Remedial impact of hesperidin on dichlorvos-occasioned pulmonary dysfunction in male Wistar rats

[*1,2]Akamo, A.J., [2]Ojelabi, A.O., [1]Akinsanya, M.A., [3]Taiwo, A.M., [1]Adebisi, A.A., [1]Adekunbi, T.S., [1]Adenowo[1], A.F., [1]Olagunju, B.A., [1]Anifowose, F., [1]Ajagun-Ogunleye, O.M., [4]Opowoye, I.O. and [2]Ugbaja, R.N.

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Lagos, Nigeria
[2]Clinical Biochemistry and Mechanistic Toxicology Research Cluster, Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria
[3]Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, Nigeria
[4]Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria

Abstract
Dichlorvos (DDVP) is a widely used organophosphate insecticide, but it may pose public health concerns when found in food products. Hesperidin, a citrus flavonoid, is known for its antioxidant properties and ability to maintain redox balance and cytoplasmic membrane integrity. This study evaluated the remedial effects of hesperidin on DDVP-evoked pulmonary toxicity using animal model. Forty-two rats were randomized into seven groups (6 rats/group): control, DDVP alone (8 $mg.kg^{-1}day^{-1}$); DDVP with hesperidin (50 and 100 $mg.kg^{-1}day^{-1}$); DDVP with atropine (0.2 $mg.kg^{-1}day^{-1}$); and hesperidin alone (50 and 100 $mg.kg^{-1}day^{-1}$). The rats were treated with DDVP orally for one week, followed by two weeks of hesperidin intervention. Blood and lung samples were collected post-treatment. Hesperidin significantly ($p<0.05$) reduced DDVP-induced increases in pulmonary cholesterol, phospholipids, hydrogen peroxide, nitric oxide, and malondialdehyde levels and mitigated decreases in lung triacylglycerol, reduced glutathione concentrations and activities of glutathione S-transferase, superoxide dismutase, catalase, glutathione peroxidase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase-3. Histological studies also confirmed the ameliorative effects of hesperidin on DDVP-induced pulmonary damage. Overall, hesperidin was able to counteract DDVP-induced lung impairments possibly by reversing oxidative stress and lipid dysmetabolism.

Keywords: Hesperidin, Dichlorvos, Pulmonary toxicity, Antioxidants, Dyslipidemias

Abbreviations

A	Atropine
Ach	Acetylcholine
AChE	Acetylcholinesterase
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CAT	Catalase
CHOL	Cholesterol
COPD	Chronic Obstructive Pulmonary Disease
DDVP	Dichlorvos
eNOS	Endothelial Nitric Oxide Synthase
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GST	Glutathione S-Transferase
iNOS	Inducible Nitric Oxide Synthase
LDH	Lactate Dehydrogenase
MDA	Malondialdehyde
NO	Nitric Oxide
NOS	Nitric Oxide Synthases
OPs	Organophosphates
PHLS	Phospholipids
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
TAG	Triacylglycerol
TP	Total Protein

*Corresponding Author_E-mail address: akamoaj@funaab.edu.ng
Received: May 14, 2024; Revised: June 23, 2024; Accepted: June 28, 2024

1. Introduction

The escalating demand for food production, driven by population growth has necessitated adoption of agricultural practices, such as pesticide use, to enhance crop yield and quality [1]. However, these practices present a double-edged sword for food safety. While pesticides enhance crop yields and quality, they raise concerns about potential contamination [2]. Organophosphates (OPs) constitute a significant portion (approximately 33%) of globally used pesticides, which stands at around 2 million tons annually [2-3]. Dichlorvos (DDVP), a potent broad-spectrum insecticide, exemplifies this trend. Despite being banned in developed countries for safety reasons, DDVP remains popular in various applications across agriculture, households, and veterinary medicine in many developing nations [4]. Unfortunately, underdeveloped countries often bear the brunt of pesticide poisoning incidents and fatalities due to inadequate safety practices, improper application techniques, and deficiencies in regulatory frameworks [5-6]. The affordability, accessibility, and effectiveness of OPs like DDVP pose significant health threats to both occupational workers and the general population through exposure to contaminated food and water sources [2,7]. Such exposure can lead to a myriad of acute and chronic health problems, exacerbated by the long-term persistence of pesticide residues in the environment [4, 8].

The mechanism of toxicity associated with DDVP primarily involves inhibition of acetylcholinesterase (AChE), an enzyme essential for terminating signals at nerve junctions. This inhibition leads to accumulation of the neurotransmitter acetylcholine, overstimulating cholinergic receptors and triggering harmful effects like seizures and respiratory failure [9-10]. AChE inhibition elicited myriads of consequences, including perspiration, nausea, lacrimation, bloating, loose stool, increased bronchial discharge, and mortality, which can result from disrupted parasympathetic autonomic nervous system cholinergic neurotransmission [11]. Muscle cramps, fasciculation, weakness, and flaccidity can result from motor nerve fibre effects in skeletal muscles. Cholinergic central nervous system effects include sleepiness, weariness, cognitive disorientation, migraines, seizures, unconsciousness, and mortality [12].

The mammalian brain cannot withstand more than 20% acetylcholinesterase suppression, and DDVP acute sickness mainly involves cholinesterase inhibition [13]. Furthermore, DDVP exposure has been linked to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), culminating in oxidative and nitrative stress, respectively [14], further exacerbating cellular damage. Pulmonary fibrosis, resulting in lung damage, is the most common and fatal consequence of DDVP intoxication. However, other organs, such as the liver, kidney, heart, and central nervous system, can also be damaged [15]. Given the mounting concerns over DDVP toxicity and the limitations of conventional antidotes like atropine, which are hindered by their short half-lives, challenging dosing schedules, and potential cytotoxicity [16,17], it is crucial to urgently explore alternative treatment strategies.

Hesperidin ($C_{28}H_{34}O_{15}$), a bioflavonoid found naturally in vegetables, herbs, legumes, and citrus fruits such as tangelos (*Citrus sinensis*) and synthesized artificially [18,19], holds significant promise as a potential therapeutic agent. Its antioxidant properties and ability to modulate inflammatory and lipid metabolism pathways make it a compelling candidate for mitigating DDVP-induced pulmonary toxicity [20-21] with the hope that it would abate DDVP lung toxicosis. Hence, the present study aims to assess the remedial effects of hesperidin on DDVP-evoked pulmonary dysfunction in rats.

