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The Role of Vitamin C in the Modulation of Toxicological Effect of Crude Oil Vapour on the Activities of Transaminases of Heart of Albino Rats

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Abstract: This study was conducted to assess the toxic effects of crude oil vapor and oral administration of vitamin C on rats' cellular systems using various routes of administration and the potential involvement of oxidative stress in the mechanism of action. Forty-eight albino rats were split into two main groups (24 male and 24 female): control, vitamin C, group A (25%) and group B (50%) and group C (75%) and group D (100%). Exposure to petroleum products through inhalation and oral administration of vitamin C has been linked to elevated blood pressure, but the mechanism of action has not been fully explained. As mentioned above, each group was further broken into six subgroups, each containing four rats. No therapy was administered to the control. The duration of each exposure and administration was three weeks. When compared to control groups, the results indicated that the treated groups' blood pressure and pulse rate were significantly higher ($p<0.05$). Research has demonstrated that breathing in crude oil vapor does not kill living things. This is in line with earlier research showing that rats exposed to crude oil vapor for 6–13 weeks survived. Rats' weights in the exposed and control groups were tracked and reported as weekly percentage weight gain (PWG). Growth retardation and weight loss are thought to be the result of complex interactions between different components of crude oil and a signaling pathway involving intercellular and molecular mechanisms that suppress growth stimulatory signals and stimulate growth stimulatory pathways. Severe and perhaps fatal weight loss in rats may occasionally be linked to petroleum exposure.

Keywords: *Vitamin. C, Toxicological Effect, Crude Oil Vapour, Transaminases of Heart, Albino Rat.*

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I. INTRODUCTION

It's interesting to note that according to local folklore, crude oil can be applied topically to burns, foot rot, leg ulcers, poisoning, witchcraft, gastrointestinal ailments, and reproductive issues. While Orisakwe et al. (2000) documented the analgesic effect of BLCO in comparison to aspirin and supported its traditional use for foot rot and leg ulcer pain, its nephrotoxicity was also documented based on changes in serum electrolytes, urea, and creatinine as well as pathological changes in kidney biopsies (Orisakwe et al., 2004). Furthermore, BLCO has been shown to be hematotoxic and to significantly raise aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in a dose-dependent levels, while the level of alkaline phosphatase was considerably lower than that of the controls (Orisakwe et al., 2005). In adult guinea pigs administered intraperitoneally for two days, BLCO has been demonstrated to cause changes in the amounts of calcium, cytoplasmic total hydrocarbon, and liver mitochondrial DNA (Oruambo and Jones, 2007). According to Orisakwe et al. (2004), exposure to BLCO has a negative impact on male fertility and causes significant impairment of testicular functions, including degenerative changes in seminiferous tubules and Leydig cells. Additionally, oral gavage at 200, 400, and 800 mg kg21 for a week alters antioxidant systems in a dose-dependent manner by inducing oxidative stress (Farombi et al., 2009).

II. EXPOSURE TO CRUDE OIL AND ITS TOXICITY

Exposure can occur in a variety of ways, including ingestion or absorption through the skin. By building up in tissues and organs, they can also harm human life by causing

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birth abnormalities, cancer, asthma, hormone imbalances, and damage to the brain, nerves, and liver. ulcers and skin irritation. While drivers are exposed to the smells of crude oil when they fill up at gas stations, gas station employees are more vulnerable due to their work-related exposure (Micyus et al., 2005). Even for a short time (seconds), breathing in crude oil vapor is dangerous.Vapour concentrations, measured in parts per million (ppm) or mass of total hydrocarbons per unit volume (mg/m3), are 25,000 in the air above an open barrel in an unventilated outhouse on a "hot" day, 50–320 in the air surrounding a tanker during bulk loading, and 20–200 in the air surrounding a service station gas pump during vehicle fueling (Micyus et al., 2005). The primary drawback of the studies is the lack of evaluation of exposure patterns across time and the scarcity or nonexistence of data on particular exposures. Exposure is calculated using the premise that living near possible exposure sources carries a higher risk to one's health in the majority of surveys that use individual-level data. Because lipid solubility plays a significant role in the transit of crude oil components through the cell's plasma membrane and, ultimately, in the degree of membrane disruption, the toxicity of a crude oil fraction is correlated with its hydrophobicity (Freedman, 2000). There are hints that oxidative stress may result from prolonged exposure to crude oil in humans and other animals that resemble humans. Predators that ingested polluted marine prey may be exposed to oil by ingestion of the prey, and animal species not directly affected by the oil spill may also suffer injury through the food chain (Sunmonu and Oloyode, 2007). Due to the various uses of crude oil and the ambient level, both humans and animals are becoming more exposed to these substances (Patrick-Iwuanyanwu et al., 2011). The local populace has utilized crude oil or its fraction, which is primarily taken orally, to treat a variety of illnesses over the years, including burns, foot rot, leg ulcers, poisoning, gastrointestinal issues, and even witchcraft (Orisakwe et al., 2000). Therefore, the purpose of this study was to examine the potential risks that food contaminated with crude oil poses to albino rats (rattusnovergicus) (Odo et al., 2012). Either the physical characteristics of the oil (physical contamination and suffocation) or its chemical components (toxic effects and accumulation leading to tainting) are responsible for the impacts of oil spills on aquatic life. Aquatic life may also be impacted directly by cleanup efforts or indirectly by physical harm to plant and animal habitats.

III. MATERIALS AND METHODS

➢ *Reagents*

Reagents and solvents were of analytical grade and are products of British Drug House, Poole, England.

➢ *Crude oil Used*

The crude oil was obtained from Warri refinery and Petrochemical Company (WRPC), Effurun, Delta State. Nigeria.

➢ *Experimental Rats and Treatments*

Before the experiment started, 48 albino rats (Rattus novergicus), 24 of which were male and 24 of which were female, were acquired from the Animal Holding of the Department of Anatomy University of Benin, Benin-City, Nigeria. They were given seven (7) days to acclimate. There are two main types of male and female experimental animals. Male albino rats were housed in six plastic cages with four animals each, and they were divided into six groups. The same was true for female rats. The following concentrations of crude oil (diluted with distilled water) were administered to these groups:

Table 1 Animal Groups and Treatment

Group (Male)	Treatment	Period of Treatment
1.	Control (exposed to 0% v/v crude oil)	21 days
2.	Rats Exposed (orally) to Vit. C (Ascorbic acid) only Doses of ascorbic acid: @400 mg/kg body weight	21 days
3.	Rats Exposed to 25% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days
4.	Rats Exposed to 50% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days
5.	Rats Exposed to 75% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days
6.	Rats Exposed to 100% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days

The experimental rats were fed *ad libitum* with commercial rat chow throughout the experiment period.

