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CURCUMIN REVERSES DICHLORVOS-MEDIATED ERYTHROCYTE TOXICOSIS IN RATS BY ATTENUATING OXIDATIVE STRESS AND DEPLETION OF CELLULAR ACETYLCHOLINESTERASE

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ABSTRACT

In low- and middle-income countries, including Nigeria, dichlorvos (DDVP) is a prevalent organophosphate insecticide. However, its misuse as a suicide agent and presence in food items endangers the human populace. Curcumin is known to modulate free radicals, antioxidant proteins, and lipid peroxidation. However, its effect on DDVP-induced erythrocyte intoxication remains undocumented in the scientific literature. Herein the influence of curcumin was examined on DDVP-mediated erythrocyte toxicosis in Wistar rats. Forty-two rats were randomly allocated to seven groups of six rats each: normal control, DDVP alone (20 mg/kg/day), DDVP delivered with curcumin (50 and 100 mg/kg/day) or atropine (0.2 mg/kg/day) as a reference drug, and curcumin alone (50 and 100 mg/kg/day). Rats were humanely killed after one week of gavage DDVP treatment and another two weeks of curcumin therapy; blood was collected, and erythrocytes were isolated. Sub-acute administration of curcumin markedly ($p<0.05$) attenuated DDVP-provoked augmentation in the erythrocyte concentrations of NO and malondialdehyde and the activity of GST. Curcumin abrogated DDVP-mediated decreased in erythro-

cyte GSH levels and activities of SOD, catalase, and glutathione peroxidase, acetylcholinesterase. Ultimately, curcumin ameliorated DDVP-mediated erythrocyte toxicosis via anti-oxidative and cholinergic mechanisms.

Keywords: Curcumin, dichlorvos, erythrocyte, oxidative stress, acetylcholinesterase

INTRODUCTION

Organophosphate pesticides (OPs) constitute roughly 23% of the global pesticide market, significantly impacting household, veterinary practices, and agricultural pests (insects, herbs, fungi and rodents) mitigation leading to value-added crop and animal production, health and economic growth (Leskovac & Petrović, 2023). Dichlorvos (DDVP), categorized by WHO as highly hazardous chemical and widely used OP, is particularly prevalent in low- and middle-income countries, including Nigeria (Oridupa, 2020). However, its availability and affordability have led to concerning cases of misuse, including intentional poisoning, non-occupational exposure and accidental contamination of food, posing a significant public health threat (Mfaume *et al*., 2023).

In many advanced economies, the use of OPs has been prohibited due to their detrimental effects on human health and ecosystems (Oyeyemi *et al*., 2020). Nonetheless, these chemicals continue to circulate in many underdeveloped countries, posing significant risks (Oridupa, 2020). Exposure to OPs can occur either acutely or chronically through occupational or non-occupational activities. Individuals may inadvertently ingest pesticide residues present in food such as fruits, vegetables, and grains, as well as drinking water contaminated with these substances. Exposure can also occur through the inhalation of air containing DDVP after its application in homes, or through skin contact with contaminated surfaces (Okoroiwu and Iwara, 2018). The residues of pesticides and their metabolites have the potential to contaminate soils and water sources, leading to infiltration into the food chain. This contamination can have severe repercussions on human well-being (Dwivedi *et al*., 2010; Nan *et al*., 2015; Mfaume *et al*., 2023).

The detrimental impacts of DDVP are chiefly triggered via phosphorylation and inhibition of acetylcholinesterase (AChE), an enzyme critical for neurotransmitter signaling termination at cholinergic synapses (Okoroiwu and Iwara, 2018). This inhibition leads to acetylcholine accumulation, over stimulating cholinergic receptors, and causing a cascade of harmful displays, including seizures and respiratory failure (Oridupa, 2020). Additionally, DDVP exposure has been connected to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively termed oxidative and nitrative stress, respectively (Akande and Ahmed, 2017). Both ROS and RNS contribute to membrane and cellular damage and dysfunction.

Due to their primary oxygen transport function, erythrocytes are particularly susceptible to oxidative stress. DDVP has been implicated as an agent that disrupts the antioxidant defense system within red blood cells, leading to various erythrocyte dysfunctions (Akamo *et al*., 2021). Consequently, identifying efficacious therapeutic remedy to alleviated DDVP-induced toxicosis is of critically matter.

Curcumin, a Curcuma longa rhizome polyphenol phytochemical, exhibits therapeutic potential in modulating free radical activity, boosting antioxidant enzymes, and mitigating various toxicant-elicited impairments and diseases (Messarah *et al.*, 2013; Kępińska-Pacelik & Biel, 2023). However, the potential of curcumin as an antidote against DDVP-mediated erythrocyte toxicosis remains sparsely documented in the scientific literature.

