# L-Arginine Supplementation Mitigates Dichlorvos-Induced Haematocardiotoxicity, and Oxidative Stress in Male Wistar Rats

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## Abstract

Due to its toxicity, dichlorvos—a common organophosphate pesticide—poses significant risks to human health. This study utilized male Wistar rats to explore the potential protective effects of L-arginine supplementation against dichlorvos-induced toxicity, focusing on cardiotoxicity, haematotoxicity and oxidative stress. The rats were divided into four groups: Control, L-arginine (L), Dichlorvos (D), and L-arginine + Dichlorvos (L + D). Dichlorvos was administered to the D group, L-arginine (100 mg/kg) to the L group, and both L-arginine and dichlorvos to the L + D group. The study evaluated various parameters, including cardiovascular, oxidative stress markers, and haematological indices. Significant changes in haematological parameters such as haemoglobin (Hb), haematocrit (HCT), and red blood cell count (RBC) indicated haematotoxicity after dichlorvos administration. Additionally, elevated cardiac markers, including lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB), suggested cardiotoxic effects. Exposure to dichlorvos also resulted in decreased antioxidant enzyme levels and increased oxidative stress indicators like malondialdehyde (MDA). Remarkably, L-arginine supplementation mitigated the damage caused by dichlorvos. It normalized the altered haematological parameters, demonstrating its protective effect against haematotoxicity. The rise in cardiac markers was reduced with L-arginine supplementation, indicating protection against cardiotoxicity. Moreover, L-arginine significantly decreased oxidative stress, as evidenced by lower MDA levels and restored antioxidant enzyme activity. In conclusion, L-arginine supplementation in male Wistar rats showed promising protective effects against

dichlorvos-induced cardiotoxicity, haematotoxicity and oxidative stress. This suggests that L-arginine may offer a beneficial intervention to mitigate the adverse effects of dichlorvos on blood and heart health, paving the way for potential treatments for pesticide poisoning.

## 1. Introduction

Dichlorvos, an organophosphate commercially available since 1961 [1], is extensively used in agriculture, households, aircraft, and outdoor areas. Like other pesticides, it can be absorbed through the skin, ingestion, or inhalation, leading to neurotoxicity and various adverse impacts [2]. The primary mechanism of organophosphate toxicity involves acetyl cholinesterase inhibition, resulting in the accumulation of acetylcholine and subsequent symptoms of poisoning [3]. Chronic exposure to dichlorvos has been linked to health issues, including cancer and haematological disorders [4]. Dichlorvos is a widely used organophosphate insecticide that has drawn attention from all over the world because of its detrimental effects on human health and the environment. Known by another name, DDVP, this substance is used in agriculture, public health, and household pest management. Its effects on ecosystems, biodiversity, and human health are of concern [5]. Due to its poisonous qualities, there are serious dangers to human health and the environment. When ingested, inhaled, or exposed topically, there may be acute or long-term health consequences. Due to its persistence and bio accumulative nature in the environment, studies have connected dichlorvos exposure to neurological diseases, respiratory conditions, and possible carcinogenicity [6]. Furthermore, target pests have developed a resistance to pesticides as a result of the extensive agricultural use of dichlorvos, which could worsen environmental pollution [7]. International organizations have taken regulatory measures to reduce the use of dichlorvos; nevertheless, effective enforcement is still a challenge, especially in areas with weak regulatory frameworks [8]. Governments, scientists, industry stakeholders, and civil society must work together to address these issues, putting a focus on sustainable pest management techniques and the search for safer alternatives [9]. In Nigeria, dichlorvos is commonly used as a household and agricultural pesticide under various names like Nuvan, Sniper, and "Pia-pia" [10]. Despite its widespread use, there is a lack of knowledge about the haematological impacts of dichlorvos exposure in Nigerians. Blood serves as a reliable medium for assessing health status, with various parameters indicating physiological and pathological conditions in animals [11]. Cardiovascular diseases are a major global cause of death, and there is a need to understand the chronic impacts of pesticide exposure, particularly on cardi-

ovascular health [12]. Chronic exposure to pesticides, such as organochlorines, has been associated with cardiovascular diseases through mechanisms like altered lipid metabolism [12]. Electrocardiogram (ECG) changes, including prolonged QTc interval, sinus tachycardia, and other cardiac manifestations, have been observed in acute organophosphate poisoning. Chronic pesticide exposure has been linked to non-fatal myocardial infarction and arterial peripheral disease [13]. L-arginine, an indispensable amino acid, plays a pivotal role in cardiovascular function and exhibits potential therapeutic impacts in alleviating pesticide-induced toxicity [14]. Beyond its cardiovascular contributions, L-arginine serves various essential biological functions. It contributes to acid-base balance, a clinically underappreciated aspect, as the urea cycle significantly consumes bicarbonate [15], playing a critical role in maintaining acid-base homeostasis [15]. This amino acid significantly influences the proper functioning of the cardiovascular system (CVS). Clinical studies involving hypertensive, diabetic, and healthy individuals suggest that L-arginine can regulate vascular haemostasis [15]. Supplementation with L-arginine has been shown to ameliorate the pathology of various diseases in clinical practice [14]. Vascular endothelial dysfunctions, considered pathological changes in the cardiovascular system, often coincide with a reduction in nitric oxide (NO) synthesis [16]. The oxidative stress observed in endothelial cells is associated with vascular endothelial dysfunctions and diminished NO synthesis, factors linked to the aetiology of numerous pathological alterations in the cardiovascular system [17]. The impact of L-arginine supplementation on haematological parameters, cardiovascular response, and oxidative biomarkers in male Wistar rats exposed to dichlorvos is crucial for several reasons. First, the commonly used pesticide dichlorvos has been associated with haematotoxicity, emphasizing the need to understand and mitigate its harmful impacts. Second, the potential of the essential amino acid L-arginine to combat such toxicity remains an unstudied area. Third, this research is related to the growing concern about pesticide exposure and the need to identify protective measures to protect physiological functions. Fourth, the incorporation of arginine has the potential to provide new perspectives on toxicity mechanisms related to oxidative stress, cardiovascular, and haematological responses. It may also demonstrate protective effects via mechanisms like enhanced nitric oxide bioavailability and decreased oxidative stress [18]. This study aims to investigate the impacts of L-arginine supplementation on haematological parameters, cardiovascular biomarkers, and oxidative responses in rats exposed to dichlorvos. The research addresses a critical gap in the literature and may contribute to developing strategies to mitigate the adverse effects of pesticide exposure. The findings are expected to provide insights into the protective role of L-arginine and inform future interventions for pesticide-related health issues.

