

## L-arginine supplement ameliorates dichlorvos-induced systemic inflammatory response and liver dysfunction in male wistar rats

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### ABSTRACT

Dichlorvos (DDVP), a frequently used organophosphate insecticide, has been shown to cause systemic inflammation and liver damage via oxidative stress and inflammatory pathways. L-arginine, a semi-essential amino acid, has been shown to protect against oxidative damage and inflammation in a variety of animals. The study's main goal was to raise awareness of Dichlorvos's (DDVP) harmful effects on systemic inflammation and liver function, as well as L-arginine's possible mitigating impact. In order to assess the preventive benefits of L-arginine against induced liver dysfunction and systemic inflammation, liver tissue was selected for this investigation due to its vital role in detoxification and its high susceptibility to harm from toxic chemicals such as Dichlorvos. A total of 40 adult Wistar rats were separated into four groups: control, dichlorvos only, L-arginine only, and dichlorvos plus L-arginine. Dichlorvos was provided orally at a dose of 8 mg/kg body weight, whereas L-arginine was given orally at a dose of 100 mg/kg body weight for six weeks. The study investigated systemic inflammation markers (C-reactive protein, TNF- $\alpha$ , IL-6, and Caspase 3) as well as liver function markers (ALT, AST, ALP, albumin, total protein and gamma glutamyl). The findings revealed that dichlorvos significantly ( $p < 0.05$ ) enhanced systemic inflammation and decreased ( $p < 0.05$ ) liver function when compared to the control group. However, L-arginine supplementation greatly improved these effects by significantly ( $p < 0.05$ ) lowering inflammatory indicators and restoring liver enzyme levels to normal. Histopathological findings validated L-arginine's protective function against dichlorvos-induced liver injury. These data indicate that L-arginine supplement may reduce the negative effects of dichlorvos on systemic inflammation and liver function, providing a possible therapeutic method for controlling organophosphate toxicity.

### 1. Introduction

Inflammation, a biological response orchestrated by the immune system, can arise from various sources like pathogens, damaged cells, and toxic substances such as dichlorvos. These triggers induce acute or chronic inflammatory reactions in multiple organs, potentially leading to tissue damage and diseases [8]. This process is integral to the immune system's identification and elimination of harmful stimuli, initiating the healing process. Inflammation, whether acute or chronic, involves complex interactions among various cellular and molecular components, meticulously coordinated to mount an appropriate response to inflammatory stimuli or infections [22]. Recent findings suggest that inflammation plays a pivotal role in the pathophysiology of dichlorvos toxicity in humans [9], with its manifestation preceding

immunosuppression, hypersensitivity, and autoimmunity [43], emphasizing the importance of addressing it effectively.

The liver, located in the upper right quadrant below the diaphragm, undertakes vital functions such as primary detoxification, protein synthesis, and digestive enzyme production [18]. Metabolizing carbohydrates, proteins, and fats, the liver's enzymatic products serve as sensitive indicators of abnormalities [45]. Exposure to organophosphates like DDVP can induce hepatotoxicity and impair liver function.

Dichlorophosphate (DDVP), an organophosphate pesticide, is extensively used to control pests in households, public spaces, and stored products [24]. Its efficacy extends to various organisms, making it valuable in agriculture and pest control. However, dichlorvos exposure poses health risks, leading to hepatotoxicity [7] and other adverse effects [24]. L-arginine, a basic amino acid with anti-inflammatory and

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hepatoprotective properties, has been investigated for its potential to mitigate these effects. Furthermore, as a result of the widespread use of dichlorvos in agriculture, target pests have developed pesticide resistance, potentially worsening environmental pollution [39]. International institutions have implemented regulatory steps to minimize the use of dichlorvos; however, effective enforcement remains a challenge, particularly in places with weak regulatory frameworks [42]. Governments, scientists, industry stakeholders, and civil society must collaborate to address these concerns, with a focus on sustainable pest management practices and safer alternatives [48].

Arginine, discovered over a century ago, is naturally present in our diet and plays essential roles in various biological processes [11,40]. This study aims to explore the effects of L-arginine supplementation on inflammatory and liver biomarkers in male Wistar rats exposed to dichlorvos. The use of pesticides like dichlorvos raises concerns about potential long-term health and environmental influences, making interventions such as L-arginine supplementation worth investigating.

This research hypothesizes that L-arginine supplement can attenuate systemic inflammatory responses and improve liver function in dichlorvos-exposed rats, building on the known anti-inflammatory and hepatoprotective properties of L-arginine. By exploring this hypothesis, the study not only contributes to understanding the protective role of L-arginine in pesticide toxicity but also offers insights into potential preventive strategies for individuals at risk of pesticide exposure.

## 2. Materials and methods

### 2.1. Experimental animals

The study included 40 healthy male Wistar rats weighing between 220 and 250 g, bought from a private breeder. These rats had not previously received any experimental manipulations and were judged healthy due to the lack of stress or infection indications. Before starting the tests, the rats were individually weighed following a two-week acclimatization period. They were maintained in well-ventilated plastic cages at the animal facility of the Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, with ethical approval number (ERCFBMSLAUTECH/018/01/2024). The animals in the study had access to food and water, and the methods followed the National Research Council's Guide for the Care and Use of Laboratory Animals.