2. Materials and methods

2.1 Chemicals and reagents

Dichlorvos (DDVP, CCl_2=$CHOPO(OCH_3)_2$, CAS No. 62-73-7), hesperitin-7-rhamnoglucoside ($C_{28}H_{34}O_{15}$, CAS No. 520-26-3), ketamine hydrochloride ($C_{13}H_{17}Cl_2NO$, CAS No. 1867-66-9), xylazine hydrochloride ($C_{12}H_{17}ClN_2S$, CAS No. 23076-35-9), total protein kit (TP, Randox, Cat. No. TP 245), alanine aminotransferase kit (ALT, Randox, Cat. No. AL 1200), aspartate aminotransferase kit (AST, Randox, Cat. No. AS 1202), alkaline phosphatase kit (ALP, Randox, Cat. No. AP 542), lactate dehydrogenase kit (LDH, BioSystems, COD 12580), triacylglycerol kit (TAG, Labkit, Ref. 30360), cholesterol kit (CHOL, Labkit, Ref. 30180), phospholipids kit (PHLS, Labkit, Ref. 30320), methenyl trichloride ($CHCl_3$, CAS No. 67-66-3), methyl hydroxide (CH_3OH, CAS No. CAS No.: 67-56-1), potassium monochloride (KCl, CAS No. 7447-40-7), 2,2,2-trichloroacetic acid (TCA, Cl_3CCOOH, CAS No. 76-03-9), monobasic potassium phosphate (KH_2PO_4, CAS No. 7778-77-0), dibasic potassium phosphate (K_2HPO_4, CAS No. 7758-11-4), hydrogen peroxide (H_2O_2, CAS No. 7722-84-1), p-sulphanilic acid ($NH_2C_6H_4SO_3H$, CAS No. 121-57-3), anhydrous phosphoric acid (H_3PO_4, CAS No. 7664-38-2), N-1-Naphthalenyl-1,2-ethanediamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$, CAS No. 1465-25-4), L-glutathione (GSH, $H_2NCH(CO_2H)CH_2CH_2CONH$, CAS No. 70-18-8),

dithionitrobenzoic acid (DTNB, [-$SC_6H_3(NO_2)CO_2H]_2$, CAS No. 69-78-3), 2,4-Dinitrophenyl chloride (DNCB, $C_6H_3ClN_2O_4$, CAS No. 97-00-7), tris-base ($C_4H_{11}NO_3$ CAS No. 77-86-1), hydrogen chloride (HCl, CAS No. 7647-01-0), trihydroxybenzene ($C_6H_3(OH)_3$, CAS No. 87-66-1), edetic acid ($C_{10}H_{16}N_2O_8$, CAS No. 60-00-4), diammonium dioxomolybdenum [$(NH_4)_2MoO_4$, CAS No. 13106-76-8), natrium azide (NaN_3, CAS No. 26628-22-8), 2-sulfanylidene-1,3-diazinane-4,6-dione ($C_4H_4N_2O_2S$, CAS No. 504-17-6), formalin solution (neutral buffered, 10%, CAS No. HT501128-4L), hemotoxylin ($C_{16}H_{14}O_6$•xH_2O, CAS No. 517-28-2), sodium eosine ($C_{20}H_6Br_4Na_2O_5$, CAS No. 17372-87-1), and light photomicroscope. These chemicals and reagents are products of Randox Laboratories Limited, Crumlin, UK; Biosystems Diagnostics, Costa Brava, Barcelona, Spain; Chemelex S.A. Diagnostics, Barcelona; Solarbio Science & Technology Company Limited, Beijing, China and Sigma-Aldrich Chemical Co., St. Louis, MO 63118, US. In this investigation, all compounds were pure and of analytical grade.

2.2 Animal regime

In this inquiry, pathogen-free forty-two male Wistar rats (12 weeks old, 183 ± 7 g mean weight) were secured from the College of Biosciences, Federal University of Agriculture, Abeokuta (FUNAAB). The rats were housed in adequately aired suspended plastic cages with enough aspen bedding; in temperature-moderated (29 ± 2°C) and humidity-checked (49 ± 3%) surroundings with a standard *12*:*12 light-dark* cycle. The animals were nurtured with regular rodent feed and had unrestricted access to good quality drinking water. The animals were acclimatized for a week before the start of the experiment, and they were provided with compassionate treatment according to the regulations endorsed by the FUNAAB Ethical Committee and the United States of America National Institute of Health [22]. In addition, the institution authorized the study protocol with the ethical number FUNAAB/COLBIOS/BCH/PG/17/0544.

2.3 Experimental layout

Randomization was used to allocate forty-two acclimatized rats into seven groups of six animals as follows:

- Group 1 (control): 2 mL/kg of distilled water (7 days) + 2 mL/kg of olive oil (14 days)
- Group 2: 8 mL/kg of DDVP (7 days) + 2 mL/kg of olive oil (14 days)
- Group 3: 8 mL/kg of DDVP (7 days) + 0.2 mL/kg of atropine (14 days)
- Group 4: 8 mL/kg of DDVP (7 days) + 50 mg/kg of hesperidin (14 days)
- Group 5: 8 mL/kg of DDVP (7 days) + 100 mg/kg of hesperidin (14 days)
- Group 6: 2 mL/kg of distilled water (7 days) + 50 mg/kg of hesperidin (14 days)
- Group 7: 2 mL/kg of distilled water (7 days) + 100 mg/kg of hesperidin (14 days)

All administrations were completed between 8:00 a.m. and 9:00 a.m. daily. Hesperidin (50 and 100 $mg.kg^{-1}day^{-1}$) and DDVP (8 $mg.kg^{-1}day^{-1}$) corresponds to one-tenth of oral LD50, according to earlier studies [23-25]. Similarly, sub-acute treatment durations for DDVP and hesperidin were picked based on a previous experiment [23-25].

2.4 Collection and processing of samples (blood and lung)

Twenty-four hours after the last intervention (day twenty-two), rats were anesthetized intraperitoneally with 100 mg kg^{-1} day^{-1} and 10 mg kg^{-1} day^{-1} of ketamine and xylazine, respectively as designated by Wellington et al. [26]. Blood was collected through retro-orbital perforation veins into 10 mL **lithium heparinized** tubes. P**lasma** was obtained by centrifugation for 15 minutes for biochemical investigations. The rats were then humanely dissected, and the lungs were excised and washed in 1.15% of ice-cold potassium chloride medium to clear blood stains, blotted dry with blotting paper, and weighed. A slice (0.2 g) of the lungs tissue was homogenized in 1.8 mL of potassium phosphate solution (0.1 M, pH 7.4, 2°C) i.e. (10% w/v) to form 10% homogenate and spun for 15 min at 10,000 x g to obtain lung homogenate. The supernatant was extracted at 4°C and stored at -20°C for biochemical assays. In addition, 10% formalin was used to fix a piece of the lung for histomorphological investigation.

2.5 Biochemical assays

Total protein content, the activities of ALT, AST, ALP, and LDH were determined in lung homogenate and plasma according to the instructions provided by the Randox/Biosystem kits [27, 28]. According to the LABKIT handbook, lung triacylglycerol, cholesterol, and phospholipids concentrations were determined [27]. Standard methods were used to determine reduced glutathione (GSH), glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) [31-35]. Lipid peroxidation in the lungs was determined by measuring malondialdehyde (MDA) concentration [36].

2.6 Histological studies

Slices of lung slices were preserved in 10% formalin and then encased in paraffin wax. The lung tissue was cut into sections of 5 μm thick and then mounted on slides. These sections were stained with hematoxylin and eosin to identify and analyze the morphology of cells using a light photomicroscope.

2.7 Statistical analysis

Quantitative variables were expressed as mean ± S.E.M. of six replicates per group. ANOVA was utilized to determine group homogeneity while Duncan Multiple Range Test separated diverse groups. A 0.05 p-value was acknowledged as significant. All statistics were done with SPSS 20.0 and graphs were generated with GraphPad Prism 8.0.

