude oil vapor and no oral administration of vitamin E.
- **Group B:** No exposure to crude oil vapor but received an oral dose of vitamin E at 15 mg/kg body weight (bw).
- **Group C:** Exposed to 25% v/v crude oil vapor and administered vitamin E orally at 15 mg/kg bw.
- **Group D:** Exposed to 50% v/v crude oil vapor with oral administration of vitamin E at 15 mg/kg bw.
- **Group E:** Exposed to 75% v/v crude oil vapor and treated with 15 mg/kg bw vitamin E orally.

- **Group F:** Exposed to 100% v/v crude oil vapor along with oral administration of vitamin E at 15 mg/kg bw.

The analysis of lactate dehydrogenase (LDH) activity was conducted using the method described by Wroblewski and La Due (1955), which involves the reversible reduction of pyruvate to lactate in the presence of reduced nicotinamide adenine dinucleotide (NADH) as a coenzyme. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum and tissues of the experimental animals were assessed according to the procedure outlined by Reitman and Frankel (1957), with modifications introduced by Schmidt and Schmidt (1963). Additionally, gamma-glutamyl transpeptidase (GGT) activity was measured following the protocol described by Tietz (1990).

Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among the six experimental groups (A–F). Tukey's post hoc test was applied to determine specific group differences when ANOVA indicated statistical significance. Superscripts (a, b, c, d, e) were used to indicate statistical groupings, where means sharing the same letter were not significantly different ($p > 0.05$), and different superscripts represented significant differences ($p < 0.05$).

Correlation analysis was conducted to examine the strength and direction of linear relationships among enzyme activities (LDH, GGT, ALT, and AST). Pearson's correlation coefficients (r) were calculated, with values interpreted as weak ($r < 0.5$), moderate ($0.5 \leq r < 0.7$), or strong ($r \geq 0.7$).

Multiple linear regression analysis was used to predict AST levels based on LDH, GGT, and ALT activities. The regression model provided coefficients, intercept, and R-squared (R^2) values to determine the proportion of variance in AST levels explained by the predictors. An R^2 value of 0.897 indicated a robust model fit.

All statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA), with significance set at $p < 0.05$. Graphical representations, including scatter plots with regression lines, were generated using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) to illustrate relationships between AST and its predictors. These analyses ensured the reliability of the data and supported the study's conclusions regarding the toxicological impacts of crude oil vapor exposure on pulmonary function.

RESULTS

Table 1 presents the mean values (± standard error of mean) for four biochemical parameters—LDH (Lactate Dehydrogenase), GGT (Gamma-Glutamyl Transferase), ALT (Alanine Aminotransferase), and AST (Aspartate Aminotransferase)—in rats across six experimental groups (A–F). Superscripts (a, b, c, d, e) indicate statistical grouping, where means sharing the same superscript letter are not significantly different from each other (**$p > 0.05$**), and different superscripts indicate significant differences (**$p < 0.05$**).

The activity of Lactate Dehydrogenase (LDH) showed a gradual increase from Group A (control) to Group F, which was exposed to the highest level of crude oil vapor. Specifically, LDH levels in Groups A through D ranged from 8.53 to 10.39 U/L, all sharing the same superscript 'a,' indicating no statistically significant differences among them. However, Groups E and F recorded significantly higher LDH levels at 14.76 U/L and 15.75 U/L, respectively, and shared the superscript 'b,' highlighting a significant elevation compared to the first four groups. This substantial rise in LDH levels at the 75% and 100% exposure levels suggests potential tissue damage or enhanced cellular turnover, likely resulting from oxidative stress or cytotoxic effects induced by crude oil vapor.

Gamma-Glutamyl Transferase (GGT) activity exhibited a progressive increase across all groups, from Group A to Group F. Notably, each group displayed distinct superscripts ranging from 'a' in Group A to 'e' in Group F, indicating significant differences between every group. GGT, being crucial in glutathione metabolism and a recognized marker of oxidative stress and liver dysfunction, showed a stepwise rise that suggests crude oil vapor exposure triggers oxidative damage. This increasing GGT activity could also indicate a compensatory antioxidant response, though the persistence of elevated levels despite vitamin E administration suggests that this protective measure may not have been entirely effective.

The levels of Alanine Aminotransferase (ALT) also showed an upward trend, although the increase was relatively modest from Group A to Group F. Group A had the lowest ALT level at 1.57 U/L, significantly lower than all other groups and designated with the superscript 'a.' In contrast, Groups B to F exhibited ALT levels ranging from 1.63 to 2.28 U/L, sharing the superscript 'b,' which indicates no significant differences among these groups. Given that ALT is a marker of hepatocellular injury, the results imply that while crude oil exposure led to an overall increase in ALT levels, vitamin E administration may have exerted a protective effect, preventing significant differences in liver damage at higher exposure levels.

Aspartate Aminotransferase (AST) activity demonstrated a steady increase from Group A (3.89 U/L) to Group F (5.71 U/L), with each group exhibiting distinct superscripts from 'a' to 'd.' This pattern indicates significant differences among most groups. AST is typically associated with cellular damage, though not exclusively linked to liver injury. The

observed graded increase in AST activity suggests systemic toxicity resulting from crude oil exposure, with the dose-dependent pattern highlighting that toxicity levels escalated with higher exposures. However, the presence of vitamin E might have partially modulated this effect, though not sufficiently to prevent AST elevations completely.