### 2.2. Dosage and administration

The rats were randomly assigned to four groups. Group A (control) received 0.5 mL of distilled water, group B (DDVP only) was given 8 mg/kg body weight of dichlorvos orally, group C (DDVP + L-arginine) was given dichlorvos 8 mg/kg body weight as well as 100 mg/kg of L-arginine daily for six weeks, and group D (L-arginine only) was given 100 mg/kg of L-arginine daily for six weeks. The doses and routes of administration for dichlorvos and L-arginine are as previously described by Saka et al., [33] and Saka et al., [34] respectively with slight modification. Without the possible complications of multiple dosing, a single dosage of L-arginine was employed to examine its early protective effects on systemic inflammation and liver dysfunction, enabling a clear evaluation of its initial impact in response to Dichlorvos exposure [32].

### 2.3. Method of Euthanasia

To euthanasia the Wistar rats, isoflurane was used. The animals were first placed in a sealed chamber and gradually subjected to increasing quantities of isoflurane vapor and euthanasia was confirmed using thoracotomy. This procedure reduced pain and discomfort while conforming to humane euthanasia criteria [1].

### 2.4. Collection of blood samples

Following the experiment, blood samples were obtained following an overnight fast [17,15]. Blood was collected via cardiac puncture with a 2 mL needle and syringe and transferred to standard vials [14].

### 2.5. Collection of tissues

Liver tissues were cleaned in normal saline and stored in labeled bottles containing formalin for subsequent analyses. The extracted tissues were maintained cool at 10 °C prior to biochemical examination [16].

### 2.6. Inflammatory studies

The enzyme-linked immunosorbent assay (ELISA) test was used to detect C-reactive protein, interleukin-6, TNF alpha and caspase 3 (Elabscience, Wuhan, China). The assay procedures were in accordance with the manufacturers' instructions and as described by Saka et al., [34].

### 2.7. Liver function assessment

An automated analyser (Mindray BS-120, Chema Diagnostica, Italy) was used to measure serum albumin, total protein, alkaline phosphatase (ALP), alkaline amino transferase (ALT), aspartate amino transferase (AST), and gamma glutamyl.

### 2.8. Histopathological analysis

Liver tissues were fixed in phosphate-buffered saline (0.1 M; pH 7.4), then thoroughly washed, dehydrated in ethanol, and embedded in paraffin wax. Haematoxylin and eosin were used to stain thin sections of liver tissue (4 mm thick). These sections were permanently fixed on slides with DPX mounts [35,5]. They were then viewed under a microscope with photography equipment (Motic Images Plus version 2.0), and photomicrographs were taken.

### 2.9. Statistical analysis

Data was reported as mean  $\pm$  standard error of the mean. The statistical differences between means were examined using one-way analysis of variance. Turkey's post hoc test was employed to detect differences between individual means. Graph Pad Prism 5 (Graph Pad Software, Inc., La Jolla, CA, USA) was used with 95 % confidence intervals and a significance level of  $p < 0.05$  for all results. Because it detects general differences between group means and explicitly identifies which pairs of means differ significantly while accounting for Type I error, ANOVA with Tukey's post hoc test is acceptable for statistical analysis.

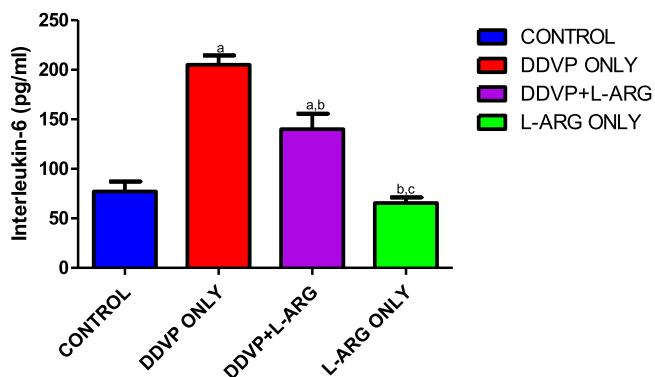
## 3. Results

### 3.1. Effect of L-arginine on Interleukin-6

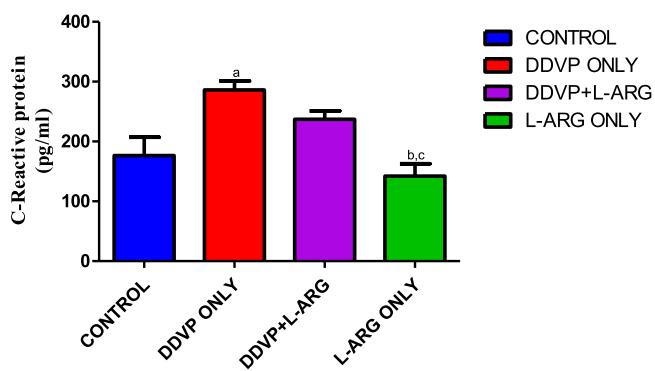
The findings depicted in Fig. 1 illustrate the influence of L-arginine on Interleukin-6 in rats exposed to DDVP. A notable rise in IL-6 was observed in DDVP-exposed rats in comparison to the control at a significance level of  $p < 0.05$ . L-arginine administration significantly lowered IL-6 levels in rats compared to those subjected to DDVP and DDVP+L-arginine ( $p < 0.05$ ).