## 2. Materials and Methods

Forty (40) healthy male Wistar rats weighing between 220 - 250 g, obtained from

a private breeder, were selected for the study. The use of Wistar rats in research offers a balance between practical considerations, genetic stability, and biological relevance, making them valuable models for a wide range of scientific investigations. These rats had not undergone any prior experimental interventions and were considered healthy based on the absence of stress or infection indicators. Before initiating the experiments, the rats were individually weighed after a period of acclimatization lasting two weeks. They were housed in well-ventilated plastic cages at the animal facility of the Department of Physiology, Faculty of Basic Medical Sciences, LAUTECH, Ogbomoso, Oyo State. During the research, the animals were provided with both standard diet and water under 12 hours light/12 hours dark cycle; temperature range; 25 ± 2°C and 60% - 65% humidity. The experimental protocols adhered to the guidelines outlined in the National Research Council's Guide for the Care and Use of Laboratory Animals.

## 2.1. Arrive Statement

All animal experiments comply with the ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments and the National Research Council's Guide for the Care and Use of Laboratory Animals.

## 2.2. Ethical Approval

Ethical approval was sought and given to conduct this study from Ethics and Review Committee of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso with a reference number FBMS/AECP/092/23.

## 2.3. Dosage and Administration

A low dose of 100 mg/kg was used to determine the dose administered to each animal [19] [20]. A low dose of 100 mg/kg was used to calculate the dosage given to each animal. Based on the formula weight (g) × dosage (100 mg/1000 g), the computation was performed with an average weight of 250 g for the rats. The amount of L-arginine given was calculated based on the fact that one 500 mg capsule of L-arginine includes ten rats totaling 2500 g in weight. The proportion for 2500 g (×) of L-arginine was calculated using 1000 g containing 100 mg of L-arginine. As a result, the group of 10 male Wistar rats received 250 ml of L-arginine. Consequently, 25 mg of L-arginine were given to each rat.

## 2.4. Chemical

DDVP (®Sniper, containing 1000 g of DDVP/Litre; manufactured by Forward (Beinaj) Hepu Pesticide Co. Limited, China from Saro Agrosciences Limited, Oyo state, Nigeria) was used. Rats in DDVP group was exposed to 50 ml DDVP/50 ml distilled water-Dichlorvos, as previously mentioned, was added to

an open container to facilitate the odour's easy dispersion into and out of it, preventing caged animals from inhaling it. Inside a container was the metal cage in order to avoid body contact, the container was positioned within the box at a corner so that the animals could not pour out its contents [19] [20].

## 2.5. Determination of Body Weight

Rats were weighed before dosing and weekly during the experiment using a scale. The weight of the animal is necessary to determine the dose of medicine administered to the animal (Salter, England).

## 2.6. Grouping

They were divided into four groups of ten (10) animals each as follows:

**Group A** (control group): They received only food and water throughout the study.

**Group B** (only dichlorvos): This group was exposed to dichlorvos by inhalation for 10 minutes in a desiccator containing 2 ml of dichlorvos, which was moistened daily for three weeks without treatment.

**Group C** (dichlorvos (DDVP) + L-arginine): This group was exposed to dichlorvos in the same way as group B and treated with oral L-arginine for six weeks.

**Group D** (only L-arginine): This group received only one supplemental dose of L-arginine per day for six weeks.

#### Route of drug administration

Dichlorvos was administered by inhalation while L-arginine was given by oral route.

## 2.7. Collection of Blood Samples

Rats were sacrificed using ketamine as a sedative [21]. Blood samples were collected by cardiac puncture into plain bottles, allowed to clot at 40°C for 1 h, and centrifuged at 3000 × g for 15 min to obtain serum samples for biochemical analysis. The obtained serum samples were stored at −20°C until assayed [22].

## 2.8. Collection of Tissues

The animals were dissected; kidney and heart tissues were removed and washed in an ice cold trichloroacetic acid and rinsed with 1.15% KCl and blotted [23]. The samples were stored in a standard bottle containing formalin and normal bottles containing normal saline and were kept cold at −4℃ before biochemical analysis [23]-[25].

## 2.9. Haematological Parameters

The process followed the instructions provided in the Haematology Analyser manual (Sysmex Kx21). Various blood parameters, such as total red blood cell

(RBC) count (× 10/uL), hemoglobin content (Hb; g/dL), hematocrit (HCT; %), total white blood cell (WBC) or leukocyte count (× 10/uL), lymphocyte count (LYM; × 10/uL), and platelet (PLT) count (× 10/uL), were assessed. Additionally, mean corpuscular volume (MCV; fL), mean corpuscular hemoglobin (MCH; pg), and mean corpuscular hemoglobin concentration (MCHC; %) were calculated as part of the analysis.

## 2.10. Cardiovascular Parameters

### Electrocardiography (ECG) on Male Wistar Rats

The rats underwent intramuscular anaesthesia using a combination of ketamine and xylazine at 2.5 mg/kg, inducing muscle relaxation for several minutes. Subsequently, the animals were carefully positioned in a supine posture on a clean board. Wet gel was applied to the skin surface of both arms and legs, and electrodes were meticulously affixed to these coated areas. The ECG amplifier was then linked to the monitor, and the recorded data were repeatedly collected and saved for all animals.

The trachea tube was then secured in place by wrapping two loops of thread around it. The carotid artery was meticulously isolated from the vagus nerve, exteriorized, and cannulated using a PE50 cannula filled with 1% normal saline. The pressure transducer (model 7 d, grass instrument Massachusetts, USA) was connected to the cannula via a 3-way tap, and the pressure transducer was then connected to a grass polygraph through a driver amplifier and preamplifier, and finally to an ink writing stylus. A 10- to 15-minute rest period was given to the animal before blood pressure measurements were made. The diastolic pressure (DP) plus one-third of the pulse pressure (PP) (DP + 1/3PP) were added to determine the mean arterial pressure. The difference between the diastolic and systolic pressures (measured in millibars) is known as pulse pressure [26].

## 2.11. Lactate Dehydrogenase and Creatinine Kinase

Lactate dehydrogenase and Creatinine kinase were determined colometrically by the method specified in AGAPPE kit, Switzerland.

## 2.12. Cardiac Troponin I

Cardiac troponin I was determined using rat cardiac troponin ELISA kit, Elabscience, Wuhan, China with manufacturer's instruction strictly followed.

## 2.13. Oxidative Stress Studies

Superoxide dismutase (SOD) activity was assayed in line with the protocol described by [27], reduced glutathione (GSH) activity was assayed as described by [27], Catalase (CAT) activity was determined by the protocol of [28] and Malondialdehyde (MDA) level was assayed according to the protocol described by [29].

## 2.14. Statistical Analysis

Data were recorded as mean ± standard error of the mean. Statistical differences between means were determined by one-way analysis of variance. Turkey's post hoc test was used to identify differences between individual means. Confidence intervals were set at 95%, and results with a value of $p < 0.05$ were accepted as significant in all cases (Graph Pad Prism 5, Graph Pad Software, Inc., La Jolla, CA, USA).