It was hypothesized that curcumin possesses salubrious propensities in mitigating DDVP-triggered erythrocytotoxicity in rats. Herein, assessment was made of the therapeutic influence of curcumin on DDVP-engendered erythrocyte toxicosis in Wistar rats. Precisely, the underlying mechanism through which curcumin impacts oxidative stress and cellular homeostasis in DDVP-treated rats was examined.

MATERIALS AND METHODS

Chemicals

The 2,2-dichloroethenyl dimethyl phosphate (DDVP, $C_4H_7Cl_2O_4P$, CAS No. 62-73-7), 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione ($C_{21}H_{20}O_6$, Cat No. SC8670), haemoglobin kit (Hb, Anamol, Prodect Code: LD01), 2,2,2-trichloroethanoic acid (TCA, Cl_3CCOOH, CAS No. 76-03-9), aniline-p-sulfonic acid ($NH_2C_6H_4SO_3H$, CAS No. 121-57-3), monophosphoric acid (H_3PO_4, CAS No. 7664-38-2), 2-(1-Naphthylamino)ethylamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$, CAS No. 1465-25-4), gamma-L-glutamyl-L-cysteinylglycine (GSH, $C_{10}H_{17}N_3O_6S$, CAS No. 70-18-8), 3,3'-Dithiobis(6-nitrobenzoic acid) (DTNB, $C_{14}H_8N_2O_8S_2$, CAS No. 69-78-3), 1,3-Dinitro-4-chlorobenzene (DNCB, $C_6H_3Cl(NO_2)_2$, CAS No. 97-00-7), Trometamol ($C_4H_{11}NO_3$, CAS No. 77-86-1), anhydrous hydrochloric acid (HCl, CAS No. 7647-01-0), pyrogallol ($C_6H_6O_3$, CAS No. 87-66-1), sequestrene aa [$((HOOCCH_2)_2NCH_2)_2$, CAS No. 60-00-4], diammonium molybdate, sodium nitride (NaN_3, CAS No. 26628-22-8), monopotassium dihydrogen monophosphate (KH_2PO_4, CAS No. 7778-77-0), dipotassium monohydrogen monophosphate (K_2HPO_4, CAS No. 7758-11-4), dihydro-2-thioxo-4,6(1H,5H)-pyrimidinedione ($C_4H_4N_2O_2S$, CAS No. 504-17-6), 2-(Acetylthio)-N,N,N-trimethylethanaminium iodide ($C_7H_{16}INOS$, CAS No. 1866-15-5). The organization that supplied the Abanol kit was Anamol laboratories Pvt. Ltd, Kolgaon, Maharashtra 401404, India. The supplier of curcumin was Solarbio Science & Technology Company Limited (Tongzhou District, Beijing, China). The remaining chemicals were acquired from Sigma-Aldrich Chemical Company (St. Louis, MO 63118, USA). It is essential to note that all chemicals employed in this investigation were of high purity and met the standards of analytical grade.

Animal handling

Forty-two (42) male Wistar rats (11 weeks old, 161–199 g) that were free of pathogens used in this study were obtained from the Department of Biochemistry, College of Biosciences, Federal University of Agriculture, Abeokuta (FUNAAB). The rats were kept in a temperature-regulated (28 ± 2°C) and humidity-regulated (47 ± 2%) environment with a conventional 12-hour light/12-hour dark trajectory. They were confined in appropriately ventilated, suspended plastic cages with enough aspen shavings for bedding. The experimental rats received regular rat food and access to sanitary water supply on demand. All animals underwent a seven-day acclimatization period before the experiment began.

Ethical approval

All animal procedures were conducted in accordance with the guidelines set forth in the ARRIVE - Animal Research: Reporting of In Vivo Experiments (Percie du Sert, *et al.*, 2020) and were approved by the FUNAAB Research Ethical Committee (license cryptogram: FUNAAB/COLBIOS/BCH/PG/17-0135).

Experimental design and treatment

Following a one-week acclimatization period, rats were randomly assigned to seven groups (n=6/group):

Control: Received distilled water [2 mL/kg body weight (b.wt.)] orally for seven days, followed by olive oil (2 mL/kg) for an additional fourteen days.

DDVP alone: Received DDVP (20 mg/kg) orally for seven days, followed by olive oil (2 mL/kg) for fourteen days.

DDVP + Atropine: Received DDVP (20 mg/kg) for seven days, followed by atropine (0.2 mg/kg) for fourteen days.