### 3.2. Effect of L-arginine on C-reactive protein

The findings in Fig. 2 demonstrated the effect of L-arginine on C-reactive protein (CRP) in rats subjected to DDVP. DDVP-exposed rats

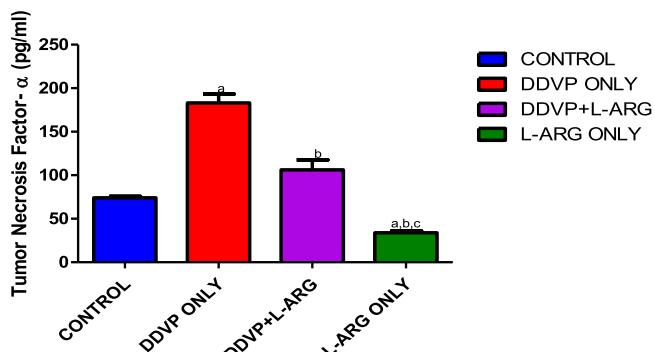


**Fig. 1.** Influence of L-arginine on Interleukin-6 of DDVP-induced and L-arginine supplemented rats. The values are reported as mean  $\pm$  SEM. a Statistically significant difference compared to control rats at  $p < 0.05$ . b Statistically significant difference compared to rats exposed to DDVP at  $p < 0.05$ . c Significant difference compared to rats treated to DDVP+L-ARG at  $p < 0.05$ . DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.



**Fig. 2.** Influence of L-arginine on C-reactive protein of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.

had significantly higher CRP levels compared to the control group ( $p < 0.05$ ). L-arginine injection significantly reduced CRP levels in rats compared to those treated to DDVP or DDVP+L-arginine ( $p < 0.05$ ).



**Fig. 3.** Influence of L-arginine on TNF alpha of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.

### 3.3. Effect of L-arginine on TNF- $\alpha$

The outcomes presented in Fig. 3 depicted the influence of L-arginine on TNF- $\alpha$  in rats exposed to DDVP. A noteworthy rise in TNF- $\alpha$  was observed in DDVP-exposed rats compared to the control at a significance level of ( $p < 0.05$ ). Nevertheless, the administration of L-arginine to DDVP-exposed rats resulted in a reduction in TNF- $\alpha$  levels compared to DDVP-exposed rats.

### 3.4. Effect of L-arginine on Caspase-3

The results shown in Fig. 4 demonstrate the effect of L-arginine on Caspase-3 in rats exposed to DDVP. Rats exposed to DDVP showed a significant increase in Caspase-3 levels compared to the control group ( $p < 0.05$ ). Nonetheless, administering L-arginine to rats resulted in a reduction in caspase-3 levels when compared to DDVP-exposed rats, though this difference was not statistically significant.

### 3.5. Effect of L-arginine on aspartate aminotransferase (AST)

Fig. 5 shows that rats exposed to dichlorvos had significantly higher levels of aspartate aminotransferase ( $p < 0.05$ ) compared to the control group. L-arginine significantly reduced aspartate aminotransferase levels ( $p < 0.05$ ) compared to DDVP-exposed rats.

### 3.6. Effect of L-arginine on alanine aminotransferase (ALT)

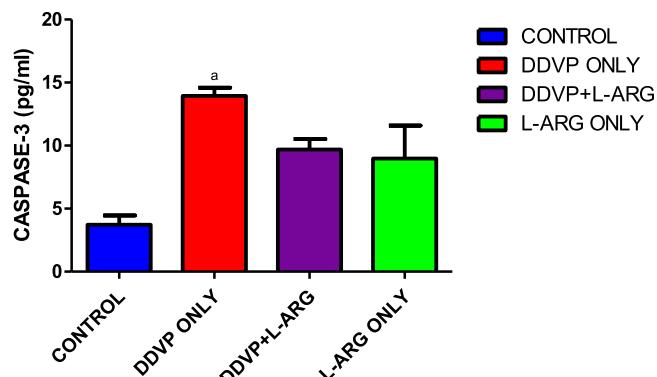
Fig. 6 shows that rats exposed to dichlorvos had a significant increase ( $p < 0.05$ ) in alanine aminotransferase levels compared to the control. Furthermore, delivery of L-arginine resulted in a non-significant decrease ( $p > 0.05$ ) in alanine aminotransferase levels as compared to those exposed to DDVP.