3. Results

The impacts of DDVP (20 mg kg^{-1} day^{-1}), DDVP combined with hesperidin supplementation (at doses of 50 and 100 mg kg^{-1} day^{-1}), or reference atropine (at a dose of 0.2 mg kg^{-1} day^{-1}), and hesperidin unaccompanied (at doses of 50 and 100 mg kg^{-1} day^{-1}), on lung activities of ALT, AST, ALP, and LDH are depicted in Figure 1. Exposure to DDVP resulted in significant decreases ($p < 0.05$) in lung ALT by 17.65%, AST by 45.91%, and LDH by 57.58% compared to the control group. However, there was no significant difference ($p > 0.05$) in ALP levels compared to the control. Post-treatment with atropine or hesperidin (at doses of 50 and 100 mg kg^{-1} day^{-1}) following DDVP exposure significantly mitigated the declines provoked by DDVP. Specifically, ALT levels decreased by 15.48%, 19.05%, and 17.86% with atropine and hesperidin treatments, respectively. AST levels decreased by 36.05%, 47.67%, and 73.26%, and LDH levels decreased by 69.54%, 67.33%, and 41.94% with the same treatments. Furthermore, in rats supplemented with hesperidin alone at doses of 50 and 100 mg kg^{-1} day^{-1}, all assayed enzyme activities were not statistically different ($p > 0.05$) from those of the standard control.

Figure 2 represents the influence of different treatments on the levels of triacylglycerol (TAG), cholesterol (CHOL), phospholipids (PHLS), and the cholesterol/phospholipids ratio in the lung. Exposure of rats to DDVP alone at a dose of 8 mg kg^{-1} day^{-1} caused a significant ($p < 0.05$) reduction ($p < 0.05$) in TAG levels by 59.27%, while CHOL and PHLS levels increased significantly ($p < 0.05$) by 184.56% and 157.36%, respectively, compared to the control group. However, the CHOL/PHLS ratio in DDVP-treated rats was not significantly different ($p > 0.05$) from that of the control group. Following supplementation with either atropine (at a dose of 0.2 mg kg^{-1} day^{-1}) or hesperidin (at doses of 50 and 100 mg kg^{-1} day^{-1}) alongside DDVP exposure, there was a significant attenuation ($p < 0.05$) of the decrease in TAG levels by 47.31%, 59.98%, and 63.89%, respectively. Additionally, these treatments significantly attenuated ($p < 0.05$) the increases in CHOL by 29.12%, 32.38%, and 35.46%, respectively, and PHLS by 29.02%, 32.21%, and 38.69%, respectively, triggered by DDVP. Moreover, when rats were solely supplemented with hesperidin, there were no significant differences ($p > 0.05$) in the levels of the assessed lipids compared to the control group of normal rats.

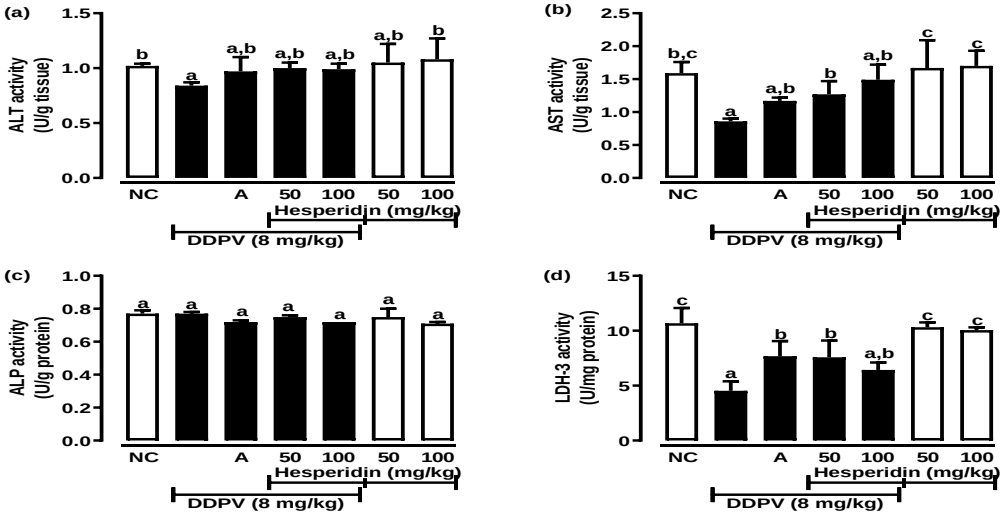

Figure 1: The effect of 14 days subacute hesperidin treatment on the activities of pulmonary ALT (a), AST (b), ALP (c), and LDH (d) in rats exposed to dichlorvos for 7 days. NC symbolizes normal control and letter 'A' symbolizes atropine. Results are expressed as mean ± SEM of six determinations. Bars with different lower case letters differ significantly ($P < 0.05$).

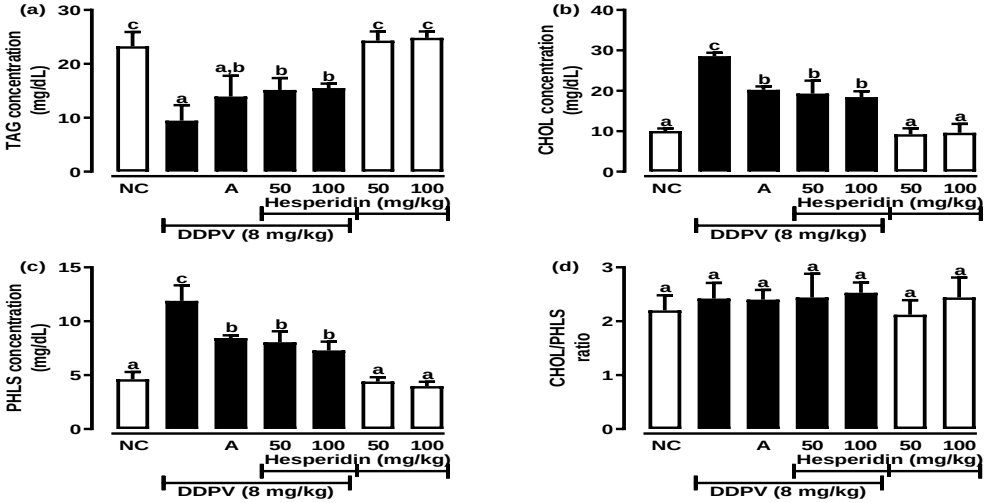

Figure 2: The effect of 14 days subacute hesperidin treatment on the concentrations of pulmonary triacylglycerol (a), cholesterol (b), phospholipids (c), and cholesterol/phospholipids ratio (d) in rats exposed to dichlorvos for 7 days. NC symbolizes normal control and letter 'A' symbolizes atropine. Results are expressed as mean ± SEM of six determinations. Bars with different lower case letters differ significantly ($P < 0.05$).

The consequences of DDVP (8 mg kg^{-1} day^{-1}), DDVP supplemented with hesperidin (50 and 100 mg kg^{-1} day^{-1}), or the reference prescription atropine (0.2 mg kg^{-1} day^{-1}), as well as hesperidin alone (50 and 100 mg kg^{-1} day^{-1}), on lung hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels are presented in Figure 3. In DDVP-treated rats, the hydrogen peroxide level (Figure 3a) surged significantly ($p < 0.05$) by an impressive 360.94%, while the nitric oxide content (Figure 3b) rose significantly ($p < 0.05$) by 110.53% compared to the control group. Conversely, after administration of atropine or hesperidin (at doses of 50 and 100 mg kg^{-1} day^{-1}) to DDVP-treated rats, there was a significant ($p<0.05$) reversal of the DDVP-induced elevations in both H_2O_2 (by 44.75%, 48.64%, and 41.53%, respectively) and NO (by 50%, 56.25%, and 52.50%, respectively) levels. Furthermore, administration of hesperidin alone (at doses of 50 mg and 100 mg kg^{-1} day^{-1}) did not show any significant ($p>0.05$) difference in H_2O_2 and NO levels relative to the control group.