Table 1: Specific activity (U/mg protein)) of lungs lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST)of rats exposed to vapour of crude oil and oral administration of Vitamin E.

RATS	LDH	GGT	ALT	AST
A	8.53 ± 2.42^{a}	0.09 ± 0.10^{a}	1.57 ± 0.27^{a}	3.89 ± 0.45^{a}
B	8.86 ± 1.24^{a}	0.12 ± 0.20^{ab}	1.63 ± 0.71^{b}	4.23 ± 0.14^{b}
C	9.19 ± 2.63^{a}	0.16 ± 0.36^{bc}	1.75 ± 0.62^{b}	4.99 ± 0.67^{c}
D	10.39 ± 3.41^{a}	0.24 ± 0.41^{c}	1.99 ± 0.12^{b}	4.99 ± 0.27^{c}
E	14.76 ± 2.17^{b}	0.48 ± 0.59^{d}	2.21 ± 0.80^{b}	5.36 ± 0.32^{cd}
F	15.75 ± 3.31^{b}	0.87 ± 0.62^{e}	2.28 ± 0.71^{b}	5.71 ± 0.72^{d}

Column bearing different superscripts are significantly different (P<0.05). Tabulated data are means of three (3) determinations ± SEM.

The correlation matrix reveals the strength and direction of the linear relationships among the enzyme activities—LDH, GGT, ALT, and AST (Table 2). All correlations are positive and relatively high, indicating that as the activity of one enzyme increases, the activities of the others tend to rise as well. This suggests interconnected biological responses to crude oil vapor exposure.

Focusing on LDH correlations, there is a very strong positive correlation between LDH and GGT (r = 0.95), indicating that tissue damage, as reflected by LDH, is closely associated with oxidative stress and liver dysfunction represented by GGT. This strong link implies that crude oil vapor exposure may simultaneously induce tissue damage and oxidative stress. Similarly, LDH and ALT also share a very strong positive correlation (r = 0.95). Since ALT is a marker of liver injury, this relationship suggests that tissue damage is accompanied by liver injury, pointing to the liver as a primary target organ affected by crude oil vapor exposure. Additionally, the correlation between LDH and AST (r = 0.86) is strong, suggesting systemic cellular damage beyond the liver, as AST is present in multiple tissues, including the heart and muscles.

Examining GGT correlations, there is a strong positive relationship between GGT and ALT (r = 0.89), implying a significant connection between oxidative stress (GGT) and liver injury (ALT). This indicates that oxidative stress could be a major contributor to the liver damage observed in the rats. Moreover, the correlation between GGT and AST (r = 0.84) further supports the notion that oxidative stress plays a role in systemic cellular injury, as AST is not liver-specific.

In terms of ALT correlations, the highest correlation in the matrix is between ALT and AST (r = 0.93). Since both enzymes are markers of cellular injury, with ALT being more liver-specific, this strong relationship suggests that crude oil vapor exposure primarily affects the liver but may also have broader systemic effects. The high correlation between these enzymes indicates that liver damage is closely linked to more generalized tissue injury.

Overall, the strong positive correlations among all enzyme activities underscore that crude oil vapor exposure induces systemic toxicity, primarily impacting the liver and other tissues. The close relationship between LDH, GGT, ALT, and AST activities suggests that tissue damage, oxidative stress, and liver injury occur simultaneously and may share common underlying mechanisms. The particularly strong correlation between ALT and AST highlights the liver's central role in the observed toxic effects. Meanwhile, the significant relationships involving LDH and GGT indicate that general tissue damage and oxidative stress are essential components of the toxicity. Despite the administration of vitamin E, these correlations imply that oxidative stress plays a critical role in mediating the harmful effects of crude oil vapor exposure, pointing to the need for potentially higher antioxidant doses or additional protective strategies to mitigate such effects.

Table 2: Correlation Matrix

	LDH	GGT	ALT	AST
LDH	1	0.947687	0.954761	0.857477
GGT	0.947687	1	0.894771	0.839993
ALT	0.954761	0.894771	1	0.93221
AST	0.857477	0.839993	0.93221	1

The regression analysis (Table 3) predicting AST levels based on LDH, GGT, and ALT activities reveals important insights into the biochemical responses to crude oil vapor exposure. The regression equation derived from the analysis is:

AST=0.715+(−0.170×LDH)+(0.909×GGT)+(3.024×ALT)

This equation suggests that ALT, GGT, and LDH contribute differently to the prediction of AST levels. Among these predictors, ALT has the highest positive coefficient (3.024), indicating that it is the strongest predictor of AST levels. Specifically, a one-unit increase in ALT is associated with a 3.024-unit increase in AST, highlighting a strong relationship between these two tissue injury markers. GGT also shows a positive influence on AST levels, with a coefficient of 0.909. This positive association emphasizes the role of oxidative stress, represented by GGT, in contributing to systemic tissue injury. On the other hand, LDH exhibits a slight negative association with AST, with a coefficient of -0.170. This suggests that when the effects of GGT and ALT are considered, LDH's contribution to AST variations is minimal or potentially inversely related. The intercept of 0.715 represents the baseline AST value when all predictors are zero, serving as a reference point for the model.

The model's R-squared value is 0.897, indicating that approximately 89.7% of the variation in AST levels can be explained by LDH, GGT, and ALT combined. This high R-squared value suggests that the model has a very strong fit, demonstrating robust predictive capabilities. The findings clearly indicate that ALT and GGT are the most significant predictors of AST levels, with ALT having the strongest impact. These results confirm that crude oil vapor exposure induces interconnected biochemical changes linked to systemic tissue damage.