## 3. Results

### 3.1. Impact of L-Arginine on Haematological Parameters

#### 3.1.1. Impact of L-Arginine on White Blood Cell (WBC)

A noteworthy elevation ($p < 0.05$) in white blood cell (WBC) count was observed in rats exposed to DDVP as opposed to the control group. Nevertheless, supplementation with L-arginine resulted in a substantial reduction ($p < 0.05$) in WBC count compared to rats exposed to DDVP. Refer to Table 1 for details.

#### 3.1.2. Impact of L-Arginine on Red Blood Cell (RBC)

The outcomes depicted in Table 1 indicate an insignificant decline in red blood cell (RBC) count in rats exposed to DDVP compared to the control group. Nonetheless, supplementation with L-arginine resulted in a non-significant elevation ($p > 0.05$) in RBC count when compared to DDVP-exposed rats.

#### 3.1.3. Impact of L-Arginine on Haemoglobin (HGB)

The findings presented in Table 1 reveal an insignificant reduction in haemoglobin (HGB) levels in rats exposed to DDVP in comparison to the control group. Nevertheless, the introduction and application of L-arginine led to a significant increase ($p < 0.05$) in HGB levels when contrasted with DDVP-exposed rats.

#### 3.1.4. Impact of L-Arginine on Haematocrit (HCT)

The data depicted in Table 1 illustrates an insignificant reduction in haematocrit (HCT) in rats exposed to DDVP as opposed to the control rats. However, the administration of L-arginine resulted in a non-significant rise in HCT when compared to rats exposed to DDVP.

#### 3.1.5. Impact of L-Arginine on Mean Corpuscular Volume (MCV)

The findings presented in Table 1 indicate an insignificant decline ($p > 0.05$) in mean corpuscular volume (MCV) in rats exposed to DDVP compared to the control group. However, the administration of L-arginine resulted in a non-significant increase ($p > 0.05$) in MCV when compared to DDVP-exposed rats.

#### 3.1.6. Impact of L-Arginine on Mean Corpuscular Haemoglobin (MCH)

The outcomes presented in Table 1 demonstrate a statistically significant reduction

**Table 1.** Impact of L-arginine on haematological parameters of dichlorvos-exposed rats.

| Parameters | CONTROL | DDVP ONLY | L-ARG+DDVP | L-ARG CONTROL |
|---|---|---|---|---|
| WBC (uL$^{-1}$) | 4900 ± 426.6 | 7875 ± 377.3$^a$ | 5900 ± 228.0$^b$ | 5740 ± 370.9$^b$ |
| RBC (uL$^{-1}$) | $8.34 \times 10^6$ ± 160799 | $7.75 \times 10^6$ ± 477081 | $8.52 \times 10^6$ ± 120277 | $8.72 \times 10^6$ ± 231283 |
| HGB (dL$^{-1}$) | 11.72 ± 0.29 | 10.92 ± 0.43 | 11.78 ± 0.07 | 12.16 ± 0.27 |
| HCT (%) | 44.70 ± 0.84 | 42.95 ± 1.91 | 43.70 ± 0.89 | 47.10 ± 0.83 |
| MCV (fl) | 55.28 ± 0.74 | 52.70 ± 0.51 | 53.02 ± 0.31 | 52.24 ± 0.95 |
| MCH (pg) | 15.05 ± 0.27 | 13.65 ± 0.06$^a$ | 13.78 ± 0.05$^a$ | 14.33 ± 0.13$^{ab}$ |
| MCHC (dL$^{-1}$) | 27.03 ± 0.34 | 25.63 ± 0.13 | 26.23 ± 0.09 | 25.73 ± 0.17 |
| PLT (uL$^{-1}$) | 892000 ± 18713 | 773500 ± 27864 | $1.2 \times 10^7$ ± 74818$^{ab}$ | 845750 ± 24430$^c$ |

Values are presented as Mean ± SEM. $^a$Indicate a noteworthy distinction when compared to the control at a significance level of $p < 0.05$. $^b$Indicate a significant distinction when compared to rats exposed to DDVP at $p < 0.05$. $^c$Indicate a significant distinction when compared to rats exposed to DDVP + L-ARG at $p < 0.05$. ***DDVP-Dichlorvos L-ARG = L-arginine.***

($p < 0.05$) in mean corpuscular haemoglobin (MCH) in rats exposed to DDVP compared to the control group. Nevertheless, the administration of L-arginine resulted in a significant increase ($p < 0.05$) in MCH when compared to DDVP-exposed rats.

### 3.1.7. Impact of L-Arginine on Mean Corpuscular Haemoglobin Concentration (MCHC)

The findings depicted in Table 1 reveal a statistically significant decrease ($p < 0.05$) in mean corpuscular haemoglobin concentration (MCHC) in rats exposed to DDVP compared to the control group. However, the administration of L-arginine led to a non-significant increase in MCHC when compared to DDVP-exposed rats.

### 3.1.8. Impact of L-Arginine on Platelets (PLT) in Dichlorvos-Exposed Rats

The outcome depicted in Table 1 indicates a statistically insignificant reduction in platelet (PLT) levels in rats exposed to DDVP compared to control rats. Nevertheless, the administration of L-arginine significantly elevated ($p < 0.05$) PLT levels when compared to rats exposed to DDVP.

## 3.2. Impact of L-Arginine on Cardiovascular Function

### 3.2.1. Impact of L-Arginine on Systolic Pressure in Dichlorvos-Exposed Rats

Rats exposed to DDVP exhibited a statistically insignificant increase ($p > 0.05$) in systolic pressure compared to the control group. However, the administration of L-arginine led to a significant reduction ($p < 0.05$) in systolic pressure compared to rats exposed to DDVP, as depicted in Table 2.

### 3.2.2. Impact of L-Arginine on Diastolic Pressure in Dichlorvos-Exposed Rats

Rats exposed to DDVP demonstrated a statistically significant increase ($p < 0.05$)

in diastolic pressure compared to the control group. However, the administration and treatment with L-arginine resulted in a significant decrease (p < 0.05) in diastolic pressure compared to rats exposed to DDVP, as illustrated in Table 2.

### 3.2.3. Impact of L-Arginine on Heart Rate in Dichlorvos-Exposed Rats
Rats exposed to DDVP exhibited a statistically significant increase (p < 0.05) in heart rate compared to the control group. Nevertheless, the administration and treatment with L-arginine led to a non-significant decrease in heart rate compared to rats exposed to DDVP, as illustrated in Table 2.