DDVP + Curcumin-50: Received DDVP (20 mg/kg) for seven days, followed by curcumin (50 mg/kg) for fourteen days.

DDVP + Curcumin-100: Received DDVP (20 mg/kg) for seven days, followed by curcumin (100 mg/kg) for fourteen days.

Curcumin-50 alone: Received distilled water (2 mL/kg) for seven days, followed by curcumin (50 mg/kg) for fourteen days.

Curcumin-100 alone: Received distilled water (2 mL/kg) for seven days, followed by curcumin (100 mg/kg) for fourteen days.

Olive oil was used to dissolve the curcumin, hence groups I and 2 received the olive oil. All administrations occurred daily between 7:30 and 8:30 AM via oral gavage. The rationale for the sub-acute dosing regimen (one week of DDVP and two weeks of curcumin) aligns with prior studies investigating DDVP (Nwamba *et al.*, 2018) and curcumin (Forouzanfar *et al.*, 2020). The DDVP dose (20 mg/kg) represents one-quarter of the reported oral LD50 (Nwamba *et al.*, 2018), and the curcumin doses (50 and 100 mg/kg) were selected based on their established beneficial effects (Forouzanfar *et al.*, 2020).

Blood collection and erythrocyte isolation

Twenty-four hours after the last curcumin administration (day 22), rats were euthanized according to the protocol established by Wellington *et al.* (2013) following anesthesia with intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood was collected via retro-orbital bleeding into 10 mL non-anticoagulant tubes. To isolate erythrocytes, whole blood was allowed to stay at room temperature (28 ± 2°C) and plasma was separated by centrifugation at 3,000 x g for 15 minutes. The clear supernatant (plasma) was carefully removed for subsequent enzyme analysis. The buffy coat, enriched with leukocytes, was eliminated using suction. The remaining erythrocyte pellet was re-suspended and washed twice with ice-cold sodium phosphate-buffered saline (PBS, 8.1 mM, pH 7.4) at 5,000 rpm for 10 minutes each centrifugation step. This double washing step ensured thorough removal of contaminating leukocytes, resulting in a highly purified erythrocyte population. The washed erythrocytes were lysed in an isotonic Tris-HCl solution (pH 7.6) for downstream biochemical assays. Lysates were then stored at -20°C until analysis.

Biochemical analysis

Oxidative stress analysis

Nitric oxide (NO) determination

Nitric oxide (NO) levels were indirectly assessed by measuring nitrite (NO^-) concentration, a stable oxidation product of NO. En-

dothelial nitric oxide synthase (eNOS) catalyzes the conversion of L-arginine to L-citrulline and NO in the presence of oxygen and NADPH:
$2L\text{-arginine} + 6O_2 + 3NADPH + 3H^+ \rightarrow 2L\text{-citrulline} + 6NO + 6NADP^+ + 9H_2O$
NO is rapidly oxidized to NO_2^- and nitrate (NO_3^-) by cellular oxygen. NO_3^- can be further reduced to NO_2^- by NADPH-dependent nitrate reductase. The Griess reagent assay exploits the reaction between NO_2^- and sulfanilamide to form a diazonium salt, which subsequently couples with N -(1-naphthyl) ethylenediamine to generate a red-violet azo dye [NO2− + sulfanilamide/ sulfanilic acid → diazonium salt, + N-(1-naphthyl)ethylenediamine → azo dye].. Absorbance of this dye solution, measured at 520 nm, is directly proportional to the NO_2^- concentration, which reflects cellular NO production as described by Sreejayan & Rao (1997).

Antioxidant enzyme activity and reduced glutathione levels assays

The levels of reduced glutathione (GSH), and the activities of glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined using standard spectrophotometric methods.

- ***Reduced Glutathione (GSH):*** Ellman's reagent [5,5'-dithiobis(2-nitrobenzoic acid), DTNB] was used to quantify GSH. DTNB reacts with GSH to form a yellow chromophore, 2-nitro-5-thiobenzoic acid (TNB): 2GSH + DTNB → GSSG + TNB. The increased yellow intensity of TNB at 420 nm is directly proportional to GSH concentration as described by Rahman *et al.* (2006).

- ***Glutathione-S-transferase (GST):*** GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. GST conjugates GSH with CDNB to form a yellow chromophore, CDNB-SG conjugate: CDNB + GSH → CDNB-SG conjugate + HCl. The increased absorbance at 340 nm reflects GST activity as described by Habig *et al.* (1974).