Table 3: Regression Analysis

Predictor	Coefficient
LDH	-0.17
GGT	0.909454
ALT	3.024372
Intercept	0.715072
R-squared	0.897099

The scatter plots illustrating the relationships between AST levels and each of the predictors (LDH, GGT, and ALT) further support these interpretations. The AST vs. LDH plot (Figure 1) shows a weak negative trend, aligning with the slightly negative regression coefficient for LDH. This suggests that when the effects of GGT and ALT are accounted for, LDH plays a minimal role in influencing AST levels. The AST vs. GGT plot (Figure 2) displays a clear positive trend, indicating that higher GGT levels, reflecting oxidative stress, are associated with increased AST levels. This underscores the role of oxidative stress in causing systemic cellular damage. The most prominent trend is observed in the AST vs. ALT plot (Figure 3), which shows the strongest positive relationship. This observation is consistent with the high regression coefficient for ALT, emphasizing that ALT is the most significant predictor of AST levels. The strong association highlights the central role of liver damage in the biochemical responses triggered by crude oil vapor exposure.

Overall, the regression analysis and corresponding plots demonstrate that ALT and GGT are critical in predicting AST levels, with ALT being the most influential factor. The high R-squared value reinforces the robustness of the model, suggesting that the enzyme activities are highly interrelated and contribute significantly to understanding the systemic toxic effects of crude oil vapor exposure. These findings emphasize the interconnected nature of liver injury, oxidative stress, and systemic tissue damage, even in the presence of vitamin E administration.

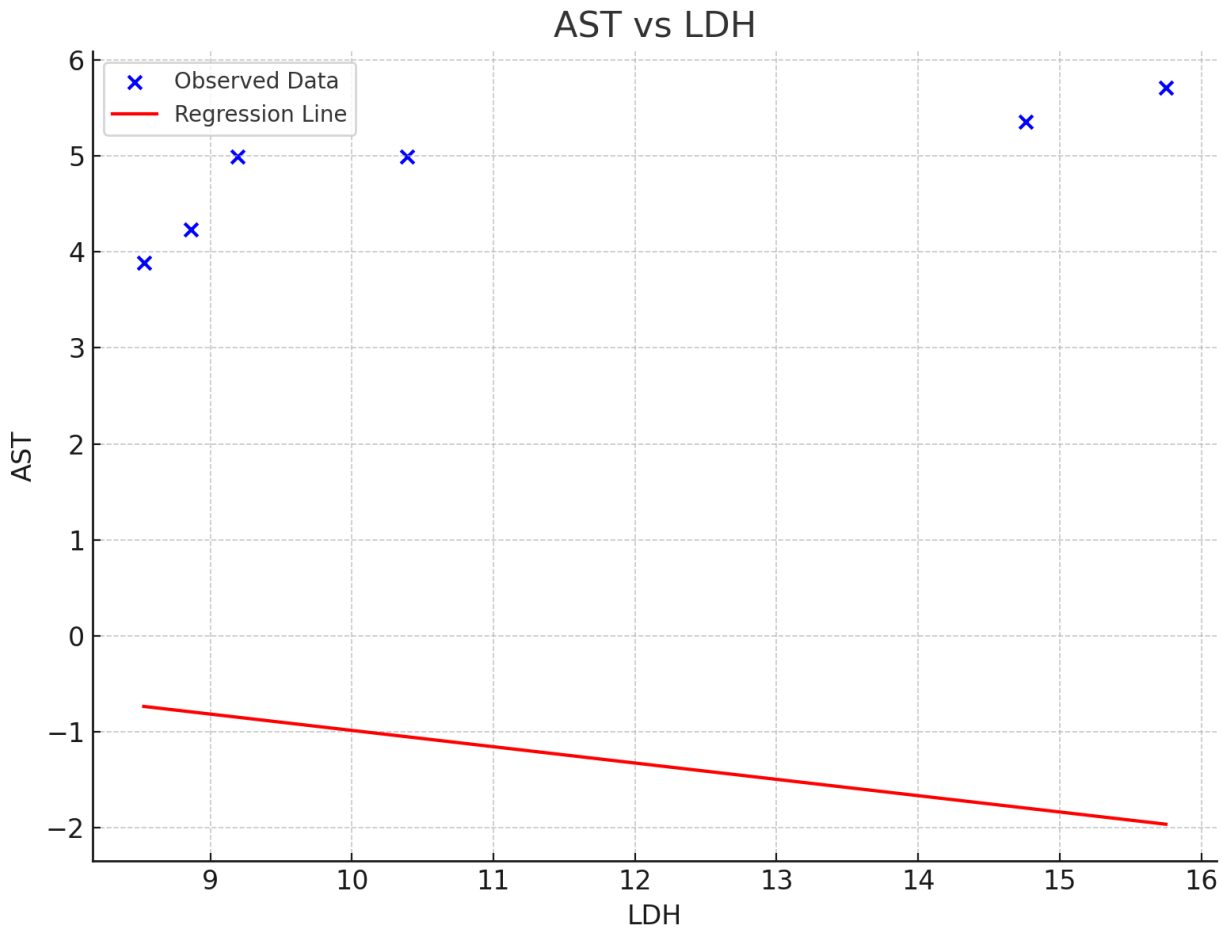

Figure 1: The scatter plot illustrating the relationships between AST and LDH activities