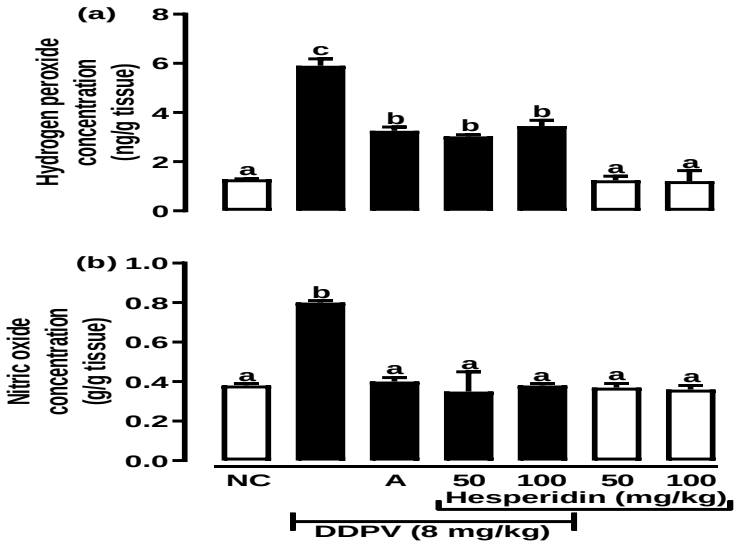

Figure 3: The effect of 14 days subacute hesperidin treatment on the concentrations of pulmonary H_2O_2 (a) and NO (b) in rats exposed to dichlorvos for 7 days. NC symbolizes normal control and letter 'A' symbolizes atropine. Results are expressed as mean ± SEM of six determinations. Bars with different lower case letters differ significantly ($P < 0.05$).

Figure 4 presents the impact of various treatments on the concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) in the lung of rats exposed to DDVP. There was a significant reduction ($p < 0.05$) in lung GSH concentration (Figure 4a) by 29.11% and a significant increase ($p < 0.05$) in MDA concentration (Figure 4b) by 1227.27% compared to the control group. However, treatment with atropine or hesperidin (at doses of 50 and 100 mg kg^{-1} day^{-1}) mitigated the decline in GSH levels by 18.47%, 36.47%, and 39.07%, respectively. Moreover, the interventions reduced the DDVP-induced increase in MDA levels by 60.96%, 58.90%, and 61.64%, respectively. Notably, there were no significant differences ($p > 0.05$) in GSH and MDA between rats receiving hesperidin supplementation and the control group.

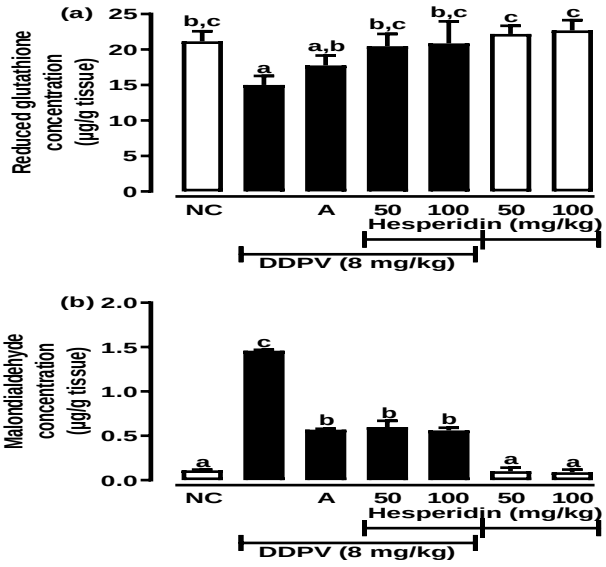

Figure 4: The effect of 14 days subacute hesperidin treatment on the concentrations of GSH (a) and MDA (b) in the lung of rats exposed to dichlorvos for 7 days. NC symbolizes normal control and letter 'A' symbolizes atropine. Results are expressed as mean ± SEM of six determinations. Bars with different lower case letters differ significantly ($P < 0.05$).

Figure 5 depicts the effect of various treatments on lung enzymatic antioxidants. DDVP exposure resulted in significant ($p < 0.05$) reduction in lung activities of glutathione S-transferase (Figure 5a), superoxide dismutase (Figure 5b), catalase (Figure 5c), and glutathione peroxidase (Figure 5d) by 60%, 52.09%, 57.62%, and 40.43%, respectively. Nevertheless, treatment with atropine or hesperidin (at doses of 50 and 100 mg kg^{-1} day^{-1}) following DDVP exposure significantly ($p < 0.05$) attenuated the decreases in GST activity by 100%, 155.26%, and 207.89%; SOD activity by 71.79%, 95.38%, and 67.18%; CAT activity by 64.79%, 74.41%, and 60.65%; and GPx activity by 25%, 21.43%, and 25.45%, respectively. Notably, there were no significant ($p > 0.05$) differences in the assayed enzymatic antioxidants between rats receiving hesperidin supplementation and the control group.

Figure 6 illustrates the effects of various treatments on lung histopathology. While no abnormalities were observed in the control group, dichlorvos caused significant effects, including necrosis, desquamation of bronchial epithelial cells, presence of mucus in the bronchial lumen (indicated by an arrow), and thickening of the interstitium due to inflammatory exudates (marked by an arrowhead). When dichlorvos was co-administered with atropine (0.2 mg/ml), thickening of the interstitium by inflammatory exudates (indicated by an arrow) and presence of inflammatory exudates in alveolar spaces were observed. Combining dichlorvos with hesperidin at 50 mg kg^{-1} day^{-1} resulted in moderate thickening of the interstitium by inflammatory exudates (indicated by an arrow) and necrosis of bronchial epithelial cells (marked by an arrowhead). At a higher dose of hesperidin (100 mg kg^{-1} day^{-1}) following dichlorvos administration, thickening of the interstitium persisted, accompanied by mucus and desquamated epithelial cells in bronchioles (indicated by an arrow) and alveolar air spaces (indicated by an arrowhead). Hesperidin alone at 50 mg kg^{-1} day^{-1} displayed interstitial thickening due to inflammatory exudates (indicated by an arrow), while hesperidin alone at 100 mg kg^{-1} day^{-1} showed moderately thickened interstitium by inflammatory exudates (shown by an arrow) and presence of mucus in the bronchial lumen (epitomized by the label). These observations stem from histological analysis of the cerebrum as part of an experimental design.