### 3.2.4. Impact of L-Arginine on P-Wave in Dichlorvos-Exposed Rats
There was a non-significant decrease in the P-WAVE among rats exposed to DDVP compared to control rats. However, the administration of L-arginine resulted in a significant increase (p < 0.05) in the P-WAVE compared to rats exposed to DDVP, as shown in Table 2.

### 3.2.5. Impact of L-Arginine on PR-Wave in Dichlorvos-Exposed Rats
Rats exposed to DDVP showed a significant increase (p < 0.05) in the PR-wave compared to control rats. Nevertheless, the administration and treatment with L-arginine led to a non-significant decrease in the PR-wave compared to rats exposed to DDVP, as depicted in Table 2.

### 3.2.6. Impact of L-Arginine on the QRS-Wave in Dichlorvos-Exposed Rats
There was a non-significant increase in the QRS-wave among rats exposed to DDVP compared to control rats. Nonetheless, the administration and treatment with L-arginine significantly raised (p < 0.05) the QRS-wave when compared to rats exposed to DDVP, as illustrated in Table 2.

### 3.2.7. Impact of L-Arginine on QT-Wave in Dichlorvos-Exposed Rats
There was a significant decrease (p < 0.05) in the QT-wave among rats exposed to DDVP compared to control rats. However, the administration and treatment with L-arginine significantly increased (p < 0.05) the QT-wave when compared to rats exposed to DDVP, as depicted in Table 2.

### 3.2.8. Impact of L-Arginine on QTC-WAVE in Dichlorvos-Exposed Rats
There was a non-significant decrease in the QTC-WAVE among rats exposed to DDVP compared to control rats. Additionally, the administration of L-arginine resulted in a non-significant reduction in the QTC-WAVE when compared to rats exposed to DDVP, as depicted in Table 2.

**Table 2.** Impact of L-arginine on cardiovascular parameters in dichlorvos-exposed rats.

| Parameters | CONTROL | DDVP ONLY | L-ARG + DDVP | L-ARG ONLY |
|---|---|---|---|---|
| SBP (mmHg) | 148.5 ± 0.65 | 156.3 ± 1.60 | 113.5 ± 0.65[ab] | 92.25 ± 4.87[abc] |
| DBP (mmHg) | 118.0 ± 0.71 | 127.0 ± 2.78[a] | 102.4 ± 1.60[ab] | 93.00 ± 1.44[abc] |
| MAP (ms) | 137.5 ± 1.64 | 112.4 ± 1.34[a] | 102.1 ± 1.94[ab] | 106.6 ± 1.86[a] |
| HR (bpm) | 217.0 ± 5.51 | 264.3 ± 6.40[a] | 258.7 ± 6.36[a] | 248.3 ± 4.84[a] |
| P-Wave (ms) | 18.25 ± 2.50 | 16.00 ± 0.82 | 29.25 ± 3.01[ab] | 19.25 ± 2.46[a] |

**Continued**

| | | | | |
|---|---|---|---|---|
| PR-Wave (ms) | 38.25 ± 3.50 | 52.60 ± 2.56 | 47.80 ± 2.52 | 46.80 ± 2.27 |
| QRS-Wave (ms) | 12.25 ± 1.03 | 12.75 ± 1.11 | 16.78 ± 0.63[ab] | 13.25 ± 0.85 |
| QT-Wave (ms) | 77.75 ± 3.88 | 59.50 ± 2.06[a] | 66.25 ± 4.50 | 77.75 ± 2.10[b] |
| QTC-Wave (ms) | 154.3 ± 5.54 | 124.5 ± 2.10[a] | 142.0 ± 9.90 | 154.3 ± 6.40[b] |

*Values are presented as Mean ± SEM. [a]Indicate a noteworthy distinction when compared to the control at a significance level of $p < 0.05$. [b]Indicate a significant distinction when compared to rats exposed to DDVP at $p < 0.05$. [c]Indicate a significant distinction when compared to rats exposed to DDVP + L-ARG at $p < 0.05$.* **DDVP-Dichlorvos L-ARG = L-arginine.**

### 3.3. Impact of L-Arginine on Cardiac Troponin I in Dichlorvos-Exposed Rats

The results depicted in **Figure 1** demonstrate a significant increase in troponin I levels in animals exposed to dichlorvos compared to the control group at $p < 0.05$. Furthermore, the administration of L-arginine led to a substantial decrease in the troponin I levels of the rats compared to those exposed to DDVP at $p < 0.05$.
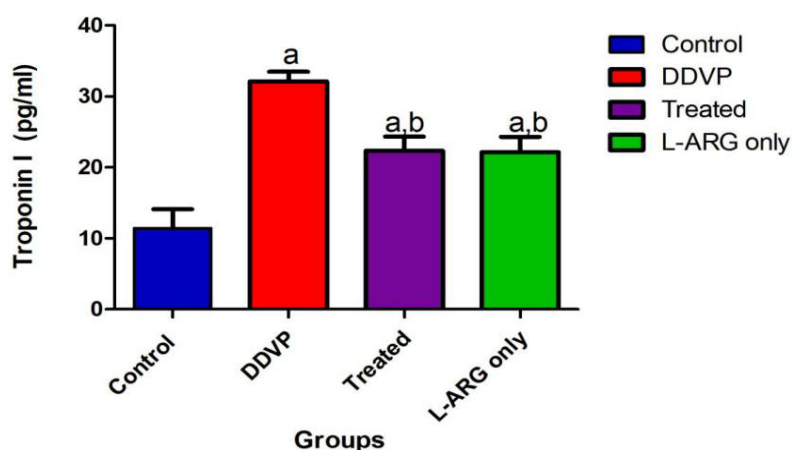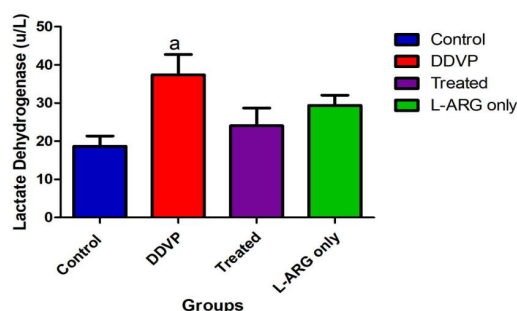


*Values are presented as Mean ± SEM. [a]Indicate a noteworthy distinction when compared to the control at a significance level of $p < 0.05$. [b]Indicate a significant distinction when compared to rats exposed to DDVP at $p < 0.05$. [c]Indicate a significant distinction when compared to rats exposed to DDVP + L-ARG at $p < 0.05$.* **DDVP-Dichlorvos L-ARG= L-arginine.**