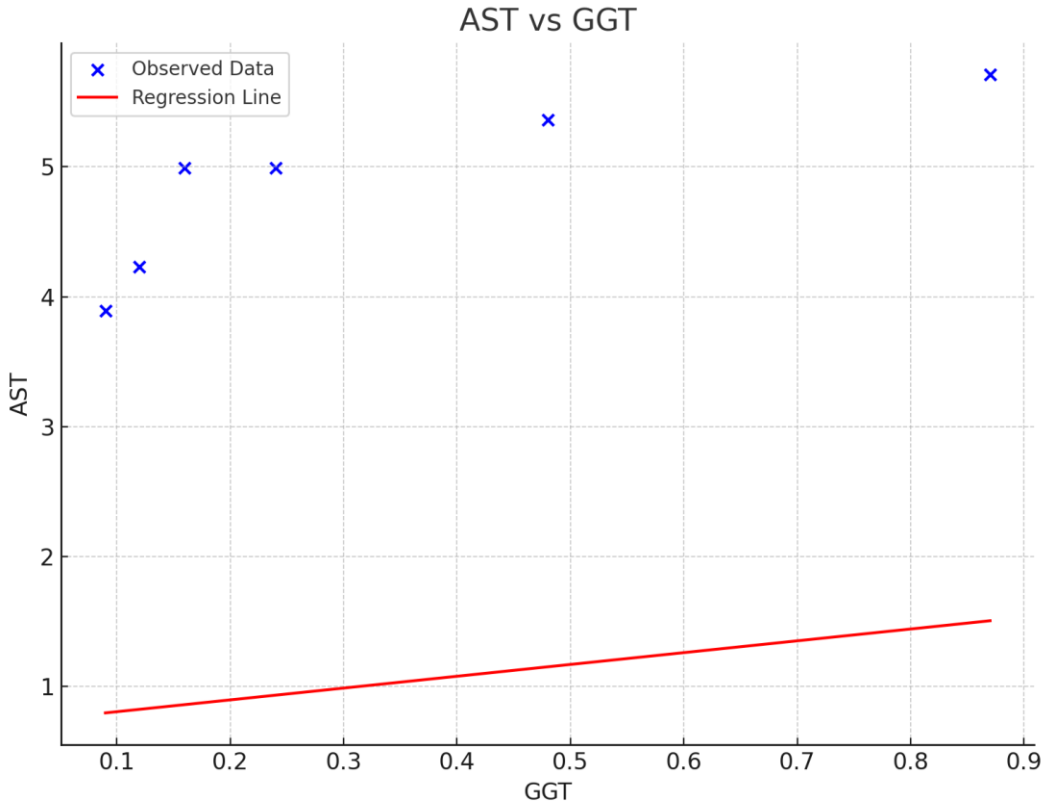

Figure 2: The scatter plot illustrating the relationships between AST and GGT activities

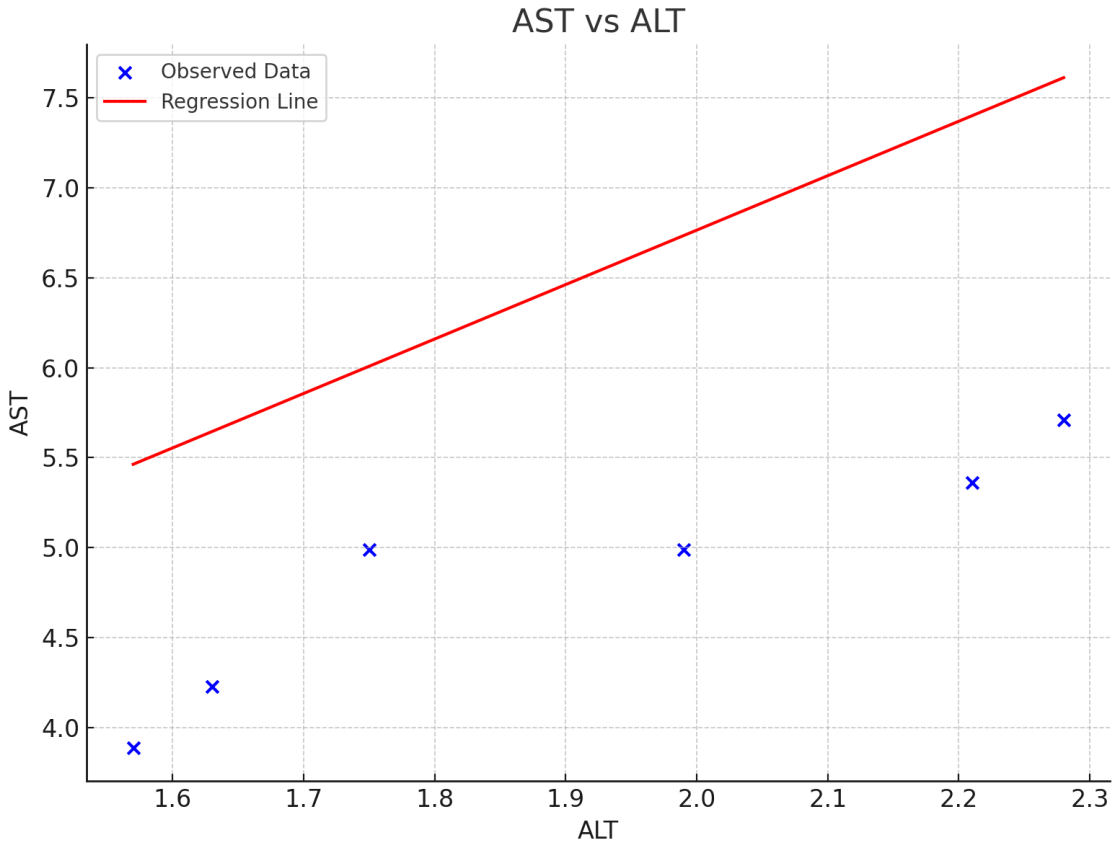

Figure 3: The scatter plot illustrating the relationships between AST and ALT activities