- ***Superoxide Dismutase (SOD):*** Pyrogallol impulsively oxidizes to semi Quinone radical, hydroxyl radical, and superoxide anion radical [$C_6H_3(OH)_3 + O_2 \rightarrow C_6H_2(OH)_2O^* + HO^* + O_2^*$-]. The superoxide anion radical then combines with pyrogallol to make brown-colored quinone, a chromophoric compound [O_2^*- + $C_6H_3(OH)_3 \rightarrow C_6H_2O_2$], enhancing the optical density. SOD mops up superoxide anion to hydrogen peroxide and oxygen ($2O_2^*$- → $H_2O_2 + O_2$). Thus, SOD strives with pyrogallol's substrate, preventing quinone formation and originating decreased optical density at 420 nm. SOD activity was assessed by monitoring its ability to inhibit the superoxide-mediated conversion of pyrogallol to a brown-colored quinone. The decrease in absorbance at 420 nm due to SOD activity is proportional to its enzymatic function (Marklund and Marklund, 1974).

- ***Catalase (CAT):*** Catalase activity was determined by measuring the decomposition of hydrogen peroxide (H_2O_2) using a potassium permanganate solution: ($2H_2O_2 \rightarrow 2H_2O + O_2$. The disappearance of H_2O_2, a substrate for catalase, leads to a decrease in the formation of a colored molybdenum complex at 405 nm. This decrease is directly proportion-

al to catalase activity. i..e. the unused H_2O_2 combined with ammonium molybdate induces a yellow chromophoric complex (H_2O_2 + ammonium molybdate → molybdenum complex). At 405 nm, the complex-occasioned declined in intensity is inversely related to the amount of unused H_2O_2 but directly related to catalase activity as described by Hadwan and Abed (2016).

- ***Glutathione Peroxidase (GPx):*** Hydrogen (organic) peroxide is reduced by glutathione peroxidase (GPx) using GSH as a substrate, resulting in the formation of alcohol and GSSG (H_2O_2 + 2GSH → $2H_2O$ + GSSG). Oxidized glutathione and 2-nitro-5-thiobenzoic acid (TNB), a yellow chromogenic compound, are generated from the reaction between the remaining GSH and DTNB [2GSH + DTNB → GSSG + TNB]. The diminution in TNB absorbance at 420 nm reflects GPx activity as described by Rotruck *et al.* (1973).

Lipid peroxidation assessment via malondialdehyde (MDA) analysis

Malondialdehyde (MDA), a prominent thiobarbituric acid reactive substance (TBARS), serves as a well-established indicator of lipid peroxidation. It reacts with thiobarbituric acid (TBA) to form a chromogenic product, a pink-colored MDA-TBA adduct (MDA + 2TBA → MDA-TBA adduct). The intensity of this chromophore, measured at 523 nm, directly correlates with MDA content and reflects the extent of lipid oxidative damage as described by Buege & Aust (1978).

Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM). Differences between experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Duncan's multiple range post-hoc test for inter-group comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Statistical analyses were performed using SPSS Statistics version 26. Graphical representations of the data were constructed using GraphPad Prism version 9.0 software. This allowed clear visualization of differences between control and treatment groups based on oxidative stress and antioxidant parameters examined.

RESULTS

Curcumin ameliorates DDVP-induced alterations in erythrocyte glutathione and glutathione S-transferase activity

DDVP exposure resulted in a marked ($p < 0.05$) decrease in GSH levels by 82.4% and a doubling of GST activity compared to the control group (Fig, 1). Both atropine and curcumin administrations demonstrated protective effects against DDVP-induced alterations. Post-atropine and post-curcumin at varying doses (50 and 100 mg/kg/day) administration to DDVP-treated rats significantly abated DDVP-induced GSH reduction by 191.0%, 225.4%, and 257.6%, respectively. Similarly, they notably reduced the DDVP-mediated elevation in GST activity by 83.3%, 83.3%, and 50.3%, respectively. Conspicuously, while protective against DDVP, curcumin alone also exhibited mild suppressive effects on GSH levels in normal rats. Compared to the control group, curcumin at both doses (50 and 100 mg/kg/day) caused statistically significant reductions in GSH levels, ranging from 16.8% to 29.4% (Fig. 1). Similarly, the lower dose of curcumin (50 mg/kg/day) displayed a statistically significant decrease in GST activity by 33.3%, while the higher dose did not differ

significantly from the control group.