**Figure 1.** Impact of L-arginine on Troponin I of dichlorvos-exposed and L-arginine supplemented rats.

### 3.4. Impact of L-Arginine on Some Glucometabolic Enzymes

#### 3.4.1. Impact of L-Arginine on Lactate Dehydrogenase (LDH) in Dichlorvos-Exposed Rats

The results illustrated in **Figure 2** revealed a significant increase in lactate dehydrogenase (LDH) levels in animals exposed to dichlorvos compared to the control group at $p < 0.05$. Additionally, the administration of L-arginine led to a non-significant decrease in the lactate dehydrogenase (LDH) levels of the rats compared to those exposed to DDVP at $p > 0.05$.
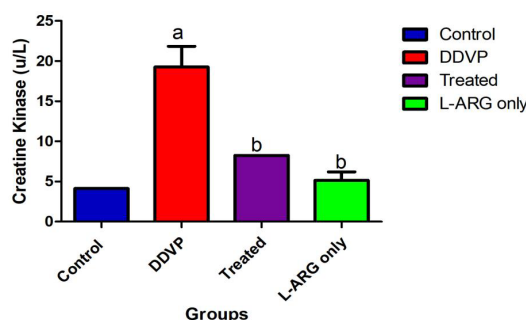
*Values are presented as Mean ± SEM. [a]Indicate a noteworthy distinction when compared to the control at a significance level of p < 0.05. [b]Indicate a significant distinction when compared to rats exposed to DDVP at p < 0.05. [c]Indicate a significant distinction when compared to rats exposed to DDVP + L-ARG at p < 0.05.* **DDVP-Dichlorvos L-ARG = L-arginine.**

**Figure 2.** Impact of L-arginine on lactate dehydrogenase of dichlorvos-exposed and L-arginine supplemented rats.

### 3.4.2. Impact of L-Arginine on Creatinine Kinase in Dichlorvos-Exposed Rats

The results depicted in **Figure 3** indicate a significant increase in creatinine kinase levels in animals exposed to dichlorvos compared to the control group at p < 0.05. Furthermore, the administration of L-arginine led to a significant reduction in creatinine kinase levels in the rats compared to those exposed to DDVP at p < 0.05.



*Values are presented as Mean ± SEM. [a]Indicate a noteworthy distinction when compared to the control at a significance level of p < 0.05. [b]Indicate a significant distinction when compared to rats exposed to DDVP at p < 0.05. [c]Indicate a significant distinction when compared to rats exposed to DDVP + L-ARG at p < 0.05.* **DDVP-Dichlorvos L-ARG = L-arginine.**

**Figure 3.** Impact of L-arginine on creatinine kinase of dichlorvos-exposed and L-arginine supplemented rats.

### 3.5. Impact of L-Arginine on MDA, CAT, SOD and GSH of Dichlorvos-Exposed Rats

The results suggest that the exposure of animals to dichlorvos significantly increased (p < 0.05) malondialdehyde levels compared to the control, as depicted in **Table 3**. Furthermore, the administration of L-arginine led to a significant decrease (p < 0.05) in malondialdehyde levels in rats when compared to those exposed to dichlorvos **Table 3**.

**Table 3.** Impact of L-arginine on MDA, CAT, SOD and GSH in dichlorvos-exposed rats.

| Parameters | CONTROL | DDVP ONLY | M-ARG + DDVP | L-ARG ONLY |
|---|---|---|---|---|
| MDA (umol/ml) | 24.2 ± 3.94 | 67.00 ± 3.94[a] | 31.04 ± 0.88[ab] | 31.04 ± 0.88[bc] |
| CAT (umol/ml) | 1.18 ± 0.08 | 0.25 ± 0.00[a] | 0.68 ± 0.08[ab] | 1.10 ± 0.08[bc] |
| SOD (umol/ml) | 42.29 ± 3.13 | 11.82 ± 1.48[a] | 24.88 ± 1.97[ab] | 41.04 ± 3.59[bc] |
| GSH (umol/ml) | 1.99 ± 0.01 | 0.92 ± 0.03[a] | 1.41 ± 0.05[ab] | 0.92 ± 0.04[ac] |

Values are presented as Mean ± SEM. [a]Indicate a noteworthy distinction when compared to the control at a significance level of $p < 0.05$. [b]Indicate a significant distinction when compared to rats exposed to DDVP at $p < 0.05$. [c]Indicate a significant distinction when compared to rats exposed to DDVP + L-ARG at $p < 0.05$. **DDVP-Dichlorvos L-ARG = L-arginine.**

In **Table 3**, the findings indicated that exposure of animals to dichlorvos caused a significant decrease ($p < 0.05$) in catalase levels compared to the control. Conversely, the administration of L-arginine resulted in a significant increase ($p < 0.05$) in catalase levels in rats when compared to those exposed to dichlorvos.

**Table 3** demonstrated that exposure of animals to dichlorvos caused a significant decrease ($p < 0.05$) in superoxide dismutase levels compared to the control. However, the administration of L-arginine resulted in a significant increase ($p < 0.05$) in superoxide dismutase levels in rats when compared to those exposed to dichlorvos.

Finally, the results presented in **Table 3** indicated that exposure of animals to dichlorvos led to a significant decrease ($p < 0.05$) in glutathione (GSH) levels compared to the control. Moreover, the administration of L-arginine resulted in a significant increase ($p < 0.05$) in glutathione levels in rats when compared to those exposed to dichlorvos.

## 4. Discussion

Dichlorvos is highly toxic and can enter the body through inhalation, dermal absorption, and ingestion [31]. Inhalation is the primary exposure route in domestic use due to dichlorvos' volatile nature. Our study found changes in haematological parameters in dichlorvos-exposed Wistar rats, indicating anaemia, contrary to previous reports [32]. The toxicants, once in the body, travel through the blood to various organs, affecting animal health. Exposure led to normocytic normochromic anaemia, possibly due to increased red blood cell destruction beyond bone marrow capacity [33]. Haematological indicators, such as platelet count, haemoglobin concentration, and erythrocyte count, were altered as a result of dichlorvos exposure. These alterations align with the haematotoxic consequences documented in the literature related to exposure to organophosphate pesticides [33]. Moreover, the observed decline in haemoglobin concentration and erythrocyte count may indicate the onset of anaemia, a known side effect of pesticide exposure [34]. However, L-arginine treatment ameliorated dichlorvos-induced anaemia, restoring blood parameters and suggesting potential im-