Group (Female)	Treatment	Period of Treatment
1.	Control (exposed to 0% v/v crude oil)	
2.	Rats Exposed(orally) to Vit. C (Ascorbic acid) only Doses of ascorbic acid: 400 mg/kg body weight	21 days
3.	Rats Exposed to 25% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days
4.	Rats Exposed to 50% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days

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5.	Rats Exposed to 75% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days
6.	Rats Exposed to 100% v/v crude oil (for 30mins Daily) with oral administration of Vit C Doses of ascorbic acid: @400 mg/kg body weight	21 days

The experimental rats were fed *ad libitum* with commercial rat chow throughout the experiment period.

➢ *Anaesthetisation of Animals and Isolation of Tissues*

Before being killed by jugular puncture, the rats were put in a jar with cotton wool that had been saturated in chloroform to induce anesthesia. The entire liver, kidney, lungs, brain, and heart were promptly dissected, removed, fat-free, blotted with sterile tissue paper, and weighed into a beaker filled with ice-cold normal saline solution. The heart puncture was used to draw the blood. Heparinized bottles were used to collect some of the blood, whereas nonheparinized bottles were used for others. After centrifuging certain blood samples in nonheparinized bottles for approximately 15 minutes at 3,500 rpm using a chilled centrifuge RC650, the serum samples were stored at -8°C until they were needed for analysis.

➢ *Preparation of Homogenate*

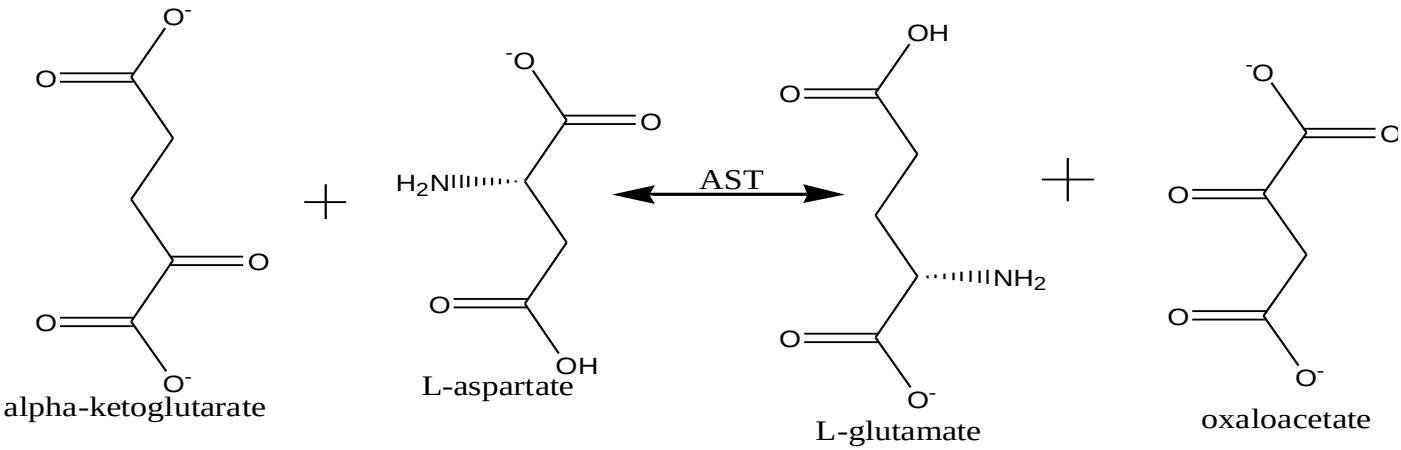
After weighing the separated tissues, a bit of each was taken out, finely diced, and then mixed with a dish of ice cubes using a pestle and mortar that had been cooled beforehand. Normal saline solution was used to dilute the tissue homogenates to a 1 in 30 dilution. Each organ's fraction was homogenized for enzyme tests and biochemical investigations. Until they were needed, the diluted homogenates were kept at -8°C.

➢ *Determination of Aspartate Aminotransferase (AST) Activity*

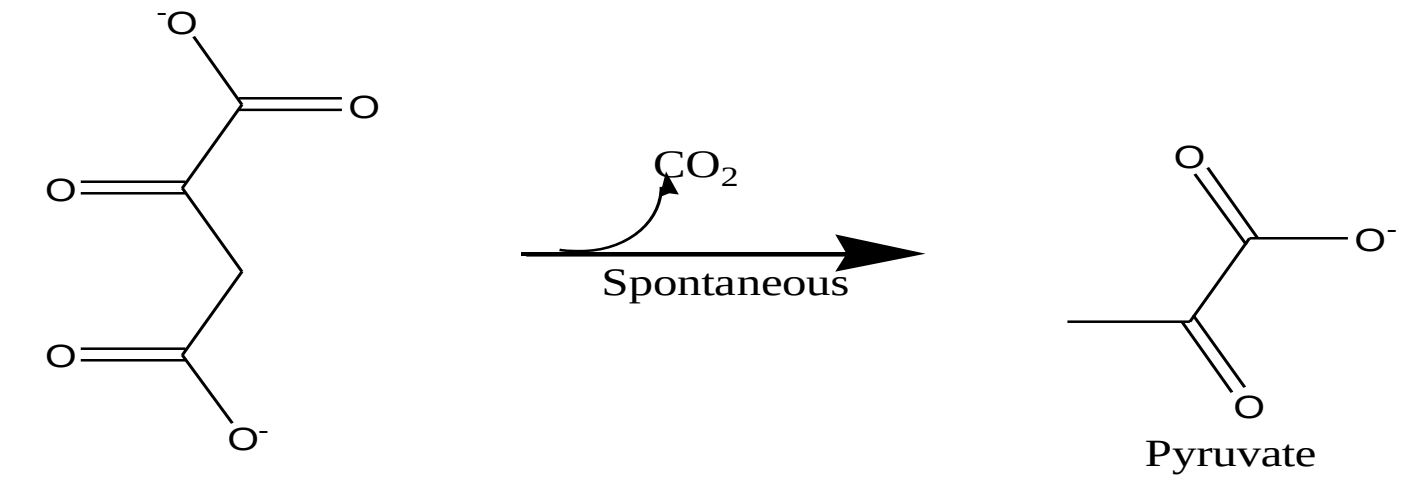
- *Principle*

The method described by Reitman and Frankel (1957) and modified by Schmidt and Schmidt (1963) was used to measure the amount of AST in the serum and tissues of experimental animals.