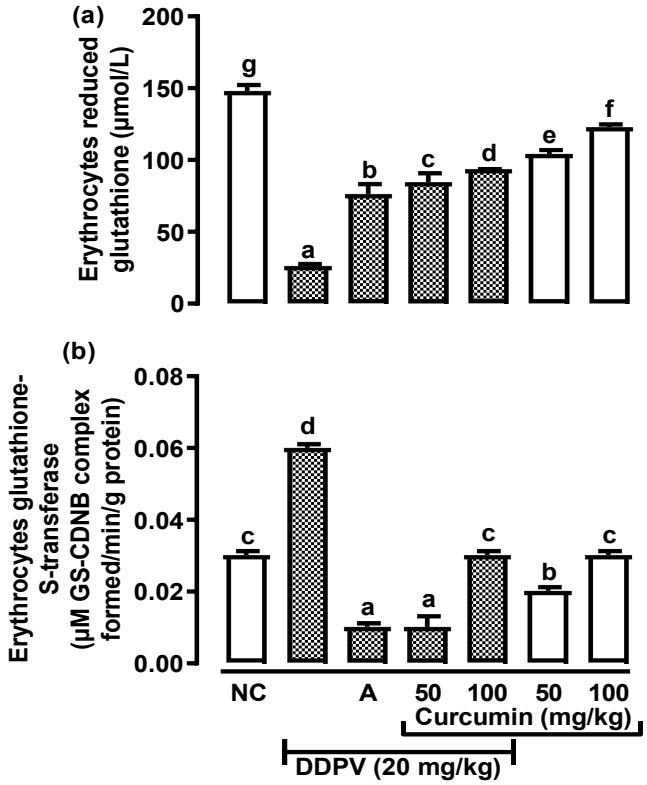

Figure 1: Effect of Curcumin intervention on: (a) reduced glutathione and (b) glutathione-s-transferase activity in DDVP-induced erythrocyte toxicosis.

NC, A, and DDPV signify normal control, atropine, and 2,2-dichlorovinyl dimethyl phosphate, respectively. Data are written as mean ± SEM (n = 6 rats/group). Bars with different alphabets differ significantly ($P < 0.05$).

Curcumin mitigates DDVP-induced reductions in erythrocyte superoxide dismutase and catalase activities

The impact of various treatments, including DDVP (20 mg/kg/day), DDVP combined with curcumin supplementation (at doses of 50 and 100 mg/kg/day), or reference atropine (at a dose of 0.2 mg/kg/day), and curcumin alone (at doses of 50 and 100 mg/kg/day), on erythrocyte SOD and catalase activities are demonstrated (Fig. 2). A significant down regulation of both superoxide dismutase (SOD, Fig. 2a) and catalase (Fig. 2b) activities following DDVP treatment compared to the control group. Compared to the control group, SOD activity declined by a staggering 89.7%, while catalase activity dropped by 87.8%. Nevertheless, these findings are complemented by the

ameliorative effects observed with atropine, as well as 50 mg/kg, and 100 mg/kg curcumin administration. The treatments significantly reversed the DDVP-induced reductions in SOD activity by 533.3%, 833.3%, and 733.3%, respectively, and catalase activity by 191.4%, 306.2%, and 483.4%, respectively. It is important to note that curcumin alone, at the administered doses, did not significantly alter SOD activity in healthy rats. However, it did cause a moderate decrease (21.2% and 26.7%) in catalase activity compared to the control group (Fig. 2).

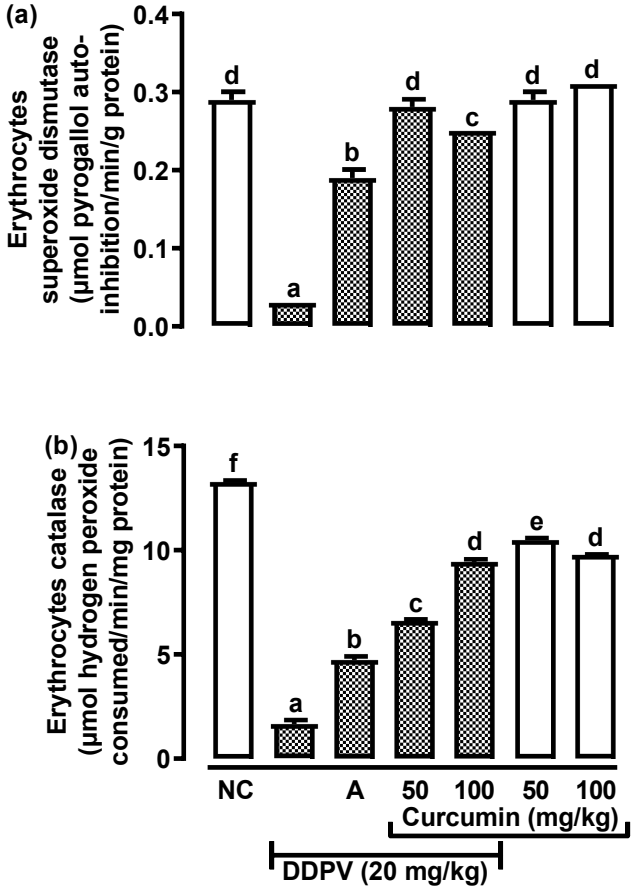

Figure 2: Effect of curcumin intervention on: (a) superoxide dismutase activity and (b) catalase activity in DDVP-induced erythrocyte toxicosis.