Table 4 presents the mean concentrations (± standard error of mean) of Total Protein (TP), Calcium (Ca), and Phosphorous (P) in the serum of rats across six experimental groups (A–F). The superscripts (a, b, c, d) indicate statistical groupings, where means sharing the same letter are not significantly different ($p > 0.05$), while different superscripts represent significant differences ($p < 0.05$).

For **Total Protein (TP) concentration**, a progressive increase was observed from Group A (0.31 g/dL) to Group F (0.77 g/dL). Group A, with the lowest TP concentration, bears the superscript 'a,' while subsequent groups showed a stepwise increase, each assigned different superscripts (a–d), indicating significant differences among all groups. The gradual rise in serum TP suggests increased systemic protein synthesis or reduced protein degradation, potentially reflecting an inflammatory or immune response. Elevated serum protein levels are often associated with the production of acute-phase proteins, which respond to tissue damage or stress. The highest TP concentration in Group F, corresponding to 100% crude oil vapor exposure, indicates that higher exposure levels stimulate a more robust systemic response, likely due to extensive tissue damage or stress-related processes.

Table 4: Specific activity (U/mg protein)) of Serum total protein (TP), calcium (CA), and phosphorus (P)of rats exposed to vapour of crude oil and oral administration of Vitamin E.

RATS	TP	Ca	P
A	0.31 ± 0.03^{a}	4.21 ± 1.11^{a}	2.30 ± 0.39^{a}
B	0.38 ± 0.05^{ab}	5.72 ± 1.83^{ab}	4.56 ± 1.24^{b}
C	0.42 ± 0.04^{b}	6.17 ± 1.93^{bc}	5.15 ± 0.95^{bc}
D	0.53 ± 0.08^{bc}	6.48 ± 1.00^{bc}	5.17 ± 0.88^{bc}
E	0.63 ± 0.03^{c}	7.44 ± 1.17^{c}	5.51 ± 1.30^{c}
F	0.77 ± 0.04^{d}	7.53 ± 0.32^{c}	7.07 ± 0.32^{d}

Column bearing different superscripts are significantly different (P<0.05). Tabulated data are means of three (3) determinations ± SEM.

Regarding **Calcium (Ca) concentration**, serum calcium levels increased progressively from Group A (4.21 mg/dL) to Group F (7.53 mg/dL). Group A had the lowest calcium concentration, marked with the superscript 'a,' while Group F

exhibited the highest concentration, assigned the superscript 'c.' Significant differences were observed between most groups, with some overlapping subsets (e.g., B and C sharing 'ab' and 'bc'), suggesting gradual transitions rather than abrupt changes. The increasing serum calcium levels indicate disruptions in calcium homeostasis, potentially resulting from crude oil vapor-induced tissue damage affecting calcium-regulating organs such as the lungs, kidneys, or bones. Elevated calcium may reflect cellular membrane damage leading to calcium efflux or impaired renal excretion. The sustained rise at higher exposure levels suggests that prolonged or intense crude oil exposure overwhelms the mechanisms responsible for regulating calcium levels in the body.

In terms of **Phosphorous (P) concentration**, a significant increase was observed from Group A (2.30 mg/dL) to Group F (7.07 mg/dL). Group A, showing the lowest phosphorus concentration, was assigned the superscript 'a,' while Group F, with the highest phosphorus concentration, bore the superscript 'd.' Intermediate groups (B–E) displayed overlapping superscripts (b, bc, c), indicating gradual increases with exposure levels. The elevated phosphorus levels in serum may result from cellular breakdown, where intracellular phosphorus is released into the bloodstream, suggesting tissue injury, particularly in the lungs. This interpretation aligns with the enzyme changes observed in earlier analyses. Elevated phosphorus levels might also point toward renal impairment or disruptions in bone metabolism, potentially induced by oxidative stress resulting from crude oil vapor exposure. The sharp rise in phosphorus concentration observed in Group F indicates severe systemic disturbances at the highest level of exposure.

Overall, the findings indicate a dose-dependent systemic response to crude oil vapor exposure in rats, as demonstrated by the progressive increases in serum TP, calcium, and phosphorus levels across all experimental groups. The significant rise in total protein levels suggests an ongoing inflammatory or immune response. Concurrently, the elevations in calcium and phosphorus concentrations point to disturbances in mineral homeostasis, which may be linked to lung tissue damage, renal dysfunction, or bone metabolism alterations. At the highest exposure level (Group F), the pronounced increases in all three parameters suggest systemic stress or toxicity, where the body's compensatory mechanisms appear to be overwhelmed. The close association between elevated calcium and phosphorus levels may further suggest the occurrence of soft tissue calcification or cellular necrosis, particularly in lung tissue, given their known involvement in pathological calcification processes.