The following equation describes how the aspartate aminotransferase catalyzes the conversion of aspartate and α-ketoglutarate into oxaloacetate and glutamate:



The unstable oxaloacetate formed is then spontaneously decarboxylated to form pyruvate as indicated in the equation:



Oxaloacetate

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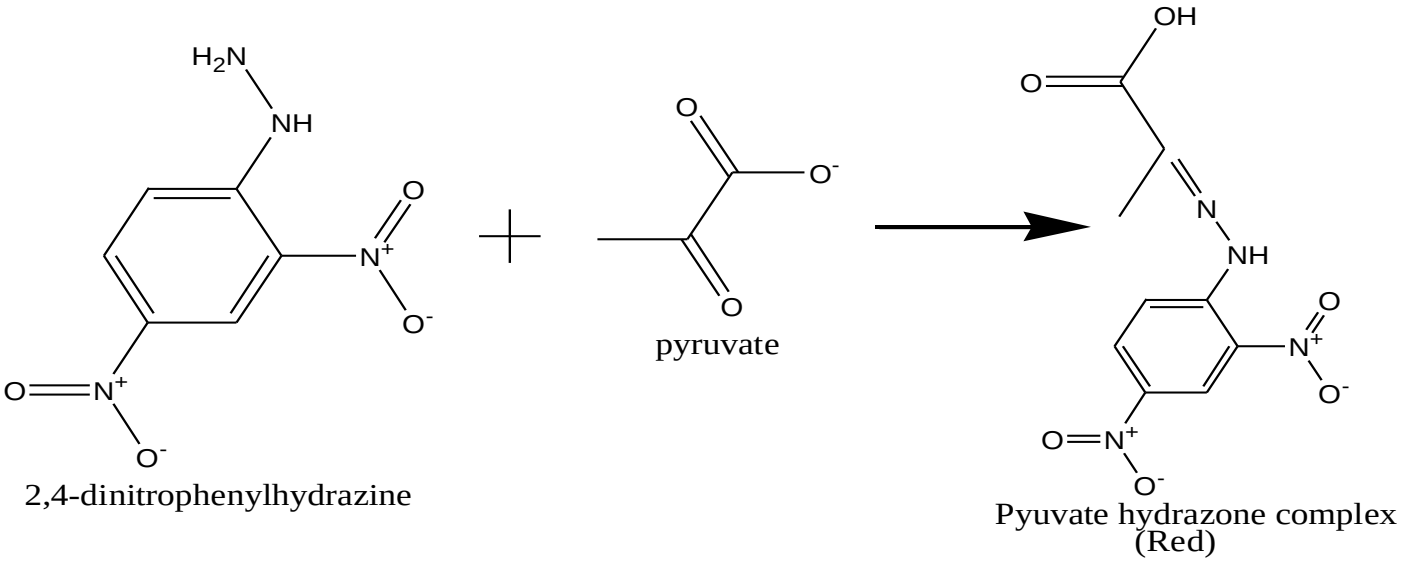
The absorbance of the red coloured complex formed from the reaction of pyruvate with p-nitrophenylhydrazine is then read at 546nm on a Spectrophotometer. The reaction is shown thus:



- *Procedure*

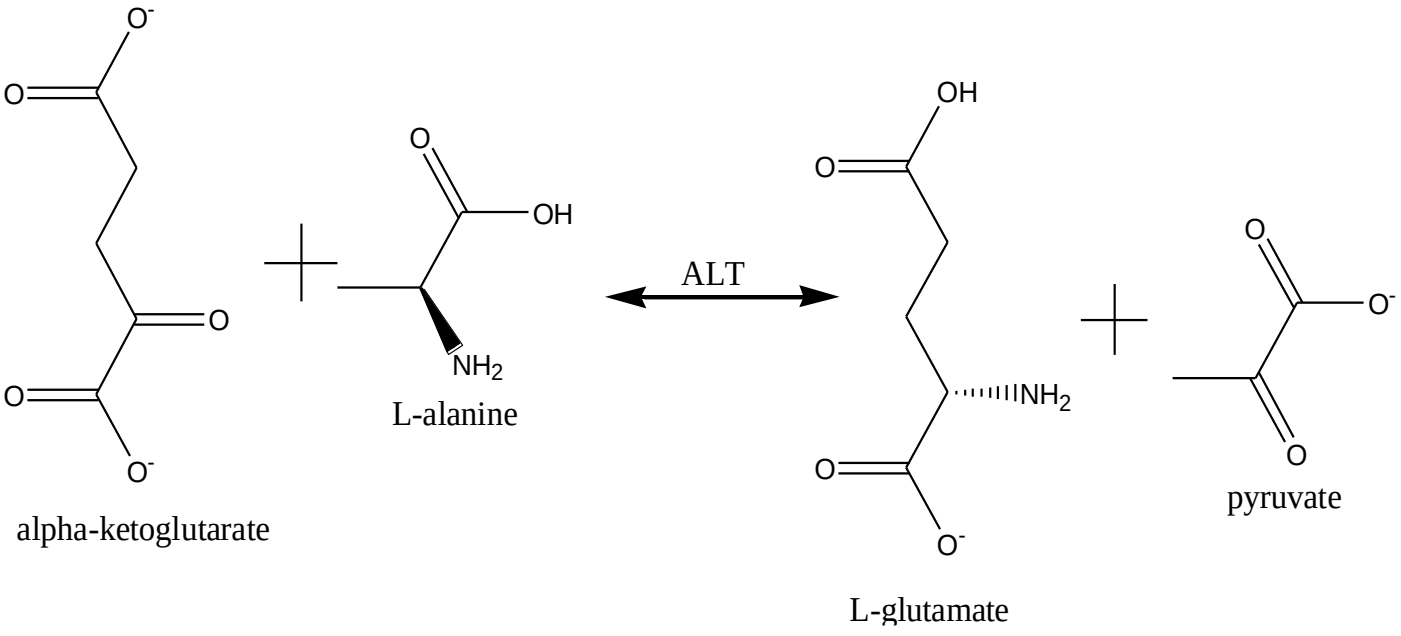
First, different volumes of pyruvate standard (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 cm3) were dispensed into separate test tubes in order to calibrate the AST standard curve. AST buffered substrate was then used to bring the volumes up to 1 cm3. After shaking, the mixture was incubated at 37°C for 30 minutes. Each test tube received 0.001M 2,4-dinitrophenylydrazine (DNPH) (1 cm3). After letting the mixture remain at 25°C for 20 minutes, 5 cm3 of 0.4N NaOH was added. Using a spectrophotometer, the mixture's absorbance was measured at 546 nm and plotted against the matching pyruvate concentration.