NC, A, and DDPV signify normal control, atropine, and 2,2-dichlorovinyl dimethyl phosphate, respectively. Data are written as mean ± SEM (n = 6 rats/group). Bars with different alphabets differ significantly ($P < 0.05$).

Curcumin mitigates DDVP-mediated inhibition of glutathione peroxidase and acetylcholinesterase activity

DDVP elicited significant ($p < 0.05$) decreases in the GPx and AChE activities compared to the control group. These reductions were

substantial, with GPx activity decreasing by 95.5% and AChE activity decreasing by 53.6%, compared to normal levels. It was, also discovered that administration of atropine (0.2 mg/kg/day), as well as 50- and 100 mg/kg/day curcumin to DDVP-treated rats significantly ($p<0.05$) improve DDVP-elicited GPx reduction by 841.7%, 866.7%, and 1016.7%, respectively, and also attenuated DDVP-incited AChE reduction by 43.0%, 23.5%, and 60.9%, respectively. Interestingly, even without prior DDVP exposure, curcumin alone (at both doses) caused a slight but significant decrease in both GPx (48.7% versus 49.4%) and AChE (8.6% versus 13.2%) activity compared to normal levels.

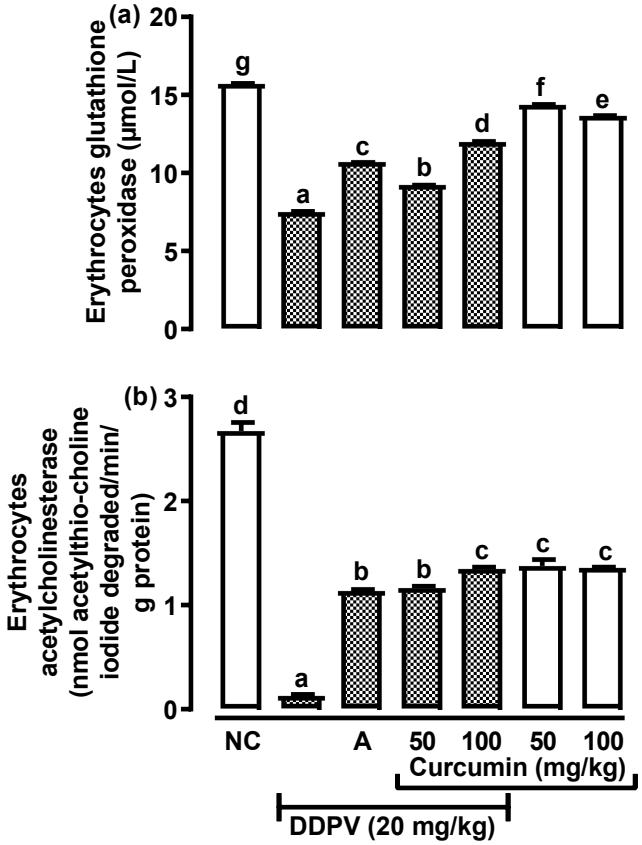

Figure 3: Effect of curcumin intervention on (a) glutathione peroxidase and (b) acetylcholinesterase activity in DDVP-induced erythrocyte toxicosis.

NC, A, and DDPV signify normal control, atropine, and 2,2-dichlorovinyl dimethyl phosphate, respectively. Data are written as mean ± SEM (n = 6 rats/group). Bars with different alphabets differ significantly.

Protective effects of curcumin on DDVP-triggered nitric oxide and malondialdehyde elevation

DDVP elicited significant ($p < 0.05$) NO and MDA levels elevation compared to the control group. These elevations were 4.4- and 2.7 times higher for NO (Fig. 4a) and MDA (Fig. 4b), respectively, compared to the normal control. Administration of atropine and curcumin (50- and 100 mg/kg) to DDVP treated rat significantly ($p<0.05$) attenuated DDVP-induced NO elevation by 36.8%, 46.5%, and 52.8%, respectively, and also attenuated DDVP-induced MDA elevation by 35.5%, 34.3%, and 39.6%, respectively., There was no significant difference ($p > 0.05$) in the levels of NO and MDA between the two doses of curcumin-only groups (50- and 100 mg/kg) and normal control.