### 3.7. Effect of L-arginine on alkaline phosphatase (ALP)

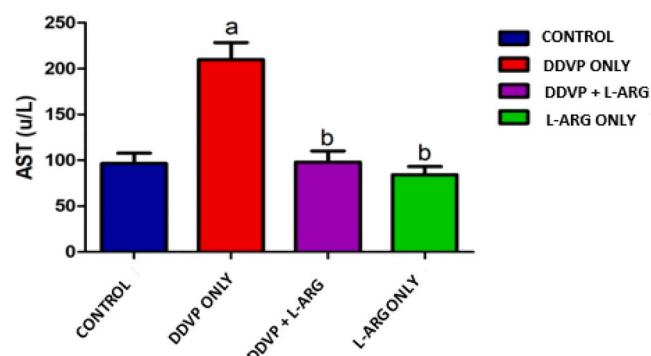
Fig. 7 shows that rats exposed to dichlorvos had significantly higher ( $p > 0.05$ ) alkaline phosphatase levels than the control group. Furthermore, delivery of L-arginine resulted in a substantial rise ( $p > 0.05$ ) in alkaline phosphatase levels compared to those exposed to DDVP.

### 3.8. Effect of L-arginine on bilirubin

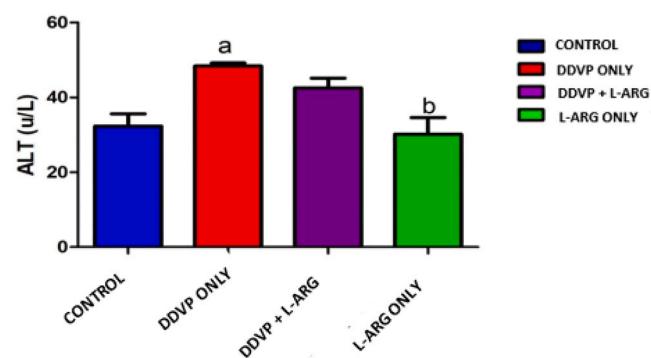
The findings from Fig. 8 demonstrated that subjecting animals exposed to dichlorvos showed a significant increase ( $p > 0.05$ ) in bilirubin levels compared to the control group. Furthermore, administering L-arginine significantly reduced ( $p < 0.05$ ) bilirubin levels compared to



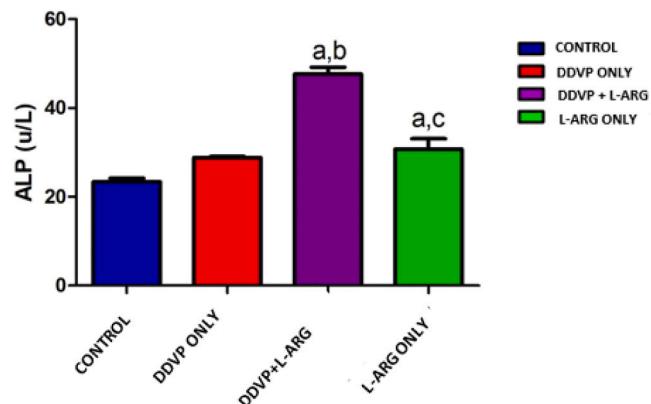
**Fig. 4.** Influence of L-arginine on Caspase-3 of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.



**Fig. 5.** Influence of L-arginine on AST of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.



**Fig. 6.** Influence of L-arginine on ALT of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.

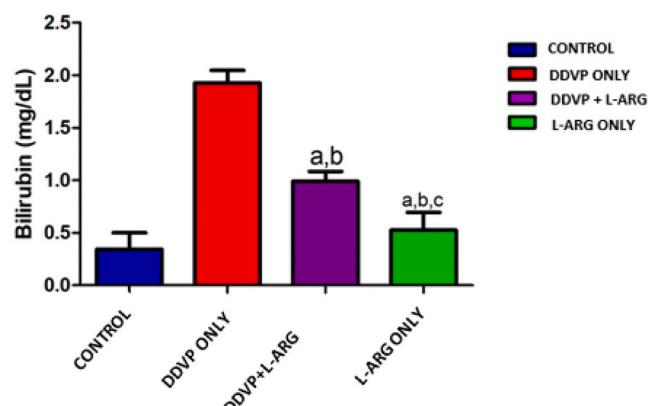


**Fig. 7.** Influence of L-arginine on ALP of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.

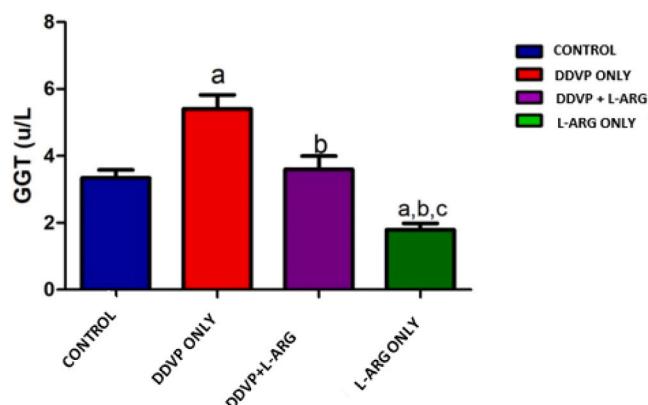
those exposed to DDVP.