After a 1 in 30 dilution, 0.5 cm3 of AST buffered substrate and 0.1 cm3 of tissue homogenates were dispensed into test tubes to measure the aspartate aminotransferase activity in the serum and tissues of experimental rats. After giving the mixture a good shake and incubating it for 30 minutes at 37°C, 0.5 cm3 of 0.001M DNPH was added, and it was let to stand for 20 minutes at 25°C. After adding 0.4N NaOH (5 cm3), the absorbance was measured at 546 nm five minutes later.

➢ *Determination of Alanine Aminotransferase (ALT) Activity*

- *Principle*

The method described by Reitman and Frankel (1957) and modified by Schmidt and Schmidt (1963) was used to measure the activity of ALT in the serum and tissues of experimental animals. As demonstrated, alanine aminotransferase catalyzes the conversion of alanine and α-ketoglutarate into pyruvate and glutamate:



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As explained in Section, the technique uses spectrophotometry to detect the absorbance of the red complex that results from the interaction of pyruvate with 2,4-dinitrophenylhydrazine.

➢ *Above*

- *Procedure*

With the exception of using ALT buffered substrate rather than AST buffered substrate, the steps for calibrating the ALT standard curve and determining ALT activity were as outlined in Section ABOVE.

IV. RESULTS

The specific activity of alanine transaminase (ALT) in the heart and serum of rats exposed to crude oil fumes and oral vitamin C co-administration is shown in Figure 1. When the concentration of crude oil rose in comparison to the Control, the female rat's heart's ALT activity significantly decreased ($p<0.05$), but the male rat showed no such pattern, with ALT activities of rats exposed to varying concentrations of crude oil vapor showing no significant difference ($p>0.05$). Serum ALT activity of both male and female rats exposed to crude oil vapor at different concentrations rose significantly ($p<0.05$) in comparison to the Control.

In general, the rats' heart ALT dropped considerably ($p<0.05$) when crude oil content rose in comparison to the control group (Figure 2). In contrast, the rat population's serum ALT rose noticeably ($p<0.05$) in relation to the control when crude oil content rose (Figure 3). Figure 4 illustrates the co-administration of vitamin C orally with 232 crude oil. For both male and female rats, higher crude oil concentrations were associated with a generally significant increase ($p<0.05$) in cardiac AST activity. However, for both male and female rats, a notable rise in AST activity was observed in the serum as the crude oil content rose.

A higher concentration of crude oil vapor also resulted in a decrease in cardiac AST activity ($p<0.05$) in a population of rats that included both male and female rats (Figure 5). Rats exposed to 100%, 75%, and 50% crude oil vapor had cardiac AST activity levels of roughly 200%, 170%, and 150%, respectively. Aspartate transaminase (AST) specific activity in the serum of rats exposed to crude oil fumes and oral vitamin C co-administration is shown in Figure 6. As the crude oil concentration rose in comparison to the control, serum AST activity rose significantly ($p<0.05$), in opposition to Figure 5. Rats exposed to 100%, 75%, and 50% crude oil fumes had serum AST levels that were three times, two and a half times, and two times higher than those of the control group, respectively.