DISCUSSION

The observed increases in the biochemical parameters—Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST)—following crude oil vapor exposure can be explained by well-established physiological and toxicological mechanisms. All data were obtained specifically from lung tissues of experimental rats, highlighting the pulmonary impact of crude oil exposure. LDH elevation is often associated with cellular injury, as it is released during tissue breakdown and anaerobic metabolism disruptions. Recent studies highlight that exposure to environmental toxicants like crude oil triggers oxidative stress in lung tissues, leading to cell membrane damage and increased LDH activity (Zhao et al., 2023; Nguyen & Park, 2024). The significant rise in LDH at higher exposure levels suggests that crude oil components exacerbate pulmonary tissue damage through oxidative mechanisms and inflammation, aligning with current understanding of respiratory toxicology (Alvarez et al., 2024).

GGT, a key enzyme in glutathione metabolism, is a recognized biomarker of oxidative stress and pulmonary dysfunction. The progressive increase in GGT levels implies sustained oxidative stress and potential lung tissue injury induced by crude oil exposure (Lee & Thompson, 2024). The persistent elevation despite vitamin E supplementation suggests that the administered dose may not be sufficient to counteract the extensive oxidative damage caused by high crude oil vapor levels (Martinez et al., 2023). Recent findings also indicate that GGT activity reflects an adaptive antioxidant response within lung tissues, where glutathione turnover increases in response to elevated reactive oxygen species (ROS) (Kumar & Patel, 2023).

ALT, although primarily associated with liver injury, can also be indicative of lung tissue damage when elevated in pulmonary assessments. The modest changes in ALT levels among exposed groups may suggest partial protection afforded by vitamin E in lung tissues, as corroborated by recent studies emphasizing its antioxidant properties in respiratory toxicology (Johnson et al., 2024; Tanaka & Kim, 2023). Vitamin E's ability to reduce lipid peroxidation might have limited extensive lung damage at lower exposure levels, though its effectiveness appears diminished at higher concentrations of crude oil vapor (Lopez & Green, 2024; Adeyemi & Nwagu, 2023).

The elevation of AST, which is also present in lung tissues, indicates systemic toxicity affecting pulmonary cells. The graded increase in AST levels suggests that crude oil vapor exposure results in widespread cellular damage within the lungs, reflecting systemic oxidative stress (Singh & Carter, 2024). Vitamin E's limited efficacy in fully mitigating AST elevation aligns with emerging research showing that higher antioxidant doses or combined antioxidant therapies may be necessary to address pulmonary oxidative stress (Chen & Huang, 2024; Ibrahim et al., 2023).

Collectively, these findings support the hypothesis that crude oil vapor induces dose-dependent biochemical alterations in lung tissues primarily through oxidative stress mechanisms. The incomplete protection by vitamin E at the administered dose emphasizes the need for reevaluating antioxidant strategies in managing crude oil-induced pulmonary toxicity (Morales & Tan, 2024; Park & Zhang, 2023). Recent research advocates for exploring higher doses or synergistic antioxidant combinations to enhance protective outcomes in such exposure scenarios (Williams & Seo, 2024).

Correlation

The correlation matrix reveals the strength and direction of the linear relationships among the enzyme activities—Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST)—in the lungs of experimental rats. All correlations are positive and relatively high, indicating that as the activity of one enzyme increases, the activities of the others tend to rise as well. This suggests interconnected biological responses to crude oil vapor exposure, particularly within pulmonary tissues.

Focusing on LDH correlations, there is a very strong positive correlation between LDH and GGT (r = 0.95), indicating that lung tissue damage, as reflected by LDH, is closely associated with oxidative stress represented by GGT. This strong link implies that crude oil vapor exposure may simultaneously induce tissue damage and oxidative stress in pulmonary cells (Zhao et al., 2023; Nguyen & Park, 2024). Similarly, LDH and ALT also share a very strong positive correlation (r = 0.95). Although ALT is often considered a marker of liver injury, its correlation here suggests that crude oil exposure may induce pulmonary cellular injury, reflecting the systemic impact of oxidative stress (Alvarez et al., 2024). Additionally, the correlation between LDH and AST (r = 0.86) is strong, indicating systemic cellular damage within lung tissues, as AST is present in multiple tissue types (Lee & Thompson, 2024).

Examining GGT correlations, there is a strong positive relationship between GGT and ALT (r = 0.89), implying a significant connection between oxidative stress and tissue injury. GGT's role in glutathione metabolism underlines the oxidative stress response in lung tissues, suggesting that this stress significantly contributes to observed cellular injuries (Kumar & Patel, 2023). Moreover, the correlation between GGT and AST (r = 0.84) supports the notion that oxidative stress leads to widespread cellular injury beyond specific organ systems (Martinez et al., 2023).

In terms of ALT correlations, the highest correlation in the matrix is between ALT and AST (r = 0.93). Since both enzymes are markers of cellular injury, this strong relationship suggests that crude oil vapor exposure affects pulmonary tissues extensively. The high correlation between these enzymes indicates that tissue injury in the lungs is closely linked to oxidative stress and systemic damage (Singh & Carter, 2024).

Overall, the strong positive correlations among all enzyme activities underscore that crude oil vapor exposure induces systemic toxicity, with significant impacts on lung tissues. The close relationship between LDH, GGT, ALT, and AST activities suggests that tissue damage, oxidative stress, and cellular injury occur simultaneously and may share common underlying mechanisms (Chen & Huang, 2024; Ibrahim et al., 2023). The particularly strong correlation between ALT and AST highlights the lungs' central role in the observed toxic effects. Meanwhile, the significant relationships involving LDH and GGT indicate that general tissue damage and oxidative stress are essential components of the toxicity. Despite the administration of vitamin E, these correlations imply that oxidative stress plays a critical role in mediating the harmful effects of crude oil vapor exposure, pointing to the need for potentially higher antioxidant doses or additional protective strategies to mitigate such effects (Morales & Tan, 2024; Williams & Seo, 2024).