mune system improvement [35]. Epidemiological evidence links pesticide exposure, including dichlorvos, to cardiovascular diseases (CVD) [13]. Dichlorvos accumulates in organs, causing toxic impacts [20]. The cardiovascular response was also significantly altered by dichlorvos exposure, as shown by changes in blood pressure, heart rate, and electrocardiographic parameters. These results are consistent with earlier research showing the cardiotoxic effects of pesticides, including organophosphates, which can cause arrhythmias and interfere with autonomic control [36]. According to [37], sympathetic overactivity is a common reaction to stress caused by pesticides, which could explain the observed rise in heart rate and blood pressure. Blood pressure associations with pesticides vary, with some studies reporting positive links [38], while others show negative associations [39]. Dichlorvos exposure increased blood pressure in rats, but L-arginine treatment alleviated this impact. This strongly agrees with a previous study [17]. The cardiovascular system's homeostasis of vascular tone, interactions between the vascular wall and circulating blood cells (primarily leukocytes and thrombocytes), and vascular structure all depend critically on the NO produced by eNOS in response to stimulation of mechanoreceptors by the flowing blood's shear stress. These functions have been thoroughly addressed in recent years [40]. One of the main pathogenic factors in the development of vascular disorders such as atherosclerosis, hypertension, and diabetic angiopathy is impaired NO formation or function in the vasculature [40]. Conversely, it has been demonstrated that a primary factor contributing to the loss of vascular resistance in septic shock is the overproduction of NO by iNOS [41]. Consequently, L-arginine-dependent metabolic pathways are important determinants of a number of pathological diseases, and L-arginine plasma content is strictly regulated. DVPP-exposed rats displayed elevated heart rate and ECG abnormalities, consistent with the cholinergic impacts of dichlorvos [13]. L-arginine treatment reduced heart rate and mitigated ECG changes, suggesting its cardio-protective potential. Histopathological examination revealed dichlorvos-induced heart damage, which L-arginine partially alleviated. Biomarkers such as CK-MB, LDH, and Troponin I indicated cardiac injury, with L-arginine reducing their levels. L-arginine, a semi-essential amino acid, plays roles in protein synthesis, immune function, and vasodilation through nitric oxide production [42]. Supplementation decreases cardiac injury markers and could potentially enhance antioxidant defense [43]. Our research showed that after being exposed to dichlorvos, there was a significant rise in oxidative stress indicators, such as malondialdehyde (MDA) levels, and a decrease in antioxidant enzyme activity. According to [34], these results point to increased lipid peroxidation and compromised antioxidant defense mechanisms, both of which are signs of oxidative stress. According to [44], oxidative stress is a critical factor in the pathophysiology of pesticide-induced toxicity, as it leads to tissue damage and malfunction. It's interesting to note that in the parameters under investigation, L-arginine supplementation had protective benefits against dichlorvos-induced toxicity. Strong

antioxidant and vasodilatory qualities of L-arginine, a precursor of nitric oxide (NO), may mitigate the oxidative stress and vascular dysfunction brought on by pesticide exposure [43]. Furthermore, it has been demonstrated that supplementing with L-arginine increases NO bioavailability, which enhances endothelial function and improves cardiovascular health [45]. Supplementation with L-arginine may lessen the negative effects of dichlorvos on haematological markers and cardiovascular response by reducing oxidative stress and maintaining cardiovascular homeostasis. L-arginine, as well as the expression and activity of the cationic amino acid transporters (CAT), which are in charge of bringing L-arginine into the cell, are prerequisites for appropriate endogenous iNOS activity during inflammation [46]. Serum arginine levels in healthy humans vary from 60 to 100 µmol [47] and comparable values for rats are reported [48]. Depletion of available substrate, restricted availability, or disruption of CAT activity can result in reduced endothelium iNOS-derived NO production and reduced resistance to atherosclerosis.

## 5. Conclusion

In summary, this investigation explored the impact of L-arginine supplementation on male Wistar rats exposed to dichlorvos. The findings indicated significant alterations in blood parameters, cardiac metrics, and oxidative stress responses due to dichlorvos exposure. Notably, L-arginine treatment demonstrated efficacy in ameliorating anaemia and cardiac issues induced by dichlorvos, suggesting its potential therapeutic utility. Additionally, the study highlighted that L-arginine supplementation influenced antioxidant enzyme levels, mitigating pesticide-induced damage in rats. The results suggest that L-arginine may offer a beneficial intervention to alleviate the adverse impacts of dichlorvos on blood and heart health, paving the way for potential treatments for pesticide poisoning.

## 6. Clinical Significance

Findings on L-arginine's protective role against dichlorvos-induced haemotoxicity have broad implications for pesticide-related health issues, especially in agricultural workers and those exposed environmentally. Pesticides globally pose significant public health risks, including haematological, cardiovascular, and neurological disorders. Understanding pesticide toxicity mechanisms and interventions is vital. L-arginine supplementation could protect against haematotoxicity and cardiovascular issues in dichlorvos exposure. This offers therapeutic and preventive potential, especially for acute poisoning or chronic exposure situations. Clinical studies on L-arginine's efficacy in pesticide-exposed populations may reveal insights into its antioxidant and cardiovascular benefits. Preventive measures, including safety protocols and consuming L-arginine-rich foods, are crucial in reducing pesticide risks. Advocating for safer pesticide practices and educating the public, including agricultural workers, is essential. Collaborative efforts among medical professionals, scientists, policymakers, and

agricultural stakeholders are necessary to develop comprehensive strategies addressing pesticide-related health issues. This study demonstrates L-arginine's efficacy in mitigating dichlorvos-induced cardiac and blood parameter changes, suggesting its therapeutic potential. L-arginine supplementation also modulates antioxidant enzyme levels, mitigating pesticide-induced harm. These findings underscore L-arginine's promise in treating pesticide poisoning, advancing potential interventions for pesticide-exposed individuals.

## Author Contribution Statement

Saka, W.A., Igbayilola, Y.D., Lawan, H.J., Muftaudeen, T.K., Adejumo, R., Alu, T.D., Ikuomola, M., Ojelere, J.T. and Adegoke, V.O.: Designed and conceived the investigations; carried out the investigation; analyzed and interpreted the data; contributed kits, equipment, tools for data analysis and wrote the manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] WHO/IPCS (1989) Dichlorvos. World Health Organization, 79, 73.

[2] Mourad, L.A. (2005) Adverse Impact of Insecticides on the Health of Palestian Farm Workers in Myeloma and Occupation. *American Journal of Industrial Medicine*, **23**, 629-39.

[3] Harlin, K.S. and Dellinger, J.A (1993) Retina, Brain and Blood Cholinesterase Levels in Cats in Pesticide Plant on Workers' Serum and Erythrocyte Cholinesterase Activity. *International Journal of Occupational Medicine and Environmental Health*, **20**, 381-385.

[4] Joshaghani, H.R., Ahmadi, A.R. and Mansourian, M.R. (2007) Impacts of Occupational Exposure Locally Formulated Pesticide (Ota-Piapia) Utilized in Northeastern Nigeria. *Scientific Research and Essay*, **5**, 49-54.