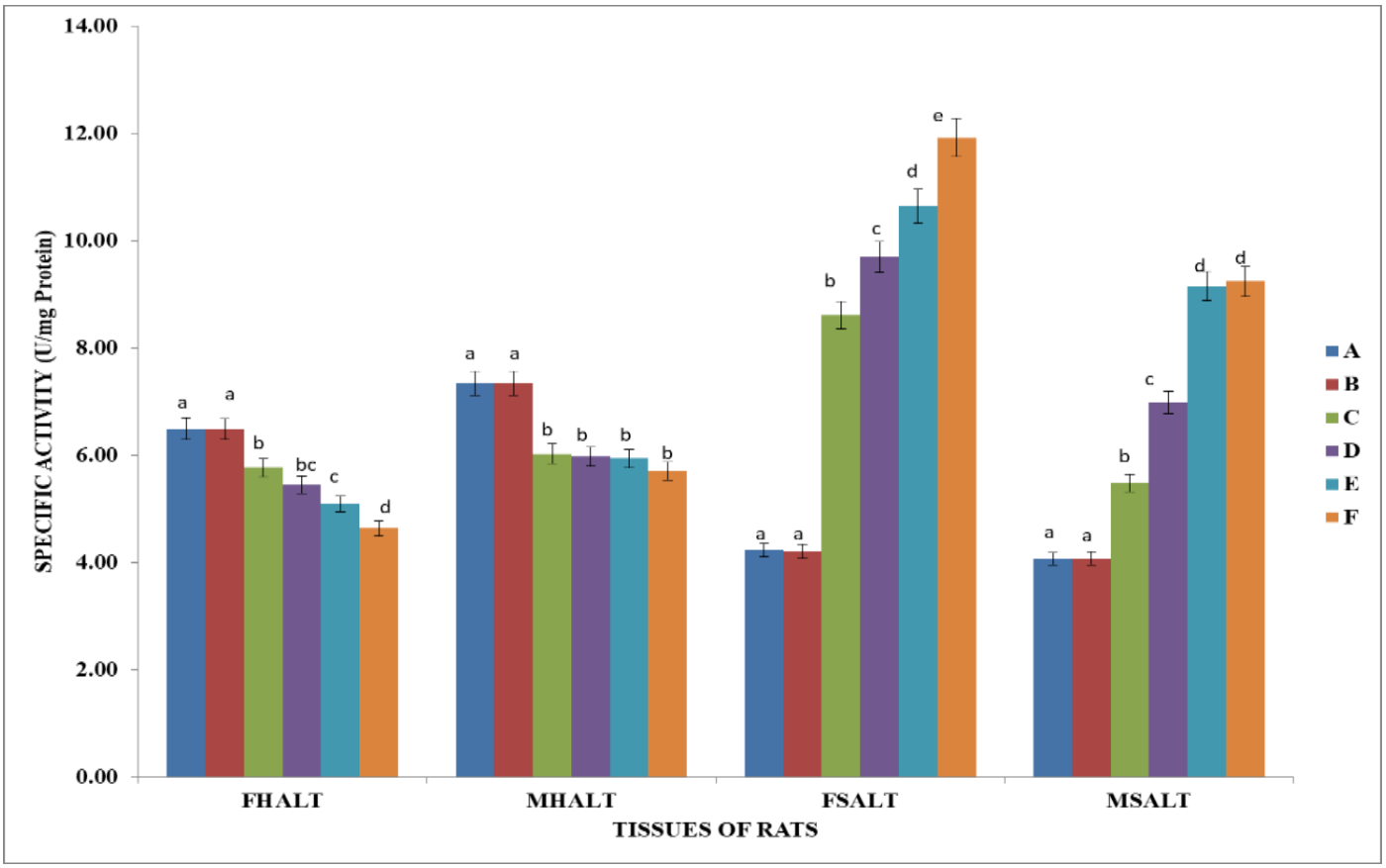

Fig 1 *Alanine transaminases (ALT) of heart and serum of rats*: Specific activity of alanine transaminase (ALT) of heart and serum of rats exposed to vapour of crude oil and oral co-administration of vitamin C. Bars in the same group bearing different superscripts are significantly different ($P<0.05$). Plotted data are means of three (3) determinations ± SEM.

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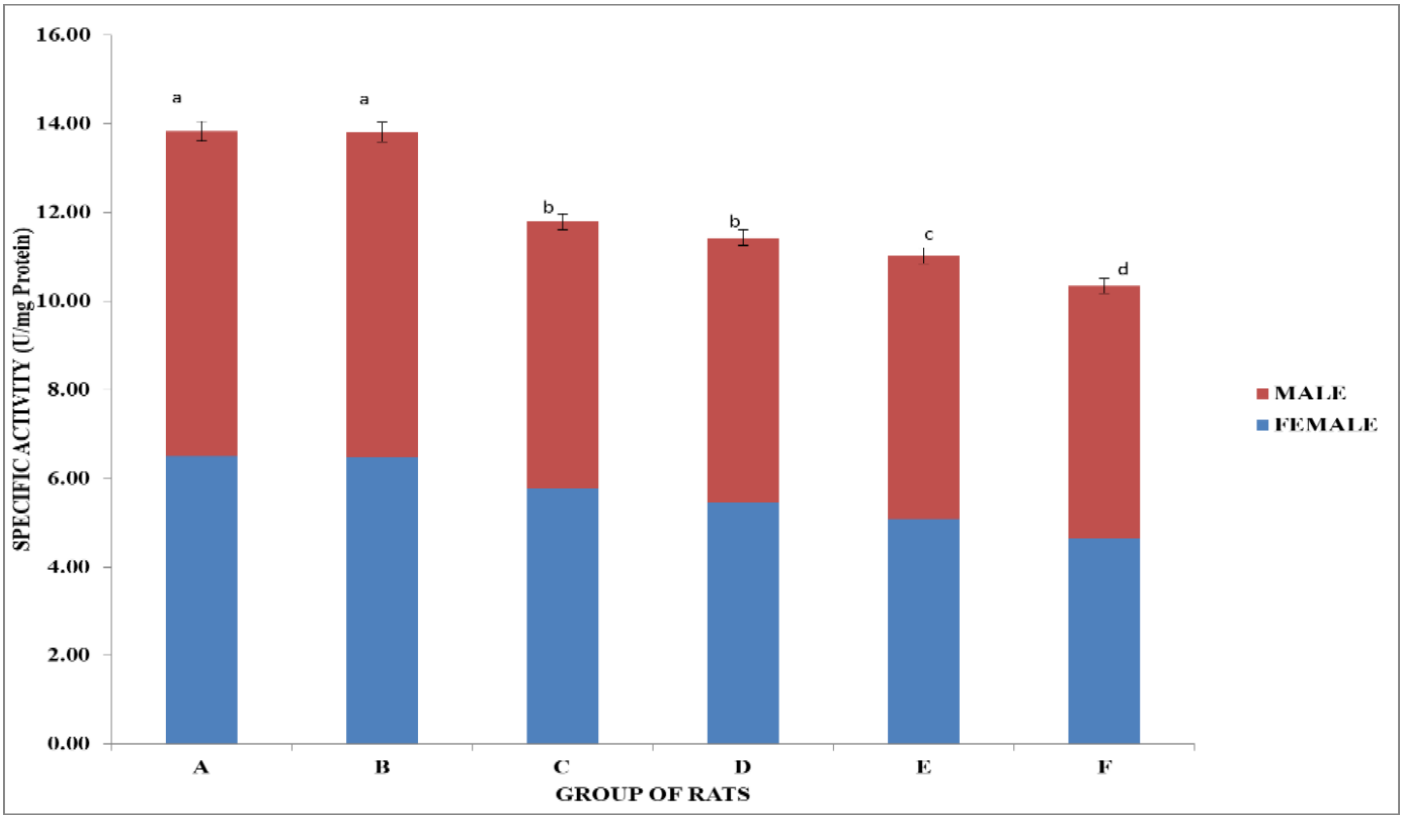

Fig 2 *Alanine transaminases (ALT) of heart of rats:* Specific activity of alanine transaminase (ALT) of heart of rats exposed to vapour of crude oil and oral co-administration of vitamin C. Bars bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