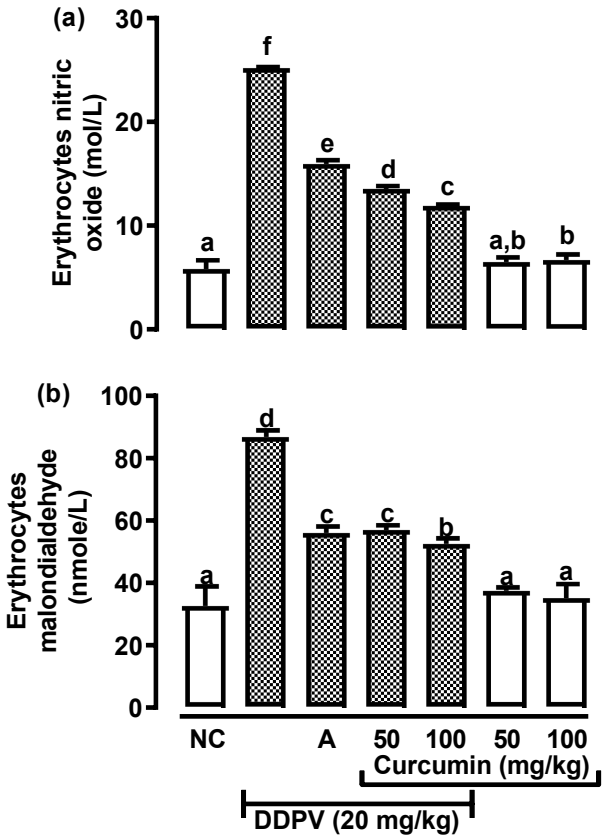

Figure 4: Effect of curcumin intervention on (a) nitric oxide and (b) malondialdehyde levels in DDVP-induced erythrocyte toxicosis.

NC, A, and DDPV signify normal control, atropine, and 2,2-dichlorovinyl dimethyl phosphate, respectively. Data are written as mean ± SEM (n = 6 rats/group). Bars with different alphabets differ significantly ($P < 0.05$).

DISCUSSION

The study investigated the potential of curcumin to alleviate the toxic effects of 2,2 -dichlorovinyl dimethyl phosphate (DDVP) on the erythrocytes in rats. The results demonstrate that DDVP exposure significantly reduced the levels of glutathione (GSH) and the activities of enzymes involved in antioxidant defense, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Conversely, DDVP increased the activity of glutathione S-transferase (GST) and the levels of malondialdehyde (MDA) and nitric oxide (NO), all indicative of oxidative stress. These findings are consistent with previous studies, suggesting that DDVP disrupts erythrocyte function and integrity through oxidative damage (Akande & Ahmed, 2017; Oridupa, 2020; Mfaume *et al.*, 2023).

Curcumin or reference drug atropine administration, effectively reversed these negative effects. They significantly increased GSH levels and the activities of SOD, catalase, and GPx, while simultaneously reducing GST activity, MDA levels, and NO production. This multifaceted response suggests that curcumin's protective effects stem from its potent antioxidant properties (Kępińska-Pacelik and Biel, 2023).

Curcumin likely exerts its antioxidant effects through several mechanisms. As a polyphenolic compound, it can directly scavenge free radicals and reactive oxygen species (ROS) generated by DDVP exposure. Additionally, curcumin may up regulate the expression of antioxidant enzymes like SOD, catalase, and GPx, thereby replenishing cellular antioxidant defenses depleted by DDVP (Messarah *et al.*, 2013; Suwarta *et al.*, 2022). Furthermore, curcumin's ability to reduce MDA levels suggests that it may inhibit lipid peroxidation, a key marker of oxidative damage.

Our findings also revealed that curcumin reversed DDVP-mediated inhibition of acetylcholinesterase (AChE) activity, an enzyme crucial for normal nervous system function. This suggests that curcumin may offer additional benefits beyond its antioxidant properties, potentially mitigating the neurotoxic effects of DDVP exposure (Abu-Taweel, 2016; Akinyemi *et al.*, 2017; Farkhondeh *et al.*, 2020).

While both curcumin and atropine effectively ameliorated DDVP-induced erythrocyte toxicity, our data suggests that curcumin might be a more potent therapeutic agent. Notably, curcumin displayed a greater ability to restore the activity of antioxidant enzymes compared to atropine. Additionally, curcumin did not significantly alter the activity of GST under normal conditions, unlike atropine, which caused a slight decrease. This suggests that curcumin might exhibit a more targeted approach to mitigating oxidative stress compared to atropine. Our observation is in consonant with a prior study that curcumin protect against oxidative stress more than atropine (Yadav *et al.*, 2012).