[5] Juraske, R., Mutel, C.L., Stoessel, F. and Hellweg, S. (2020) Comprehensive Environmental Assessment of Organic Pesticides: A Case Study with Neem Oil. *Environmental Science & Technology*, **54**, 176-184.

[6] United Nations Environment Programme (2019) Global Chemicals Outlook II—From Legacies to Innovative Solutions: Implementing the 2030 Agenda for Sustainable Development.
https://www.unenvironment.org/resources/report/global-chemicals-outlook-ii-legacies-innovative-solutions

[7] Wang, J., Cheng, S., Zhang, L., Guo, J. and Zheng, X. (2020) Behavior and Effects of Dichlorvos Exposure on Honeybees: A Review. *Environmental Pollution*, **263**, Article ID: 114616.

[8] World Health Organization (2019) The Public Health Impact of Chemicals: Knowns and Unknowns.

[9] Zhang, W., Wang, X., Ma, Z., Wang, X., Yuan, Y., Wang, L. and Lu, X. (2020) Oc-

currence, Distribution and Risk Assessment of Organophosphate Esters in Sediments from the Yellow Sea. *Environmental Pollution*, **267**, Article ID: 115490.

[10] Kosuru, R., Singh, B., Lakshmikanthan, S., Nishijima, Y., Vasquez-Vivar, J., Zhang, D.X. and Chrzanowska, M. (2021) Distinct Signaling Functions of Rap1 Isoforms in NO Release from Endothelium. *Frontiers in Cell and Developmental Biology*, **9**, 687598. https://doi.org/10.3389/fcell.2021.687598

[11] Taiwo, V.O. and Anosa, V.O. (1995) Fibrinogen, Leukocyte and Haematocrit Values of Cattle with Various Disease Conditions. *Tropical Veterinary*, **13**, 51-57.

[12] Patel, C.J., Cullen, M.R., Ioannidis, J.P. and Butte, A.J. (2012) Systematic Evaluation of Environmental Factors: Persistent Pollutants and Nutrients Correlated with Serum Lipid Levels. *International Journal of Epidemiology*, **41**, 828-843. https://doi.org/10.1093/ije/dys003

[13] Wahab, A., Hod, R., Ismail, N. and Omar, N. (2016) The Impact of Pesticide Exposure on Cardiovascular System: A Systematic Review. *International Journal of Community Medicine and Public Health*, **3**, 1-10. https://doi.org/10.18203/2394-6040.ijcmph20151542

[14] Rashid, J., Kumar, S.S., Job, K.M., Liu, X., Fike, C.D. and Sherwin, C.M.T. (2020) Therapeutic Potential of Citrulline as an Arginine Supplement: A Clinical Pharmacology Review. *Pediatric Drugs*, **22**, 279-293. https://doi.org/10.1007/s40272-020-00384-5

[15] Chin-Dusting, J.P.F., Willems, L. and Kaye, D.M. (2007) L-Arginine Transporters in Cardiovascular Disease: A Novel Therapeutic Target. *Pharmacology & Therapeutics*, **116**, 428-436. https://doi.org/10.1016/j.pharmthera.2007.08.001

[16] Arishe, O., McKenzie, J., Priviero, F., Ebeigbe, A.B. and Webb, R.C. (2019) L-Arginase Induces Vascular Dysfunction in Old Spontaneously Hypertensive Rats. *Journal of African Association of Physiological Science*, **7**, 119-127.

[17] Arikawe, A.P., Udenze, I.C., Olusanya, A.W., Akinnibosun, O.A., Dike, I. and Duru, B.N. (2019) L-Arginine Supplementation Lowers Blood Pressure, Protein Excretion and Plasma Lipid Profile in Experimental Salt-Induced Hypertension in Pregnancy: Relevance to Preeclampsia. *Pathophysiology*, **26**, 191-197. https://doi.org/10.1016/j.pathophys.2019.02.002

[18] Gupta, R.K., Patel, A.K., Shah, N., Chaudhary, A.K., Jha, U.K., Yadav, U.C.S. and Kumar, S. (2020) Arginine: A Potential Candidate in the Therapeutics of COVID-19. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, **10**, 170-175.

[19] Luciano, R., Rodrigo, L. and Flávio, A.S. (2006) Impact of Oral Arginine Administration over BP and Cardiac Parameters in Rats Submitted to Chronic Inhibition of Nitric Oxide Synthesis. *Revista Brasileira de Medicina do Esporte*, **12**, 4-6.

[20] Saka, W.A., Ayoade, T.E., Akhigbe, T.M. and Akhigbe, R.E. (2020) *Moringa oleifera* Seed Oil Partially Abrogates 2,3-Dichlorovinyl Dimethyl Phosphate (Dichlorvos)-Induced Cardiac Injury in Rats: Evidence for the Role of Oxidative Stress. *Journal of Basic and Clinical Physiology and Pharmacology*, **32**, 237-246. https://doi.org/10.1515/jbcpp-2019-0313

[21] Igbayilola, Y.D. and Gujja, M.G. (2024) Alpha-Amylase and Alpha-Glucosidase Upregulated Glucose Homeostasis in High-Fat Fed Wistar Rats Supplemented with Cocoa Flavonoid-Rich Aqueous. *Food Bioscience*, **59**, Article ID: 104070. https://doi.org/10.1016/j.fbio.2024.104070

[22] Igbayilola, Y.D., Morakinyo, A.O. and Ranloye, B.O. (2021) Leptin-Resistance Induced Hyperphagia and Diminished Oxidative Balance in Offspring of Dams Exposed to Perinatal Protein Restriction. *African Journal of Biomedical Research*, **24**,

451-458.

[23] Igbayilola, Y.D., Morakinyo, A.O. and Iranloye, B.O. (2021) Adverse Effects of Perinatal Protein Restriction on Glucose Homeostasis in Offspring of Sprague-Dawley Rats. *Scientific African*, **14**, e01036. https://doi.org/10.1016/j.sciaf.2021.e01036

[24] Igbayilola, Y.D., Morakinyo, A.O. and Iranloye, B.O. (2021) Adverse Effects of Peri-Natal Protein Restriction on Regulators of Lipid Metabolism and Hepatic Function in Offspring of Sprague-Dawley Rats. *Nigerian Journal of Experimental and Clinical Bioscience*, **9**, 74-81.

[25] Igbayilola, Y.D., Aina O.S, Aikpitanyi, I., Williams, O.D. and Olaoye, F.A. (2023) Glucometabolic Response to Perinatal Walnut (*Juglans regia* L.) Supplemented Diet in Offspring of Sprague-Dawley Rats. *Singapore Journal of Scientific Research*, **13**, 79-87.