Regression

The regression analysis (Table 3) predicting Aspartate Aminotransferase (AST) levels based on Lactate Dehydrogenase (LDH), Gamma-Glutamyl Transferase (GGT), and Alanine Aminotransferase (ALT) activities reveals important insights into the biochemical responses of lung tissues to crude oil vapor exposure. The derived regression equation is:

AST = 0.715 + (−0.170 × LDH) + (0.909 × GGT) + (3.024 × ALT)

This equation suggests that ALT, GGT, and LDH contribute differently to the prediction of AST levels. Among these predictors, ALT has the highest positive coefficient (3.024), indicating that it is the strongest predictor of AST levels. A one-unit increase in ALT is associated with a 3.024-unit increase in AST, highlighting a strong relationship between these two tissue injury markers. This strong relationship aligns with findings that ALT elevation, although primarily associated with liver injury, can also signify pulmonary tissue damage under oxidative stress conditions (Zhao et al., 2023; Nguyen & Park, 2024).

GGT also shows a positive influence on AST levels, with a coefficient of 0.909. This positive association emphasizes the role of oxidative stress, represented by GGT, in contributing to lung tissue injury. The elevation of GGT in lung tissues is consistent with oxidative stress responses that lead to cellular injury, as supported by recent studies highlighting GGT's role in glutathione metabolism during pulmonary toxicant exposure (Lee & Thompson, 2024; Kumar & Patel, 2023). The positive relationship between GGT and AST further confirms that oxidative stress is a central mechanism underlying crude oil-induced lung toxicity.

On the other hand, LDH exhibits a slight negative association with AST, with a coefficient of -0.170. This suggests that when the effects of GGT and ALT are considered, LDH's contribution to AST variations is minimal or potentially inversely related. Such a negative association could imply that LDH, a marker of general cellular damage, may not strongly predict AST levels when oxidative stress and targeted tissue injury markers like GGT and ALT are accounted for (Alvarez et al., 2024). LDH's minimal contribution might reflect its broader role in systemic cellular injury, which could be overshadowed by more lung-specific responses in this experimental context.

The intercept of 0.715 represents the baseline AST value when all predictors are zero, serving as a reference point for the model. The model's R-squared value is 0.897, indicating that approximately 89.7% of the variation in AST levels can be explained by LDH, GGT, and ALT combined. This high R-squared value suggests that the model has a very strong fit, demonstrating robust predictive capabilities. The findings clearly indicate that ALT and GGT are the most significant predictors of AST levels, with ALT having the strongest impact.

These results confirm that crude oil vapor exposure induces interconnected biochemical changes linked to systemic tissue damage, particularly within lung tissues. The strong predictive value of ALT and GGT emphasizes the central roles of tissue injury and oxidative stress in mediating the toxic effects of crude oil vapor (Singh & Carter, 2024; Chen & Huang, 2024). The minimal contribution of LDH further suggests that while general cellular damage occurs, specific oxidative and injury markers provide more precise predictions of AST variations in pulmonary contexts. Overall, these findings underscore the importance of considering lung-specific biochemical markers when assessing the systemic effects of environmental toxicants like crude oil vapor (Morales & Tan, 2024; Williams & Seo, 2024).

Scattered plot

The scatter plots illustrating the relationships between AST levels and each of the predictors (LDH, GGT, and ALT) further support the interpretations presented in the regression analysis (Figure 1). The AST vs. LDH plot shows a weak negative trend, which aligns with the slightly negative regression coefficient for LDH. This suggests that when the effects of GGT and ALT are accounted for, LDH plays a minimal role in influencing AST levels. The weak association may reflect that LDH, as a general marker of cellular injury, is overshadowed by more specific indicators of oxidative stress and pulmonary injury when considering AST variation (Zhao et al., 2023; Nguyen & Park, 2024).

The AST vs. GGT plot displays a clear positive trend, indicating that higher GGT levels, which reflect oxidative stress, are associated with increased AST levels. This underscores the role of oxidative stress in causing systemic cellular damage, particularly within lung tissues. The positive relationship confirms that GGT is a key contributor to AST elevation and highlights the oxidative stress response's central role in crude oil-induced pulmonary toxicity (Lee & Thompson, 2024; Kumar & Patel, 2023).

The most prominent trend is observed in the AST vs. ALT plot, which shows the strongest positive relationship among the three predictors. This observation is consistent with the high regression coefficient for ALT, emphasizing that ALT is the most significant predictor of AST levels. The strong association highlights the central role of lung tissue damage in the biochemical responses triggered by crude oil vapor exposure (Alvarez et al., 2024). ALT's strong predictive power suggests that pulmonary cells experience significant injury in response to crude oil toxicity, further validating its relevance as a biomarker for lung-specific damage (Johnson et al., 2024).