### 3.9. Effect of L-arginine on gamma glutamyl transferase (GGT)

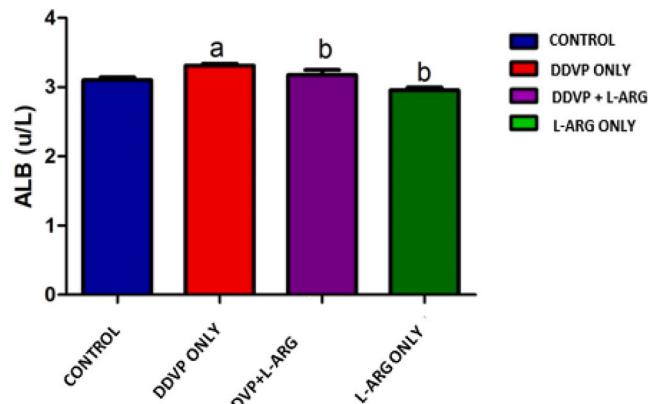
The outcomes depicted in Fig. 9 demonstrated a notable rise ( $p < 0.05$ ) in gamma glutamyl transferase levels in Animals exposed to dichlorvos were compared to a control group. L-arginine delivery



**Fig. 8.** Influence of L-arginine on bilirubin of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.



**Fig. 9.** Influence of L-arginine on GGT of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.



**Fig. 10.** Influence of L-arginine on ALB of DDVP-induced and L-arginine supplemented rats. Description of trends: See Fig. 1. DDVP- Dichlorvos L-ARG= L-arginine. DDVP- Dichlorvos: Dichlorvos exposure. L-ARG= L-arginine: L-arginine supplementation.

reduced gamma glutamyl transferase levels significantly ( $p < 0.05$ ) when compared to DDVP exposure.

### 3.10. Effect of L-arginine on albumin (ALB)

**Fig. 10** shows that rats treated to dichlorvos had significantly higher albumin levels ( $p < 0.05$ ) than the control group. L-arginine significantly reduced albumin levels ( $p < 0.05$ ) compared to those exposed to DDVP.

#### A. CONTROL

A micrograph of a liver sample treated with Haematoxylin and Eosin reveals a slightly congested vessel (indicated by a white arrow), with hepatocyte morphology appearing normal (pointed out by a blue arrow). Additionally, the sinusoids display a normal appearance without any signs of infiltration (highlighted by a slender arrow).

#### B. DICHLORVOS

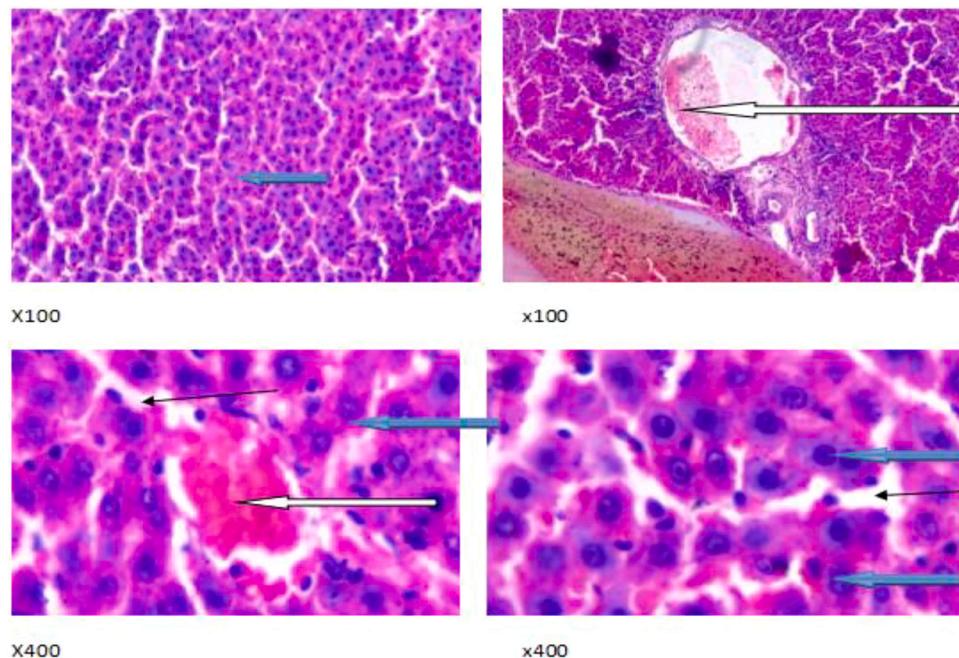
A micrograph of a liver tissue slice treated with Haematoxylin and Eosin illustrates central venules with moderate congestion (indicated by a white arrow) and a notable perivascular infiltration of inflammatory cells (highlighted by a black arrow). The hepatocytes exhibit a normal morphology (pointed out by a blue arrow), while the sinusoids show a mild infiltration (indicated by a slender arrow).