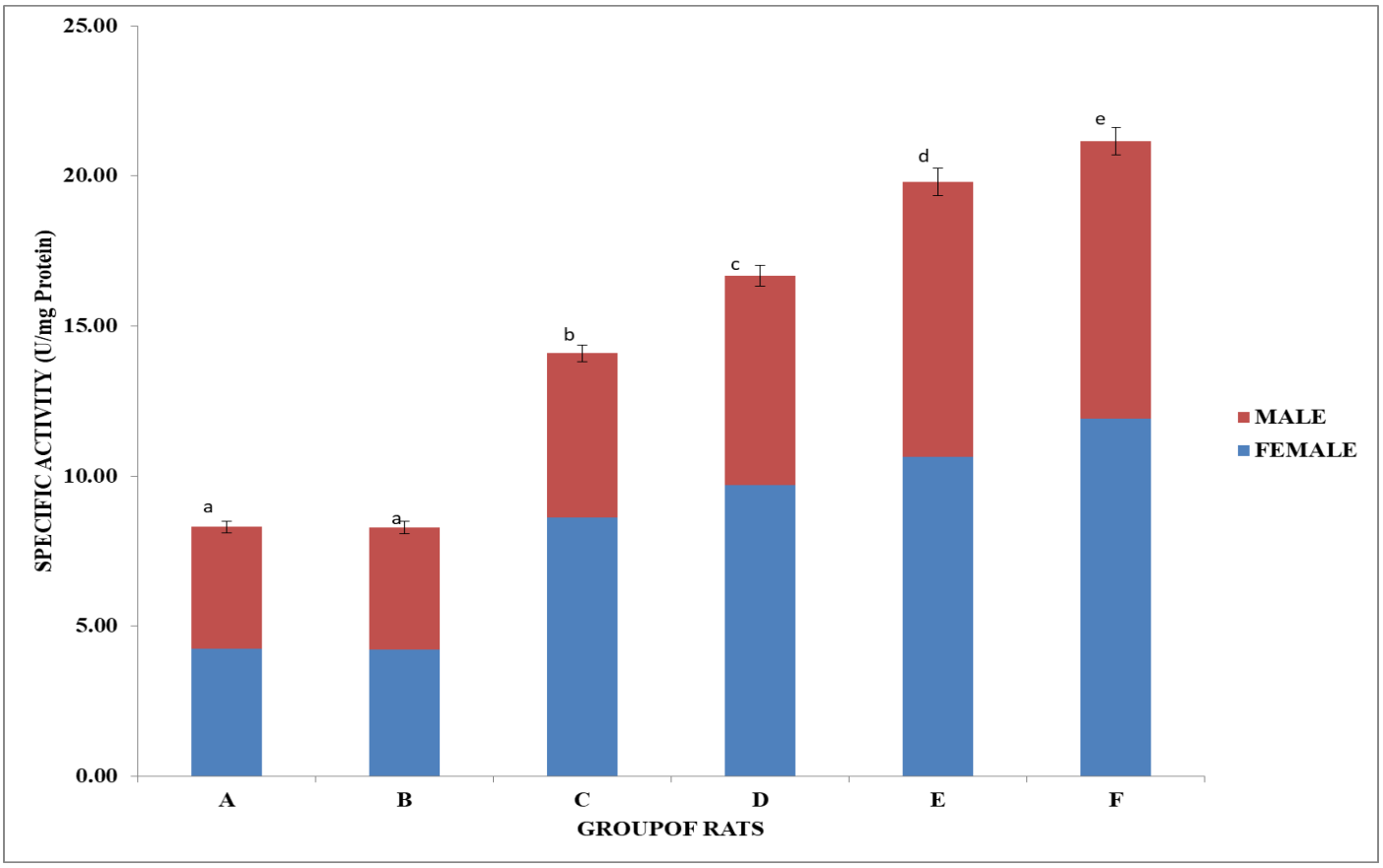

Fig 3 *Alanine transaminases (ALT) of serum of rats:* Specific activity of alanine transaminase (ALT) of serum of rats exposed to vapour of crude oil and oral co-administration of vitamin C. Bars bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

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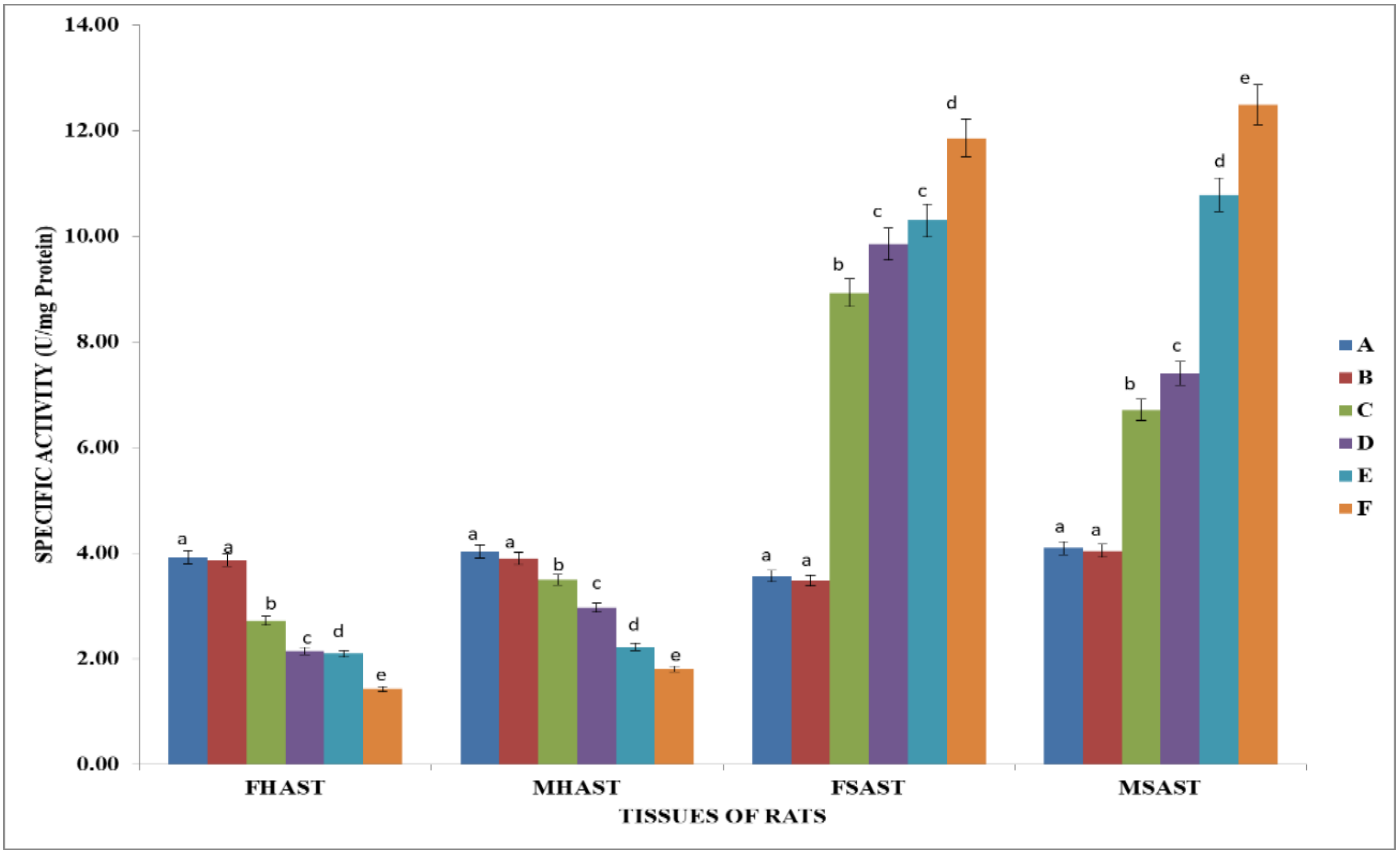