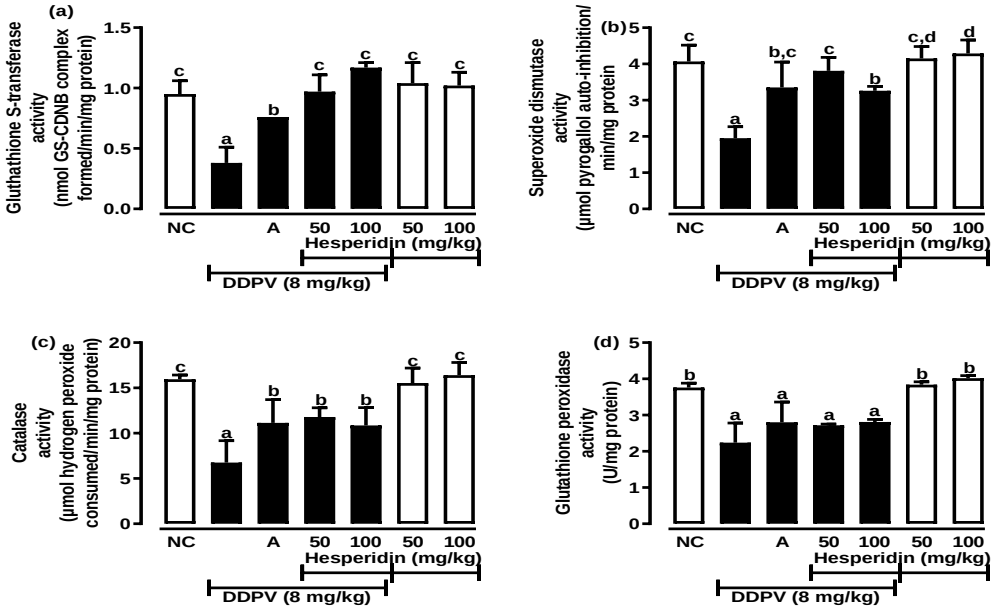

Figure 5: The effect of 14 days hesperidin subacute treatment on selected pulmonary enzymatic antioxidants activities GST (a), SOD (b), catalase (c) and GPx (d) in rats exposed to dichlorvos for 7 days. NC symbolizes normal control and letter 'A' symbolizes atropine. Results are expressed as mean ± SEM of six determinations. Bars with different lower case letters differ significantly ($P < 0.05$).

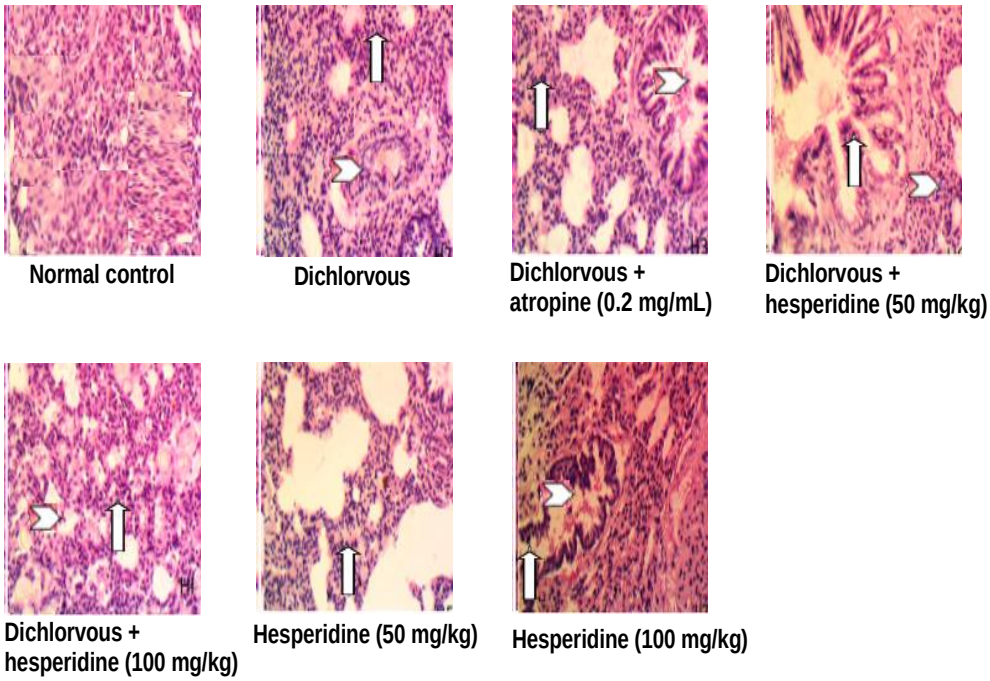

Figure 6: Photomicrographs (x 400) depicting the influence of 14 days of subacute hesperidin administration on dichlorvos-treated rat lung architecture.

Figure 7 illustrates the proposed mechanism of remedial impact of hesperidin on dichlorvos-evoked pulmonary dysfunction in rats. Exposure of experimental animals to DDVP led to derangement of enzyme action via leakage as well as generation of ROS and RNS. These reactive species caused cellular damage and malfunction by inducing oxidative stress and lipid dyshomeostasis, which were attenuated by hesperidin.

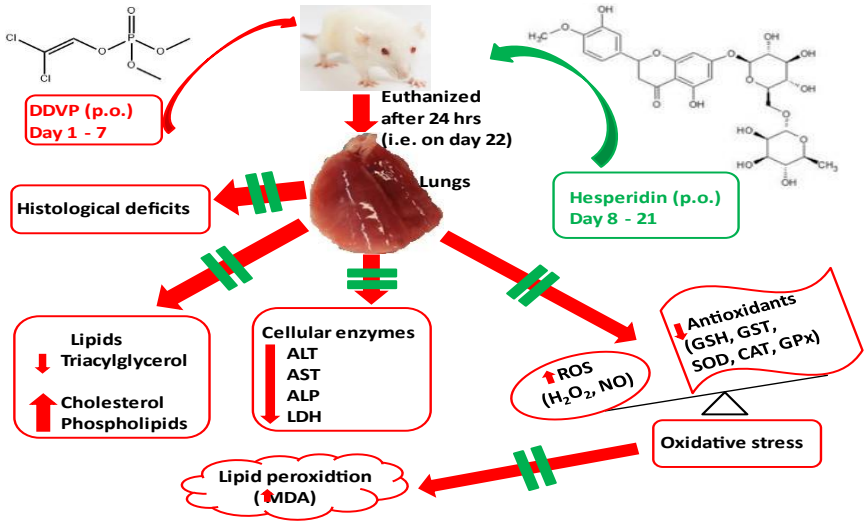

Figure 7: Graphical abstract and the proposed mechanism of the remedial impact of hesperidin on dichlorvos-evoked pulmonary dysfunction in rats. Red arrows signify the reaction pathways of the negative impact of DDVP, while the green symbol exemplified the inhibition by hesperidin intervention.

4. Discussion

The current research examined possible remedial impact of hesperidin on dichlorvos-occasioned lung perturbation in rat models. The data elucidated the complications underlying the mechanisms by which DDVP exerts its toxicosis and the therapeutic potential of atropine, a known AChE inhibitor, and hesperidin, a bioflavonoid with antioxidant and anti-inflammatory properties, in ameliorating its negative repercussions.

Findings from this study revealed that DDVP remarkably triggered deleterious effects by altering lung ALT, AST, and LDH enzymatic actions. These enzymes maintain cellular integrity, and their diminution indicates DDVP-enhanced impaired cellular integrity, membrane permeability, and lung injury. Our results aligned with prior investigations [10, 37], further emphasizing DDVP multi-organ toxicity. Interestingly, post-administration of atropine and hesperidin annulled the DDVP-engendered reductions in enzyme actions, suggesting probable adjunctive therapeutic strategies and mitigatory function against DDVP-prompted impaired cellular integrity, membrane permeability, and lung injury. Hesperidin has been reported to reverse leakage enzyme perturbation [38, 39].