- C. A photomicrograph of a liver sample, stained with Haematoxylin and Eosin, depicts central venules with moderate congestion (indicated by a white arrow). The hepatocytes exhibit normal morphology (highlighted by a blue arrow), while the sinusoids show a moderate hemorrhagic appearance (indicated by a slender arrow).
- D. A photomicrograph of a liver sample, stained with Haematoxylin and Eosin, depicts central venules with moderate congestion (indicated by a white arrow). The hepatocytes exhibit normal morphology (highlighted by a blue arrow), while the sinusoids show a moderate hemorrhagic appearance (indicated by a slender arrow).

## 4. Discussion

Exposure to dichlorvos, an organophosphate pesticide, elevates inflammatory cytokines such as TNF- $\alpha$ , CRP, caspase-3, and IL-6, which contribute to chronic conditions like atherosclerosis, diabetes, and cancer by activating pro-inflammatory pathways (NF- $\kappa$ B, MAPK) [2] and causing oxidative stress, with documented toxic effects on vital organs and systems, including the liver, kidneys, brain, and immune system ([10]; Okorowu; [27] and [25]). Dichlorvos toxicity is caused by the suppression of acetylcholinesterase (AchE), which is considered to originate from acetylcholine buildup and overstimulation of parasympathetic neurones [21]. This stimulation caused sweating, nausea, lacrimation, vomiting, diarrhea, profuse bronchial secretions, and mortality [3,49]. Other musculoskeletal and nervous system side effects include muscle spasms, discomfort, weakness, lethargy, drowsiness, exhaustion, mental confusion, headache, seizures, coma, and even death ([47]; Nwakwo et al., 2019). This study highlights a significant reduction in blood inflammatory markers in rats receiving L-arginine supplementation, suggesting its vital role in tissue repair, immune function, and nitric oxide production, which improves vascular health and cytokine activity under stress conditions; additionally, biochemical markers of liver function, essential for metabolism and glucose regulation, can reflect its protective effects [23,37,45].

This study found that rats exposed to dichlorvos had greater levels of AST, ALT, ALP, and GGT than control rats, indicating hepatic impairment. Hepatocytes release the liver enzymes AST and ALT into the extracellular space [19], hence they are frequently utilized as indicators of liver disease [6]. This is similar with prior findings [20]. Alanine transaminase (ALT) is present in the kidneys, heart, muscles and in higher concentrations in the liver than in other body tissues. ALT functions exclusively in the cytoplasm and facilitates the transamination reaction. In comparison to other human tissues such as the liver, skeletal muscle, and kidney, the heart contains the most AST [41,44]. Bilirubin, a catabolic product of hemoglobin produced in the reticuloendothelial system, is first released unconjugated. When it enters the liver, it is converted into conjugated forms of bilirubin—mono- and diglucuronides—by the enzyme UDP-glucuronyl transferase [26,31]. ALP can be found in the small intestine mucosa epithelium, the kidney's proximal convoluted tubule, bone, liver, and placenta. It transports



**Plate 1.** Histological examination of the liver in Control rats.

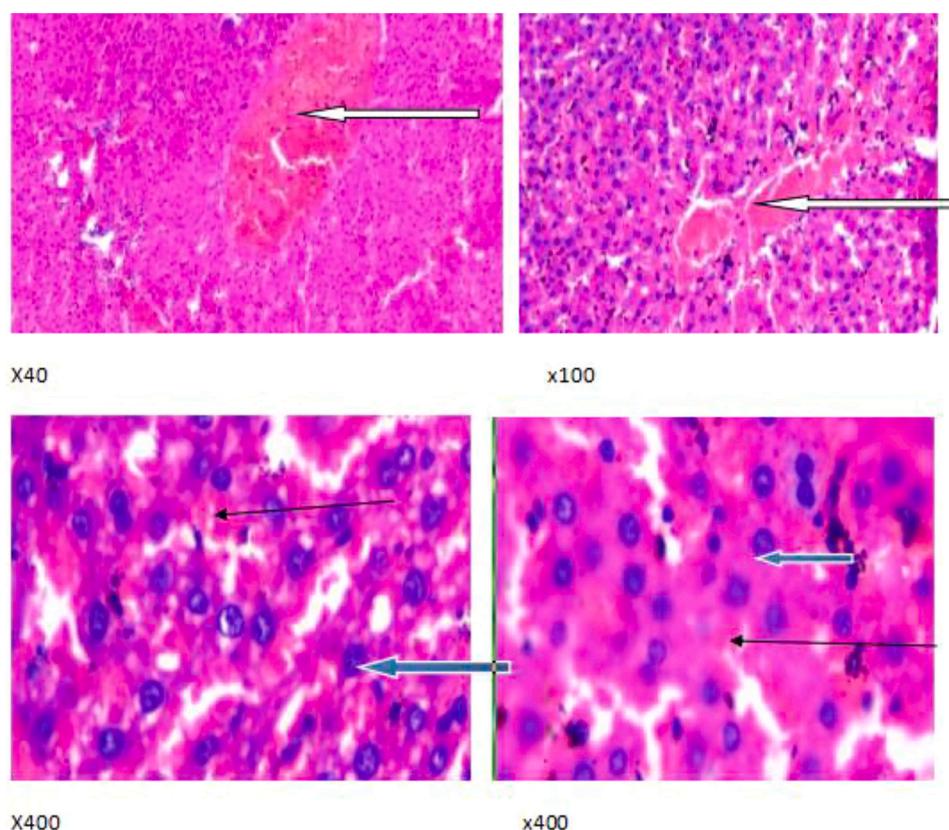


Plate 2. Histological examination of the liver in rats exposed to DDVP.

