ISSN:
Print - 2277 - 0593
Online - 2315 - 7461

Journal of Natural Science, Engineering and Technology

ERYTHROCYTE MEMBRANE-STABILITY AND PROPHYLACTIC POTENTIALS OF CHRYSIN AND RUTIN ON TRICHLOROETHYLENE-ENGENDERED ERYTHROCYTOTOXICITY IN WISTAR RATS

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ABSTRACT

Trichloroethylene (TCE) is a halogenated alkene solvent linked to several cancers and autoimmune diseases in humans and animals and is a contaminant of the air, water, and soil. Chrysin and rutin are known to modulate oxidants, antioxidants, and lipotoxicity. However, their mechanistic effect on TCE-prompted red blood cell injury remains undetermined. This investigation hypothesized that chrysin and rutin could offer an alleviative influence on TCE-triggered erythrocyte damage. Randomization was employed to earmark forty-eight rats into eight groups (6 rats/group): control, chrysin and/or rutin (100 $mg.kg^{-1}$ day^{-1}), TCE only (1,000 $mg.kg^{-1}day^{-1}$), chrysin and/or rutin (100 $mg.kg^{-1}day^{-1}$) treated with TCE. Rats were pre-treated orally by gavage with flavonoid therapy (chrysin and/or rutin) every day for fourteen days before administering a single dosage of TCE. Twenty-four hours following TCE administration, the rats were euthanized, and their blood was taken for biochemical assays. Chrysin and/or rutin pretreatment pronouncedly ($p < 0.05$) mitigated TCE-engendered increases in lipids

(triacylglycerol and cholesterol) concentrations, reactive oxygen/nitrogen specie (NO) levels, lipid peroxidation (MDA) amounts, and myeloperoxidase activity. The two flavonoids equally attenuated TCE-invoked depletions in haemoglobin, haematocrit, and total thiol levels, antioxidant enzymes (glutathione-S-transferase, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase), and lactate dehydrogenase (LDH). Improved antioxidant status, and activities of LDH in rats pretreated with the therapeutic agents are indicators of the membrane stability potential of the agents against the cellular assaults of TCE. Therefore, this study suggests that chrysin and/or rutin pretreatment abated TCE-provoked erythrocytes perturbations in rats possibly via radical trapping, antioxidants augmentation, anti-dyslipidemic mechanisms and positive modulation of LDH activity.

Keywords: Trichloroethylene, rutin, chrysin, erythrocytes, antioxidants, dyslipidemia

INTRODUCTION

Trichloroethylene (TCE) is a chemically synthesized, non-flammable, volatile, colourless, and lipophilic halogenated alkene solvent (Dumas *et al*., 2018; Adamson *et al*., 2023). It is primarily used in manufacturing chemicals, including degreasing, metal cleaning, refrigerant, and hydrofluorocarbons (Dorsey *et al*., 2023). Its relatively short half-life and slow degradation have led to public health trepidations regarding its environmental persistence as a pervasive pollutant in the atmosphere, water, and soil (Cichocki *et al*., 2016). Occupational and non-occupational exposures occur from breathing it in indoor and outdoor air, drinking contaminated water, skin contact, eating foods cleaned or processed, consumer products such as adhesives and paint removers (Chiu *et al*., 2013; Dorsey *et al*., 2023). While advanced countries have stricter regulations on TCE usage and enhanced efforts to recognize innocuous substitutes, TCE is still commonly used in low-economy countries (Njokunwogbu *et al*., 2022).

One of the consequential targets of TCE toxicity is red blood cells, whose primary role is to transport oxygen to the visceral for the oxidation of carbohydrates to generate ATP and remove the waste product of metabolism, carbon dioxide, from the visceral for excretion. Documented investigations have established that TCE exposure induces significant decreases in erythrocyte count, hemoglobin concentration, erythrocyte morphology, and leukocyte structures (Lan *et al*., 2010; Cichocki *et al*., 2016; Ordaz *et al*., 2017; Nwaogwugwu *et al*., 2023). These dysfunctions could negatively impact the oxygen- and carbon dioxide-carrying capacity of erythrocytes, possibly resulting in tissue hypoxia, hemolysis, loss of nitric oxide, otherwise called endothelial-derived relaxing factor, declined intracellular ATP, and glycolytic enzymes (Akamo *et al*., 2021).