The dysmetabolism in lipid homeostasis may be an indication that DDVP evoked a disruption in metabolic processes within the lung tissue and could contribute partly to compromised lung function and potentially increased the risks of cardiovascular complications. This submission agrees with the previous report that DDVP stimulates dyslipidemia [40]. Remarkably, despite the DDVP-instigated perturbations in the individual lipid profile concentrations, the cholesterol/phospholipids molar ratio stayed unperturbed, signifying a prospective compensatory mechanism or a differential response to DDVP-evoked intoxication among lipid subclasses. Nonetheless, atropine and hesperidin supplementation markedly attenuated DDVP-evoked decrease in TAG. Both compounds also enhanced CHOL and PHLS levels, alluding to a promising protective role against DDVP-motivated lipid dysmetabolism [41]. Of particular interest is the fact that hesperidin supplementation alone did not elicit any significant changes in lipid levels compared to the control group. This result suggests that hesperidin may not directly impact lung lipid metabolism under normal physiological conditions but could function as a potent protective drug candidate against DDVP-triggered dyslipidemia when administered concurrently.

The present study found that DDVP exposure led to marked disruptions in various oxidative stress biomarkers in the lungs of experimental animals. Specifically, there was a remarkable augmentation in H_2O_2 and NO contents, revealing heightened oxidative stress. Conversely, DDVP incited diminutions in lung GSH content and the activities of antioxidant enzymes, including GST, SOD, CAT, and GPx, further suggesting oxidative stress. Additionally, MDA content was substantially elevated, denoting enhanced lipid peroxidation and oxidative damage. Our findings agree with prior reports that, in addition to inhibiting AChE, DDVP exerts its toxicosis via the generation of ROS and RNS, depleting antioxidant defenses and contributing to lipid peroxidation and oxidative damage in the lungs [42, 43].

The lungs are particularly vulnerable to oxidative stress-provoked injury [44]. Alterations in biomarkers such as H_2O_2, NO, GSH, SOD, CAT, and MDA can significantly impact lung function and integrity. H_2O_2 is generated in the lungs through normal cellular mitochondrial metabolism. Elevated H_2O_2 levels induce oxidative damage by oxidizing biomolecules, disrupting cellular signaling pathways, promoting inflammation, and contributing to tissue injury, ultimately compromising lung function [45]. The enhanced H_2O_2 may be attributed to disruption of mitochondrial electron transport chain, leading to electron leakage and subsequent formation of H_2O_2.

NO is produced in the lung primarily by a family of enzymes called nitric oxide synthases (NOS), including inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3). eNOS-derived NO helps regulate vascular tone, promotes vasodilation, and maintains pulmonary blood flow distribution, contributing to normal lung function [46]. While NO plays essential physiological roles in the lung, dysregulated or excessive NO production, particularly by iNOS during inflammatory conditions, can provoke detrimental effects such as oxidative stress, inflammation, and pulmonary dysfunction. It may also contribute to pulmonary vascular dysfunction and airway hyperresponsiveness, as seen in myriads of pulmonary diseases such as asthma, chronic obstructive pulmonary disease (COPD), and pulmonary hypertension [47].

GSH is a crucial antioxidant and detoxifying tripeptide in animal and human lungs. GSH depletion in the lungs of DDVP-treated rats could mediate significant consequences, including increased oxidative stress, impaired detoxification, a weakened immune response, and exacerbating lung diseases. The observed GSH decrease in the lung could be that DDVP inhibits GSH synthesis, depletes GSH via enhanced ROS/RNS generation, accelerates GSH turnover rate, or disrupts GSH recycling via glutathione inhibition [48]. Detoxification in cells, including the lungs, entails GST activity. The decline in GST activity following DDVP exposure could propagate marked drawbacks, including impaired xenobiotic conjugation detoxification. This may lead to xenobiotic accumulation in the lungs, pronounced oxidative stress, compromised lung function, susceptibility to infection, and development or exacerbation of respiratory diseases [49]. The observed diminution in lung GST activities could be credited to DDVP-induced direct GST imbibition by binding to its active site, indirect inhibition via depletion of lung GSH, an essential cofactor for GST activity, inhibition of gene coding for GST expression, or epigenetic modification, including DNA methylation and histone modification [50].

SOD and CAT protect cells, tissues, and organs, including the lungs, from oxidative stress. While SOD converts superoxide radicals (O_2^{*-}) into oxygen and H_2O_2; CAT, on the other hand, converts H_2O_2 into water and oxygen [51]. Lung SOD and CAT downregulation after DDVP exposure could trigger marked abnormalities, including accumulation of O_2^{*-} and H_2O_2, respectively. O_2^{*-} and H_2O_2-provoked cellular component oxidation enhance oxidative stress, inflammation, respiratory dysfunction, coughing, wheezing, decreased lung capacity, susceptibility to respiratory diseases and lung cancer. Various investigators have shown that OPs can evoke SOD and CAT inhibition [16]. The observed decline in lung SOD activity following exposure to DDVP may be attributed to direct inhibition of SOD activity via active site binding, indirect impact via depletion of SOD and CAT due to enhanced O_2^{*-} and H_2O_2 challenge, interruption of the SOD coding gene leading to decreased synthesis, or conformational modification of the SOD and CAT three dimensional structure and function [16, 51].

The high level of MDA observed in the lung, following exposure to DDVP, may be indication that ROS and RNS have attacked the membrane. This possibly led to lipid peroxidation and oxidative damage to lung cell membranes thereby compromising their integrity and function. The oxidative stress occasioned by elevated MDA can also invoke lung inflammatory response, exacerbating asthma or COPD, respiratory impairment, and infection susceptibility [52, 53]. The DDVP-induced elevated MDA level could be a result of enhanced free radical generation (H_2O_2 and NO) as well as the observed inhibition of GSH, GST, SOD, and CAT. The effect could also be linked to inflammation or mitochondrial dysfunction which stimulate ROS generation and oxidative stress [52, 53].

Histology assessment further validated the adverse effects of DDVP on lung tissue integrity, revealing necrosis, desquamation of bronchial epithelial cells, thickening of the interstitium, and inflammatory exudates which corroborates previous study [54]. The present study emphasized the brutality of DDVP-activated pulmonary toxicity and highlighted the urgent need for effective therapeutic antidotes.

The mechanisms by which hesperidin reverses the DDVP-prompted perturbation could be by scavenging ROS and RNS, enhancing GSH levels, up-regulation of antioxidant proteins, and inhibition of lipid peroxidation toxicosis [55-57]. Overall, this present study confirmed hesperidin as a potent therapeutic candidate against DDVP-induced pulmonary toxicity by virtue of its ability to mitigate disrupted enzymatic activities, dyslipidemia, and oxidative stress in lung tissue as well as improving histopathological outcomes.

5. 0 References

[1] Devi, P.I., Manjula, M. and Bhavani, R.V. (2022). Agrochemicals, environment, and human health. Annual Review of Environment and Resources, Vol. 47, pp. 399-421.

[2] Leskovac, A. and Petrović, S. (2023). Pesticide use and degradation strategies: Food safety, challenges and perspectives. Foods, Vol. 12, No. 14, pp. 2709-2732.