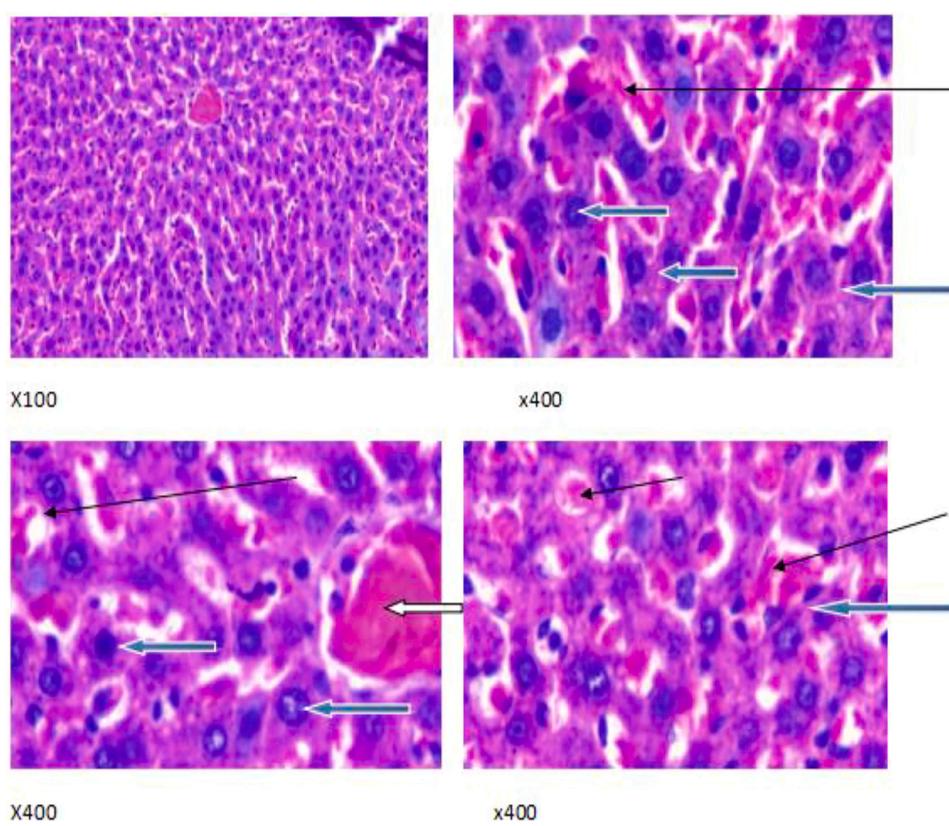
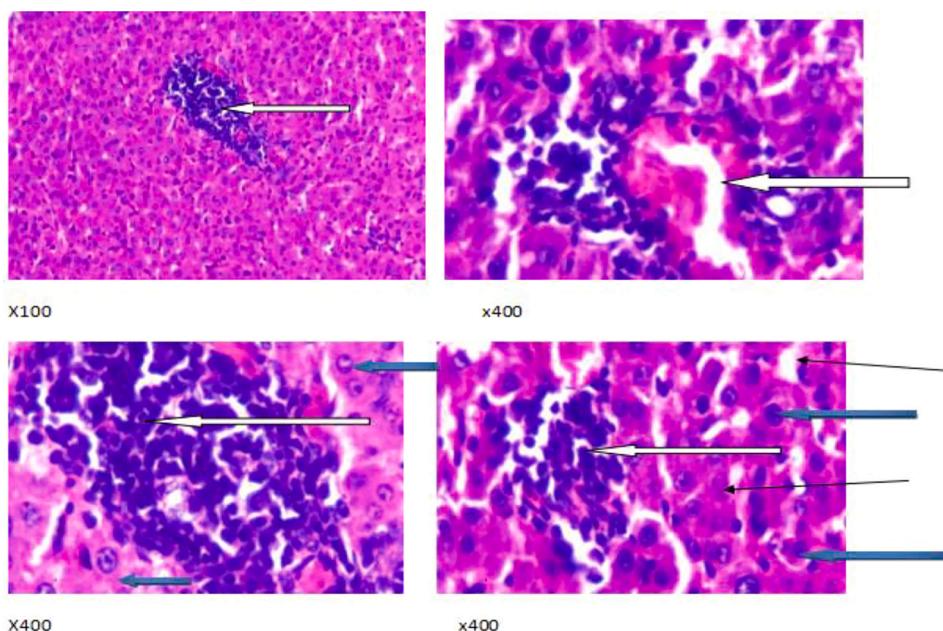


Plate 3. Histological examination of liver in rats exposed to L-arginine only.