Fig 4 *Aspartate transaminase (AST) of heart and serum of rats:* Specific activity of aspartate transaminase (AST) of heart and serum of rats exposed to vapour of crude oil and oral co-administration of vitamin C. Bars in the same group bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

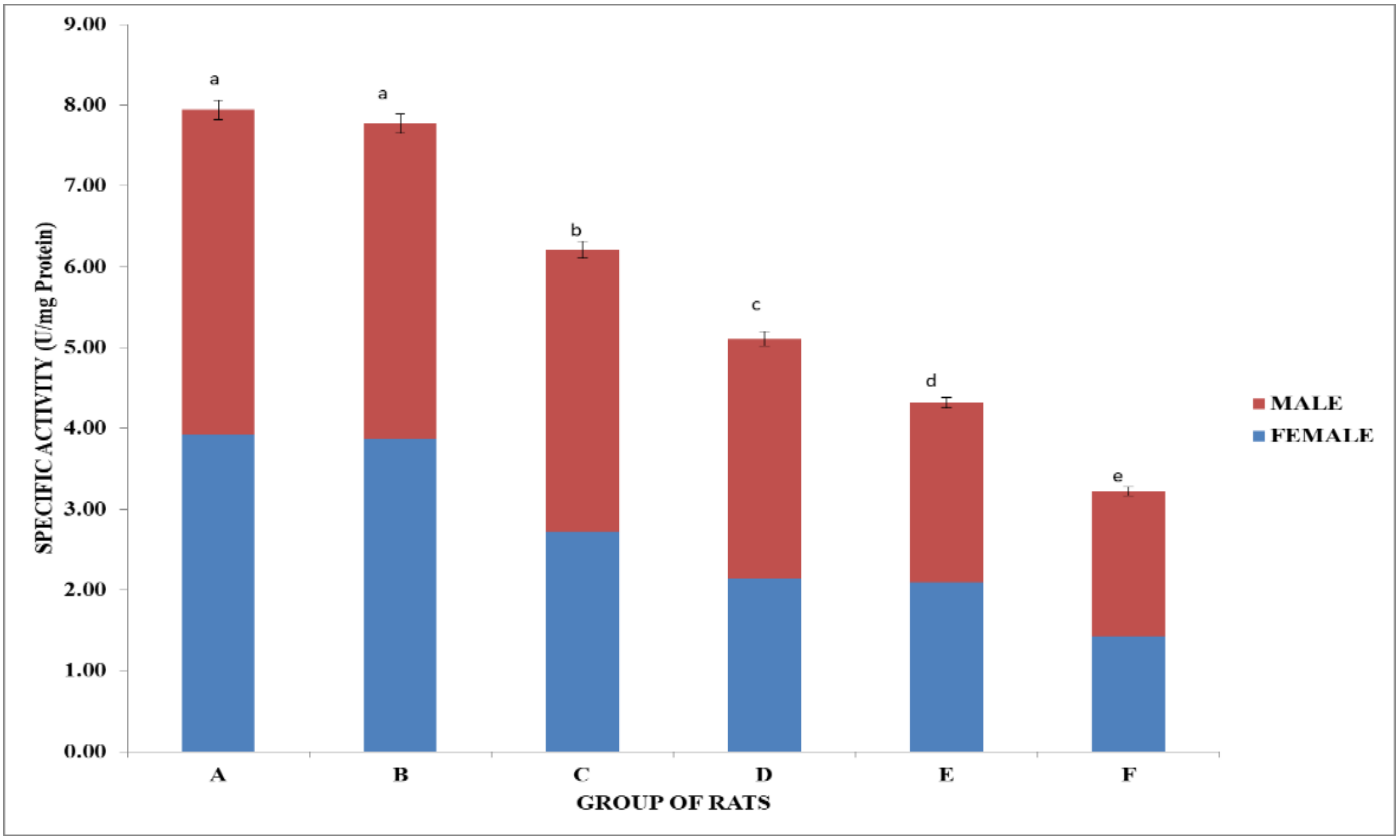

Fig 5 *Aspartate transaminase (AST) of heart of rats:* Specific activity of aspartate transaminase (AST) of heart of rats exposed to vapour of crude oil and oral co-administration of vitamin C. Bars bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