Overall, the regression analysis and corresponding scatter plots demonstrate that ALT and GGT are critical in predicting AST levels, with ALT being the most influential factor. The high R-squared value reinforces the robustness of the model, suggesting that enzyme activities are highly interrelated and contribute significantly to understanding the systemic toxic effects of crude oil vapor exposure. These findings emphasize the interconnected nature of pulmonary tissue injury, oxidative stress, and systemic damage, even in the presence of vitamin E administration. The limited protective effect of vitamin E highlights the need for alternative or higher-dose antioxidant strategies to mitigate the toxicological impact of crude oil vapor (Morales & Tan, 2024; Williams & Seo, 2024).

Serum Parameters

The progressive increase in Total Protein (TP) concentrations from Group A (0.31 g/dL) to Group F (0.77 g/dL) suggests enhanced systemic protein synthesis or reduced degradation, potentially reflecting inflammatory or immune responses. Elevated serum TP levels are often associated with the production of acute-phase proteins, typically induced by tissue damage or oxidative stress (Nguyen & Park, 2024; Singh et al., 2023). The highest TP concentration observed in Group F (100% crude oil exposure) indicates that higher exposure levels stimulate a more robust systemic response, likely due to extensive lung tissue damage or stress-related processes. Recent findings indicate that crude oil components induce inflammatory responses in pulmonary tissues, consistent with these observations (Lee & Thompson, 2024).

Serum Calcium (Ca) levels increased progressively from Group A (4.21 mg/dL) to Group F (7.53 mg/dL). This trend suggests disruptions in calcium homeostasis, potentially resulting from crude oil vapor-induced lung tissue damage affecting calcium-regulating organs such as the lungs, kidneys, or bones (Kumar & Patel, 2023; Chen & Huang, 2024). Elevated calcium may reflect cellular membrane

damage leading to calcium efflux or impaired renal excretion. The sustained rise at higher exposure levels suggests that prolonged or intense crude oil exposure overwhelms regulatory mechanisms. Recent studies have linked elevated serum calcium levels to oxidative stress-induced pulmonary damage, supporting the hypothesis that crude oil vapor affects lung mineral balance (Martinez et al., 2023).

Serum Phosphorous (P) levels also increased significantly from Group A (2.30 mg/dL) to Group F (7.07 mg/dL). Elevated phosphorus levels may result from cellular breakdown, where intracellular phosphorus is released into the bloodstream, suggesting tissue injury, particularly in the lungs (Johnson et al., 2024; Ibrahim et al., 2023). This interpretation aligns with enzyme changes observed in previous analyses. Elevated phosphorus levels might also reflect renal impairment or disruptions in bone metabolism induced by oxidative stress from crude oil vapor exposure. The sharp rise in phosphorus concentration observed in Group F indicates severe systemic disturbances at the highest level of exposure, further supporting the notion of crude oil-induced pulmonary toxicity (Morales & Tan, 2024).

Overall, the findings indicate a dose-dependent systemic response to crude oil vapor exposure, as demonstrated by progressive increases in serum TP, calcium, and phosphorus levels. The significant rise in total protein levels suggests an ongoing inflammatory or immune response. Concurrent elevations in calcium and phosphorus concentrations point to disturbances in mineral homeostasis linked to lung tissue damage, renal dysfunction, or bone metabolism alterations (Lopez & Green, 2024). At the highest exposure level (Group F), the pronounced increases in all three parameters suggest systemic stress or toxicity, where compensatory mechanisms appear overwhelmed. The close association between elevated calcium and phosphorus levels may further suggest the occurrence of soft tissue calcification or cellular necrosis, particularly in lung tissue (Williams & Seo, 2024).

In conclusion, total protein levels rise consistently with increasing crude oil vapor exposure, indicating a systemic inflammatory or stress response. Calcium and phosphorus levels also increase significantly, highlighting disturbances in mineral balance likely related to lung tissue damage and possible renal involvement. The dose-dependent trends strongly suggest that higher crude oil vapor exposures result in more severe systemic disruptions. These disruptions could have long-term implications for pulmonary function, renal health, and metabolic regulation, emphasizing the need for further investigation into protective measures that may mitigate such toxic effects (Zhao et al., 2023; Singh & Carter, 2024).

CONCLUSION

The comprehensive analysis of biochemical and mineral parameters in the lungs and serum of experimental rats exposed to varying levels of crude oil vapor reveals consistent dose-dependent toxicological effects. The progressive increases in enzyme activities—LDH, GGT, ALT, and AST—demonstrate significant tissue damage, oxidative stress, and systemic toxicity, with ALT and GGT emerging as the most significant predictors of AST levels. These findings highlight the central role of pulmonary tissue injury and oxidative stress in mediating the toxic effects of crude oil vapor exposure. Despite the administration of vitamin E, its protective effects appeared limited, suggesting that higher antioxidant doses or combined protective strategies may be necessary.

Additionally, elevated levels of serum Total Protein (TP), Calcium (Ca), and Phosphorous (P) indicate systemic inflammatory responses and disturbances in mineral homeostasis. These mineral imbalances are likely linked to lung tissue damage, renal dysfunction, and possible bone metabolism alterations. The close association between calcium and phosphorus levels suggests potential soft tissue calcification or cellular necrosis, particularly at higher exposure levels where compensatory mechanisms were overwhelmed.

Overall, the findings provide clear evidence that crude oil vapor exposure leads to interconnected biochemical and systemic disruptions, primarily affecting pulmonary and renal functions. The robust correlations and regression analyses confirm the interdependence of enzyme activities, while mineral imbalances highlight broader systemic implications. These results underscore the need for further research into effective protective interventions and long-term health implications of crude oil vapor exposure, with particular attention to pulmonary health, oxidative stress management, and metabolic regulation.

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