[26] Oloyo, A.K., Imaga, N.O.A., Fatope, Y. and Sofola, O.A. (2019) Sex Differences in Cardiac and Renal Responses to a High Salt Diet in Sprague-Dawley Rats. *Heliyon*, **5**, e01665. https://doi.org/10.1016/j.heliyon.2019.e01665

[27] Diniz Vilela, D., Gomes Peixoto, L., Teixeira, R.R., Belele Baptista, N., Carvalho Caixeta, D., Vieira de Souza, A., *et al.* (2016) The Role of Metformin in Controlling Oxidative Stress in Muscle of Diabetic Rats. *Oxidative Medicine and Cellular Longevity*, **2016**, Article ID: 6978625. https://doi.org/10.1155/2016/6978625

[28] Aebi, H. (1984) Catalase *in Vitro*. In: *Methods in Enzymology*, Elsevier, 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3

[29] Uchiyama, M. and Mihara, M. (1978) Determination of Malonaldehyde Precursor in Tissues by Thiobarbituric Acid Test. *Analytical Biochemistry*, **86**, 271-278. https://doi.org/10.1016/0003-2697(78)90342-1

[30] Bancroft, J.D. and Cook, B.C. (1984) Manual of Histological Techniques. Churchill Livingstone, 49-51.

[31] Clark, R.F. (2002) Goldfrank's Toxicological Emergencies. Organic Phosphorus Compounds and Arbamates, 1346-1360.

[32] Parrón, T., Hernández, A., Pla, A. and Villanueva, E. (1996) Clinical and Biochemical Changes in Greenhouse Sprayers Chronically Exposed to Pesticides. *Human & Experimental Toxicology*, **15**, 957-963. https://doi.org/10.1177/096032719601501203

[33] Kanu, K., Ijioma, S. and Atiata, O. (2016) Haematological, Biochemical and Antioxidant Changes in Wistar Rats Exposed to Dichlorvos Based Insecticide Formulation Used in Southeast Nigeria. *Toxics*, **4**, Article No. 28. https://doi.org/10.3390/toxics4040028

[34] Mostafalou, S. and Abdollahi, M. (2016) Pesticides: An Update of Human Exposure and Toxicity. *Archives of Toxicology*, **91**, 549-599. https://doi.org/10.1007/s00204-016-1849-x

[35] Fouad, A.A., Albuali, W.H., Al-Mulhim, A.S. and Jresat, I. (2013) Cardioprotective Effect of Cannabidiol in Rats Exposed to Doxorubicin Toxicity. *Environmental Toxicology and Pharmacology*, **36**, 347-357. https://doi.org/10.1016/j.etap.2013.04.018

[36] Golshani, S., Ansari, M., Faghir-Ghanesefat, H., Jalali, M.T. and Mohammadi, S. (2021). Cardiovascular Effects of Subchronic Exposure to Dichlorvos in Male Rats. *Environmental Science and Pollution Research*, **28**, 3114-3121.

[37] Karami-Mohajeri, S. and Abdollahi, M. (2019) Toxic Influence of Organophosphate, Carbamate, and Organochlorine Pesticides on Cellular Metabolism of Lipids,

Proteins, and Carbohydrates: A Systematic Review. *Human & Experimental Toxicology*, **38**, 963-986.

[38] Ledda, C., Fiore, M., Santarelli, L., Bracci, M., Mascali, G., D'Agati, M.G., *et al.* (2015) Gestational Hypertension and Organophosphorus Pesticide Exposure: A Cross-Sectional Study. *BioMed Research International*, **2015**, Article ID: 280891. https://doi.org/10.1155/2015/280891

[39] Suarez-Lopez, J.R., Jacobs, D.R., Himes, J.H. and Alexander, B.H. (2013) Acetylcholinesterase Activity, Cohabitation with Floricultural Workers, and Blood Pressure in Ecuadorian Children. *Environmental Health Perspectives*, **121**, 619-624. https://doi.org/10.1289/ehp.1205431

[40] Kilbourn, R.G., Szabó, C. and Trabers, D.L. (1997) Beneficial versus Detrimental Effects of Nitric Oxide Synthase Inhibitors in Circulatory Shock Lessons Learned from Experimental and Clinical Studies. *Shock,* **7**, 235-246. https://doi.org/10.1097/00024382-199704000-00001

[41] Li, X., Everson, W. and Smart, E.J. (2006) Nitric Oxide, Caveolae, and Vascular Pathology. *Cardiovascular Toxicology*, **6**, 1-14. https://doi.org/10.1385/ct:6:1:1

[42] Hogas, S., Bilha, S.C., Branisteanu, D., Hogas, M., Gaipov, A., Kanbay, M., *et al.* (2017) Potential Novel Biomarkers of Cardiovascular Dysfunction and Disease: Cardiotrophin-1, Adipokines and Galectin-3. *Archives of Medical Science*, **4**, 897-913. https://doi.org/10.5114/aoms.2016.58664

[43] Hou, Y., Zeng, Y., Li, S., Qi, L., Xu, W., Wang, H., *et al.* (2014) Effect of Quercetin against Dichlorvos Induced Nephrotoxicity in Rats. *Experimental and Toxicologic Pathology*, **66**, 211-218. https://doi.org/10.1016/j.etp.2014.01.007

[44] Lushchak, V.I. (2014) Free Radicals, Reactive Oxygen Species, Oxidative Stress and Its Classification. *Chemico-Biological Interactions*, **224**, 164-175. https://doi.org/10.1016/j.cbi.2014.10.016

[45] Chen, J., Kuhlencordt, P., Urano, F., Ichinose, H., Astern, J. and Huang, P.L. (2003) Effects of Chronic Treatment with L-Arginine on Atherosclerosis in Apoe Knockout and Apoe/Inducible NO Synthase Double-Knockout Mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **23**, 97-103. https://doi.org/10.1161/01.atv.0000040223.74255.5a

[46] Closs, E.I., Scheld, J.S. and Sharafi, M. (2000) Substrate Supply for Nitric-Oxide Synthase in Macrophages and Endothelial Cells: Role of Cationic Amino Acid Transporters. *Molecular Pharmacology*, **57**, 68-74.

[47] Preiksteinhoff, H., Zink, S., Rosen, P. and Kelm, M. (1995) Transport of L-Arginine in Arginine-Deprived Endothelial Cells. *Biochemical and Biophysical Research Communications*, **213**, 447-453. https://doi.org/10.1006/bbrc.1995.2152

[48] Nijveldt, R.J., Prins, H.A., van Kemenade, F.J., Teerlink, T., van Lambalgen, A.A., Boelens, P.G., *et al.* (2001) Low Arginine Plasma Levels Do Not Aggravate Renal Blood Flow after Experimental Renal Ischaemia/Reperfusion. *European Journal of Vascular and Endovascular Surgery*, **22**, 232-239. https://doi.org/10.1053/ejvs.2001.1444