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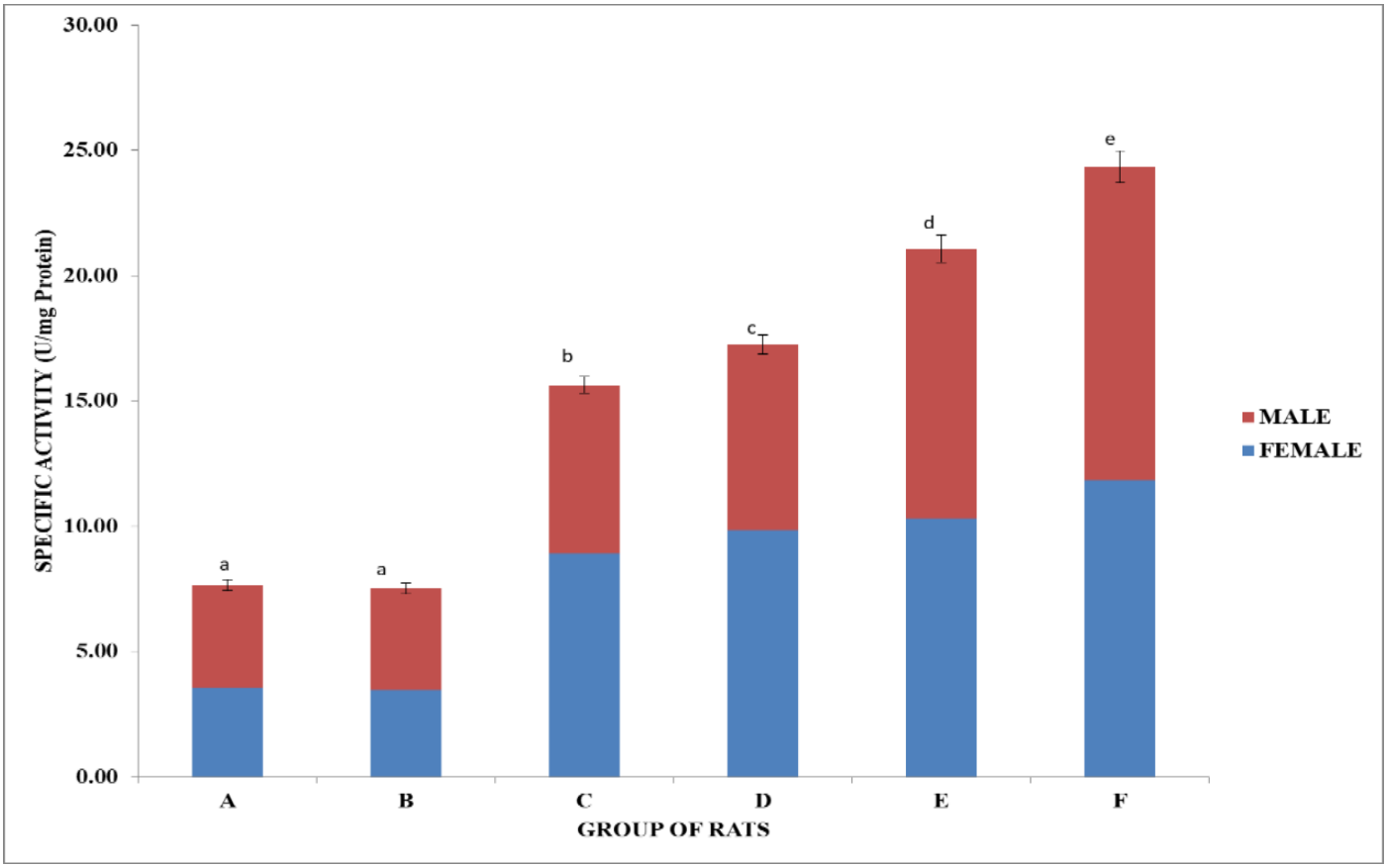

Fig 6 *Aspartate transaminase (AST) of heart of rats:* Specific activity of aspartate transaminase (AST) of serum of rats exposed to vapour of crude oil and oral co-administration of vitamin C. Bars bearing different superscripts are significantly different (P<0.05). Plotted data are means of three (3) determinations ± SEM.

V. DISCUSSION

The cardiovascular toxicity of crude oil vapor was examined in this study. The detrimental effects of internal and external stressors on the heart and circulatory system are the focus of cardiovascular toxicology. Exposure to natural items, medicinal medications, and environmental pollutants are all examples of extrinsic stress. Exposure to hazardous metabolites originating from benign substances, including those present in dietary additives and supplements, is referred to as intrinsic stress. Secondary neurohormonal disruptions, such as the overproduction of inflammatory cytokines brought on by heart pressure overload and counter-regulatory reactions to hypertension, are also included in the intrinsic exposures. Changes in biochemical pathways, abnormalities in cellular structure and function, and disease of the impacted cardiovascular system are the outcomes of these toxic exposures (James, 2015). The current study focuses on crude oil vapor as an environmental toxin and examines extrinsic stress.

The2450ctiveity in Figure 1 indicates that the male rats reached their threshold of tolerance at a crude oil concentration of 25%, whereas the female rats seem to be more sensitive to the crude oil vapor. On the other hand, male rats' serum ALT levels were comparable to those of female rats, which may indicate that male rats' hearts are more vulnerable to the toxicological effects of crude oil vapor than those of female rats. But when the general population of male and female rats was analyzed, this information was hidden (Figures 2 and 3). Both male and female rats' serum ALT levels rose as crude oil concentrations rose, indicating that enzymes from the tissues may have leaked into the blood as a result of tissue injury. Male and female rats' AST activity followed a similar trend, with the concentration of crude oil in'reasing serum activity and the heart's activity decreasing (Figures 4–6). This finding supports the hypothesis that crude oil fumes caused concentration-dependent damage to the heart and other tissues in both male and female rats. It is noteworthy to mention that the oral co-administration of vitamin C may be playing a key role in modulating the effect of the crude oil vapour in the heart as the degree of effect is curtailed when compared to that of the serum. Elevated serum activities of AST and ALT could have resulted from cellular damage which might have arisen from the toxic pollution induced by the crude oil vapour. This is because serum enzymes are cytoplasmic in nature and are only released into the blood circulation after cellular damage (Adeyemi, 2015a). It has also been reported those alterations in serum enzyme activities directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture a signal of underlying pathological process. This study's findings were in good agreement with those of other research (Adeyemi et al. 2009; Bakde and Poddar 2011). As previously noted, decreased AST and ALT activities indicate a malfunction in the metabolism of amino acids within the cell (Adeyemi et al. 2009; Bakde and Poddar 2011, Adeyemi, 2015b). The study's findings suggested that the subcellular level of crude

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oil vapor may have changed protein metabolism, among other things, which could be a sign of heart function impairment.

VI. CONCLUSION

In summary, the goal of this study was to demonstrate the chemomodulatory effect of vitamin C and the toxicological impact of crude oil fumes on the heart. According to experimental data, rats exposed to crude oil showed increased serum levels of heart transaminases (ALT and AST) and decreased heart transaminases (ALT and AST) activity proportionate to the crude oil concentration. Additionally, compared to female rats, male rats typically exhibit a little greater vulnerability. Although the exact cause of this is unknown, free radicals and sex hormones might be to blame. On the other hand, vitamin C had the ability to modulate the heart's chemo-reaction. Increasing the vitamin C dosage in future research is most likely beneficial.

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