When tested alone in healthy rats, curcumin slightly inhibited GSH, catalase, and GPx. One possible explanation is that curcumin can, under some situations, generate reactive oxygen species (ROS). In any case, the alterations were minor and clinically irrelevant.

Even more notably, curcumin abrogate DDVP-elicited toxicosis, suggesting it does not have pro-oxidant effects in pathological contexts with apparent oxidative stress.

DDVP exposure triggers reactive oxygen species (ROS) and reactive nitrogen species (RNS) which intern induced cellular damage and dysfunction via oxidative stress and ATP depletion (Figure 5).

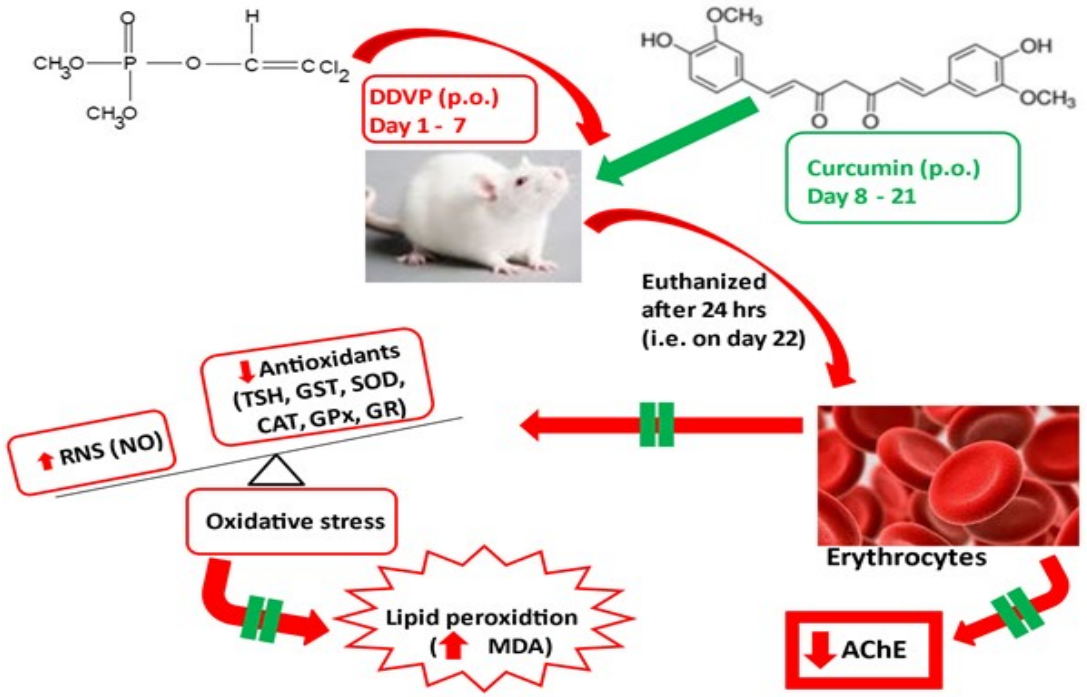

Figure 5: Probable mechanism of how curcumin reverses Dichlorvos (DDVP)-activated erythrocyte toxicosis in rats by rescinding oxidative stress and depletion of cellular acetyl-cholinesterase.

Red arrows indicates the reaction pathways of the detrimental impact of DDVP, while the green symbol typified the inhibition by curcumin intervention).

DDVP exposure triggers reactive oxygen species (ROS) and reactive nitrogen species (RNS) which in turn induced cellular damage and dysfunction via oxidative stress and ATP.

However, it is important to acknowledge that this study was conducted in an animal model, and further research is necessary to translate these findings to human applications. Future studies could explore the optimal dosage and delivery methods of curcumin for treating DDVP poisoning in humans. Additionally, investigating the molecular mechanisms underlying curcumin's protective effects at a deeper level could provide valuable insights for developing novel thera-

peutic strategies against organophosphate poisoning.

In conclusion, this study demonstrates that curcumin effectively alleviates DDVP-induced erythrocyte toxicity in rats by acting as a potent antioxidant and potentially mitigating cholinergic dysfunction (Figure 5). These mitigatory influence support the potential therapeutic application of curcumin in managing organophosphate poisoning, although further clinical trial research is warranted to establish its safety and efficacy in humans.

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