[3] Rajmohan, K.S., Chandrasekaran, R. and Varjani, S. (2020). A review on occurrence of pesticides in environment and current technologies for their remediation and management. Indian Journal of Microbiology, Vol. 60(2), pp. 125-138.

[4] Oyeyemi, W.A., Daramola, O.O., Akinola, A.O., Idris, A.O. and Aikpitanyi, I. (2020). Hepatic and reproductive toxicity of sub-chronic exposure to dichlorvos and lead acetate on male Wistar rats. Asian Pacific Journal of Reproduction, Vol. 9, No. (6), pp. 283-290.

[5] Jørs, E., Neupane, D. and London, L. (2018). Pesticide poisonings in low-and middle-income countries. Environmental Health Insights, Vol. 12, pp. 1-3.

[6] Adebisi, F., Dauda, M. and Anjorin, T. (2023). A review on global pesticide use and food contamination: African perspective. Pollution, Vol. 9, No. 4, pp. 1706-1730.

[7] Karimi-Maleh, H., Darabi, R., Baghayeri, M., Karimi, F., Fu, L., Rouhi, J. *et al*. (2023). Recent developments in carbon nanomaterials-based electrochemical sensors for methyl parathion detection. Journal of Food Measurement and Characterization, 17(5), 5371-5389.

[8] Anaduaka, E.G., Uchendu, N.O., Asomadu, R.O., Ezugwu, A.L., Okeke, E.S. and Ezeorba, T.P.C. (2023). Widespread use of toxic agrochemicals and pesticides for agricultural products storage in Africa and developing countries: Possible panacea for ecotoxicology and health implications. Heliyon, Vol. 9, No. 4, pp. 1-17.

[9] Okoroiwu, H.U. and Iwara, I.A. (2018). Dichlorvos toxicity: A public health perspective. Interdisciplinary Toxicology, Vol. 11, No. 2, pp. 129-137.

[10] Awotunsin, K.O., Oridupa, O.A., Ogunsola, J.O., Obisesan, A.D. and Saba, A.B. (2021). Simulation of hemo-and biochemical toxicities associated with chronic inhalation exposure to 2,2-dichlorovinyl dimethyl phosphate (DDVP) in Wistar rat. Environmental Toxicology and Pharmacology, Vol. 82, pp. 103547–103556.

[11] Pappano, A.J. (2018). Cholinoreceptor-activating and cholinesterase-inhibiting drugs. Basic and Clinical Pharmacology. McGraw Hill Professional, New York, NY, pp. 107-123.

[12] Sher, Y., Garcia, R. and Maldonado, J.R. (2016). Neuropsychiatric masquerades: Diagnosis and treatment. Psychiatric Times, Vol. 33, No. 2, pp. 1-10.

[13] Farkhondeh, T., Mehrpour, O., Forouzanfar, F., Roshanravan, B. and Samarghandian, S. (2020). Oxidative stress and mitochondrial dysfunction in organophosphate pesticide-induced neurotoxicity and its amelioration: A review. Environmental Science and Pollution Research, Vol. 27, pp. 24799-24814.

[14] Akande, M.G. and Ahmed, U.S. (2017). Taurine abated subacute dichlorvos toxicity. Toxicology Reports, Vol. 4, pp. 463-466.

[15] Sule, R.O., Condon, L. and Gomes, A.V. (2022). A common feature of pesticides: Oxidative stress-The role of oxidative stress in pesticide-induced toxicity. Oxidative Medicine and Cellular Longevity, Vol. 2022, pp. 1-31.

[16] Cui, T., Jiang, W., Yang, F., Luo, J., Hu, R., Cao, H. *et al*. (2021). Molybdenum and cadmium co-induce hypothalamus toxicity in ducks via disturbing Nrf2-mediated defense response and triggering mitophagy. Ecotoxicology and Environmental Safety, Vol. 228, pp. 113022–113034.

[17] Michelon, H., Larabi, I. A., Lemoine, J., Alvarez, J. C., Genevée, A., Lillo-Lelouet, A. *et al*. (2021). Atropine-induced toxicity after off-label sublingual administration of eyedrop for sialorrhoea treatment in neurological disabled patients. British Journal of Clinical Pharmacology, Vol. 87, No. 8, pp. 3364-3369.

[18] Valls, R. M., Pedret, A., Calderón-Pérez, L., Llauradó, E., Pla-Pagà, L., Companys, J. *et al*. (2021). Effects of hesperidin in orange juice on blood and pulse pressures in mildly hypertensive individuals: A randomized controlled trial (Citrus study). European Journal of Nutrition, Vol. 60, pp. 1277-1288.

[19] Binkowska, I. (2020). Hesperidin: Synthesis and characterization of bioflavonoid complex. SN Applied Sciences, Vol. 2, No. 3, pp. 445-454.

[20] Galleano, M., Pechanova, O. and G Fraga, C. (2010). Hypertension, nitric oxide, oxidants, and dietary plant polyphenols. Current Pharmaceutical Biotechnology, Vol. 11, No. 8, pp. 837-848.

[21] Mas-Capdevila, A., Teichenne, J., Domenech-Coca, C., Caimari, A., Del Bas, J.M., Escoté, X. and Crescenti, A. (2020). Effect of hesperidin on cardiovascular disease risk factors: The role of intestinal microbiota on hesperidin bioavailability. Nutrients, Vol. 12, No. 5, pp. 1488-1496.

[22] National Research Council, 2010. Guide for the Care and Use of Laboratory Animals. National Academy Press.

[23] Görmeli, C.A., Saraç, K., Çiftçi, O., Timurkaan, N. and Malkoç, S. (2016). The effects of hesperidin on idiopathic pulmonary fibrosis evaluated by histopathologial-biochemical and micro-computed tomography examinations in a bleomycin-rat model. Biomedical Research, Vol. 27, pp. 737-742.

[24] Imam, A., Ogunniyi, A., Ibrahim, A., Abdulmajeed, W.I., Oyewole, L.A., Lawan, A.H. *et al*. (2018). Dichlorvos induced oxidative and neuronal responses in rats: Mitigative efficacy of *Nigella sativa* (black cumin). Nigerian Journal of Physiological Sciences, Vol. 33, No. 1, pp. 83-88.

[25] Abolaji, A.O., Omozokpia, M.U., Oluwamuyide, O.J., Akintola, T.E. and Farombi, E.O. (2020). Rescue role of hesperidin in 4-vinylcyclohexene diepoxide-induced toxicity in the brain, ovary and uterus of Wistar rats. Journal of Basic and Clinical Physiology and Pharmacology, Vol. 31, No. 2, pp. 20180115.

[26] Wellington, D., Mikaelian, I. and Singer, L. (2013). Comparison of ketamine–xylazine and ketamine–dexmedetomidine anesthesia and intraperitoneal tolerance in rats. Journal of the American Association for Laboratory Animal Science, Vol. 52 No. 4, pp. 481-487.

[27] Tietz, N.W. (1995). Clinical guide to laboratory tests. In Clinical guide to laboratory tests, pp. 1096-1996.

[28] Burtis, C.A. and Ashwood, E.R. (1994). Tietz textbook of clinical chemistry. Amer Assn for Clinical Chemistry.

[29] Junglee, S., Urban, L., Sallanon, H. and Lopez-Lauri, F. (2014). Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. American Journal of Analytical Chemistry, Vol. 5, No. 11, pp. 730-743.