**Plate 4.** Histological examination of rats exposed to DDVP+L-arginine.

lipids in the colon and calcifies bones. The liver produces the majority of serum ALP activity, with bone accounting for 50 % [13,38,4,46].

Furthermore, there are a number of biological and methodological reasons why some results, like ALT levels, are not entirely significant in all groups compared with control. In terms of biology, the effects may be mild or take longer to show up, especially if the intervention has an indirect influence on liver function. Methodologically, statistical power may have been diminished by group variability, such as individual variations in metabolic responses or baseline ALT levels.

GGT, a microsomal enzyme is present in hepatocytes, biliary epithelial cells, renal tubules, pancreas, and the intestines [30]. Serum GGT activity is primarily attributable to the hepatobiliary system [12], but it is more prevalent in kidney tissue [36] and the results from this investigation revealed higher GTT level in rats exposed to dichlorvos which may be suggestive of dysfunction of hepatobiliary system while those treated with L-arginine was observed to have had the dysfunction of the hepatobiliary system reversed as can be seen from the significant reduction in their serum GTT level. The results of histopathological studies showed that dichlorvos caused liver damage and the observed liver damage was manifested by mild portal vein obstruction and inflammatory cell aggregation [29], consistent with a study [28].

#### 4.1. Study limitations

It is important to recognize several limitations in order to guarantee the rigor of this investigation. First, because species-specific variations in metabolism and physiology can impact reactions to Dichlorvos (DDVP) and L-arginine supplementation, the results may not be as applicable to humans if only male Wistar rats were used. Given variations in dose and metabolic processes, care must be used when extrapolating rat data to human settings. Furthermore, the precise dosage and length of exposure used in the study might not adequately represent the cumulative effects of low-dose, prolonged DDVP administration that are frequently seen in real-world situations. A more thorough understanding of these cumulative effects may be provided by future research examining a variety of dosages and longer exposure times.

Further studies using female rats would also shed important light on possible gender-specific hormonal implications on the effects of L-arginine. Lastly, a longitudinal study might assess whether L-arginine's protective effects against DDVP toxicity are maintained over time or if

adaptive processes could change how effective it is. These additions would improve knowledge of the long-term protective effects of L-arginine after exposure to DDVP.

#### 4.2. Potential application to human health

Particularly for those who are at risk of pesticide exposure, such as agricultural workers or those living in areas with heavy pesticide use, L-arginine exhibits potential as a therapeutic supplement for reducing the negative consequences of toxic exposure. If human studies verify safe, effective dosages, L-arginine may help counterbalance the toxic effects of drugs like DDVP by increasing nitric oxide (NO) production, which improves vascular function and immunological response. Its hepatoprotective and anti-inflammatory qualities also suggest that it may be used to treat liver conditions that are linked to toxicity and inflammation, such as drug-induced liver injury and non-alcoholic fatty liver disease (NAFLD), in which oxidative stress plays a major role.

L-arginine supplementation may influence dietary recommendations for groups at risk from chemical exposure or increased oxidative stress if it is proven in human trials. Foods high in L-arginine, such meat, nuts, and seeds, may provide a natural means of raising NO levels and promoting liver health. The results of the study provide a preclinical basis for upcoming clinical investigations to examine the impact of L-arginine on human inflammation and liver function. These studies, which concentrate on liver function, immunological response, and general inflammatory markers, may aid in determining the best dosage, long-term safety, and particular advantages.

#### 5. Conclusion

This preclinical study concludes that supplementing rats with L-arginine may help lessen the negative effects of dichlorvos exposure. In the context of dichlorvos poisoning, the notable decrease in inflammation-related indicators seen in rats given L-arginine suggests a possible anti-inflammatory impact. Similarly, L-arginine may reverse dichlorvos-induced liver impairment based on its beneficial effects on liver function biomarkers. Although these results demonstrate the preventive potential of L-arginine, they should be interpreted cautiously because they are based on an animal model, and more research is required to ascertain whether they apply to human health.

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## Author contribution statement

**Saka WA, IGBAYILOLA Y.D, Lawan H.J, Zakari, M.B, Awujoola, D. E, Olarinde, P.O and Adegoke, V.O:** Designed and conceptualized the researches; conducted the investigations; Analysed and interpreted data; I contributed kits, equipment, and data analysis tools, as well as writing the text.

## Author Agreement

All authors agree to be accountable for all aspects of the work and ensure that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## CRediT authorship contribution statement

**V.O Adegoke:** Investigation, Formal analysis, Conceptualization. **P. O Olarinde:** Investigation, Formal analysis, Conceptualization. **H.J Lawan:** Resources, Investigation. **D. E Awujoola:** Methodology, Investigation, Formal analysis. **W.A Saka:** Supervision, Project administration, Methodology, Investigation, Conceptualization. **Igbayilola Yusuff:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the creation of this paper, the authors used Chat GPT to obtain further information. After utilizing this tool/service, the authors examined and edited the text as needed, and they accept full responsibility for the publication's contents.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Further reading

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