[30] Sreejayan, X.X. and Rao, M.N.A. (1997). Nitric oxide scavenging by curcuminoids. Journal of Pharmacy and Pharmacology, Vol. 49, No. 1, pp. 105-107.

[31] Rahman, I., Kode, A. and Biswas, S.K. (2006). Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature Protocols, Vol. 1, No. 6, pp. 3159-3165.

[32] Habig, W.H., Pabst, M.J., Fleischner, G., Gatmaitan, Z., Arias, I.M. and Jakoby, W.B. (1974). The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proceedings of the National Academy of Sciences, Vol. 71, No. 10, pp. 3879-3882.

[33] Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, Vol. 47, No. 3, pp. 469-474.

[34] Hadwan, M.H. and Abed, H.N. (2016). Data supporting the spectrophotometric method for the estimation of catalase activity. Data in Brief, Vol. 6, pp. 194-199.

[35] Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. Science, Vol. 179, No. 4073, pp. 588-590.

[36] Buege, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. In: Methods in enzymology, Vol. 52, pp. 302-310.

[37] Ajiboye, T.O. (2010). Redox status of the liver and kidney of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) treated rats. Chemico-Biological Interactions, Vol. 185, No. 3, pp. 202-207.

[38] Nandakumar, N., Jayaprakash, R., Rengarajan, T., Ramesh, V. and Balasubramanian, M.P. (2011). Hesperidin, a natural citrus flavonoglycoside, normalizes lipid peroxidation and membrane bound marker enzymes in 7, 12-dimethylbenz (a) anthracene induced experimental breast cancer in rats. Biomedicine and Preventive Nutrition, Vol. 1, No. 4, pp. 255-262.

[39] Famurewa, A.C., Renu, K., Eladl, M.A., Chakraborty, R., Myakala, H., El-Sherbiny, M. *et al*. (2022). Hesperidin and hesperetin against heavy metal toxicity: Insight on the molecular mechanism of mitigation. Biomedicine and Pharmacotherapy, Vol. 149:112914.

[40] Jafari-Nozad, A.M., Jafari, A., Aschner, M., Farkhondeh, T. and Samarghandian, S. (2023). Curcumin combats against organophosphate pesticides toxicity: A review of the current evidence and molecular pathways. Current Medicinal Chemistry, Vol. 30, No. 20, pp. 2312-2339.

[41] Xiong, H., Wang, J., Ran, Q., Lou, G., Peng, C., Gan, Q. *et al*. (2019). Hesperidin: A therapeutic agent for obesity. Drug Design, Development and Therapy, Vol. 13, pp. 3855-3866.

[42] El-Saad, A.A., Ibrahim, M.M., Hazani, A.A. and El-Gaaly, G.A. (2016). Lycopene attenuates dichlorvos-induced oxidative damage and hepatotoxicity in rats. Human and Experimental Toxicology, Vol. 35, No. 6, pp. 654-665.

[43] Woo, S.J. and Chung, J.K. (2020). Effects of trichlorfon on oxidative stress, neurotoxicity, and cortisol levels in common carp, *Cyprinus carpio* L., at different temperatures. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, Vol. 229: 108698.

[44] Remigante, A., Spinelli, S., Straface, E., Gambardella, L., Russo, M., Cafeo, G. *et al*. (2023). Mechanisms underlying the anti-aging activity of bergamot (*Citrus bergamia*) extract in human red blood cells. Frontiers in Physiology, Vol. 14: 1225552.

[45] Bezerra, F.S., Lanzetti, M., Nesi, R.T., Nagato, A.C., Silva, C.P.E., Kennedy-Feitosa, E. *et al*. (2023). Oxidative stress and inflammation in acute and chronic lung injuries. Antioxidants, Vol. 12, No. 3, pp. 548-557.

[46] Sedoris, K.C., Ovechkin, A.V., Gozal, E. and Roberts, A.M. (2009). Differential effects of nitric oxide synthesis on pulmonary vascular function during lung ischemia-reperfusion injury. Archives of Physiology and Biochemistry, Vol. 115, No. 1, pp. 34-46.

[47] Signori, D., Magliocca, A., Hayashida, K., Graw, J.A., Malhotra, R., Bellani, G. *et al*. (2022). Inhaled nitric oxide: role in the pathophysiology of cardio-cerebrovascular and respiratory diseases. Intensive Care Medicine Experimental, Vol. 10, No. 1, pp. 28-38.

[48] Prasai, P.K., Shrestha, B., Orr, A.W. and Pattillo, C.B. (2018). Decreases in GSH: GSSG activate vascular endothelial growth factor receptor 2 (VEGFR2) in human aortic endothelial cells. Redox Biology, Vol. 19, pp. 22-27.

[49] van de Wetering, C., Elko, E., Berg, M., Schiffers, C.H., Stylianidis, V., van den Berge, M. *et al*. (2021). Glutathione S-transferases and their implications in the lung diseases asthma and chronic obstructive pulmonary disease: Early life Susceptibility. Redox Biology, Vol. 43: 101995.

[50] Sabarwal, A., Kumar, K. and Singh, R.P. (2018). Hazardous effects of chemical pesticides on human health: Cancer and other associated disorders. Environmental Toxicology and Pharmacology, Vol. 63, pp. 103-114.

[51] Abdullahi, S.N., Isa, S., Habib, A.A., Wada, F.M. and Ghali, N.M. (2022). Effect of Sniper insecticide (DDVP) on antioxidant biomarkers and histology of African catfish (*Clarias gariepinus* Burchell, 1822). Scholarly Journal of Science and Technology Research & Development, Vol. 1, pp. 6-20.

[52] Celik, I. and Suzek, H. (2009). Effects of subacute exposure of dichlorvos at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. Ecotoxicology and Environmental Safety, Vol. 72, No. 3, pp. 905-908.

[53] Hart, J.S. Lekpa, K.D. and Oghenefego, M.A. (2022). Ameliorative potentials of vitamin C against inhaled Dichlorvos lung toxicity of Wistar rats. Scholars International Journal of Anatomy and Physiology, Vol. 5, No. 3, pp. 65-69.

[54] Abdo, W., Elmadawy, M.A., Abdelhiee, E.Y., Abdel-Kareem, M.A., Farag, A., Aboubakr, M. *et al*. (2021). Protective effect of thymoquinone against lung intoxication induced by malathion inhalation. Scientific Reports, Vol. 11, No. 1, pp. 2498-24105.

[55] Wang, S., He, N., Xing, H., Sun, Y., Ding, J. and Liu, L. (2020). Function of hesperidin alleviating inflammation and oxidative stress responses in COPD mice might be related to SIRT1/PGC-1α/NF-κB signaling axis. Journal of Receptors and Signal Transduction, Vol. 40, No. 4, pp. 388-394.

[56] de Souza, A.B.F., de Matos, N.A., de Freitas Castro, T., de Paula Costa, G., Oliveira, L.A.M., Nogueira, K.D.O.P.C. *et al*. (2022). Effects *in vitro* and *in vivo* of hesperidin administration in an experimental model of acute lung inflammation. Free Radical Biology and Medicine, Vol. 180, pp. 253-262.

[57] Hosawi, S. (2023). Current update on role of hesperidin in inflammatory lung diseases: Chemistry, pharmacology, and drug delivery approaches. Life, Vol. 13, No. 4, pp. 937-948.