Humans and laboratory animals metabolize TCE via oxidation and glutathione (GSH) conjugation pathways, producing several toxicologically active metabolites, including chloral hydrate, trichloroacetic acid (TCA), and dichloroacetic acid, as well as GSH conjugation metabolites dichlorovinyl glutathione and dichlorovinyl cysteine (Cichocki *et al*., 2016). So, one of the multidimensional mechanisms underlying TCE-triggered red blood cell perturbation is the production of excessive free radicals. Free radicals damage cellular biomolecules, including proteins, lipids, and DNA (Nwaogwugwu *et al*., 2023). Erythrocytes are predominantly susceptible to oxidative damage because their enhanced polyunsaturated fatty acids are vulnerable to reactive oxygen species (ROS)-activated peroxidation (Akamo *et al*., 2021). TCE expo-

sure has been revealed to impair antioxidant homeostasis and subsequent hemolysis (Cichocki *et al.*, 2016; Nwaogwugwu *et al.*, 2023).

Research is ongoing to uncover practical strategies to mitigate the detrimental consequences of xenobiotics and environmental toxicants, including TCE. Secondary metabolites derived from plants (phytochemicals), animals, and microorganisms have materialized as promising therapeutic candidates and are employed in treating human diseases due to their broad spectrum of pharmacological activities, intrinsic and diverse array of biological activities, such as antioxidant, anti-inflammatory, and cytoprotective propensities (Haruna and Yahaya, 2021; Najmi *et al.*, 2022)

Two such phytomedicine with well-established antioxidant and cytoprotective capacities are chrysin and rutin. Chrysin, or 5,7-dihydroxy-2-phenyl-4H-chromen-4-one (MW of 254.25 g/mol), is a bioactive flavone molecule found in honey, propolis and passion flower (Mohammed *et al.*, 2023; Rao *et al.*, 2023). Rutin, or quercetin-3-O-rutinoside (MW of 610.517 g/mol), is a flavonol glycoside widely distributed throughout the plant kingdom, including fruits and vegetables, especially buckwheat, the only cereal that not only contains rutin in its seeds but has it in a high amount (Brunori *et al.*, 2018; Frutos *et al.*, 2019).

Chrysin and rutin individually have broad-spectrum biological actions such as anticancer, anti-inflammatory, antiviral, antioxidant, antihypertensive, alpha-glucosidase inhibitory actions, ant-dyslipidemia, and blood capillary strengthening potentials (Brunori *et al.*, 2018; Rao *et al.*, 2023). Chrysin and rutin have been reported to explicitly stabilize, quench, and neutralize free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS); augment the synthesis of non-enzymatic and enzymatic antioxidants, thereby assuaging the detrimental effect of oxidative stress; inhibit aromatase; and inhibit cancer chemopreventive properties through apoptosis induction and proliferation inhibition in a diverse range of human and rat cell models. Chrysin has been shown to elicit anti-inflammatory potentials and mitigate against inflammatory responses evoked by xenobiotics, including TCE (Brunori *et al.*, 2018; Frutos *et al.*, 2019; Mohammed *et al.*, 2023; Rao *et al.*, 2023).

These functions of chrysin and rutin could assuage erythrocyte deformability and enhance their oxygen/carbon dioxide transportation aptitude (Akamo *et al.*, 2021). The single or combined protective influence of chrysin and rutin against TCE-instigated erythrocytotoxicity remains fuzzy in the scientific literature. Given the well-documented detrimental consequences of TCE on red blood cells on the one hand (Nwaogwugwu *et al.*, 2023) and the advantageous antioxidant and cytoprotective capacity of chrysin and rutin on the other hand, it is hypothesized that single or combined pre-treatment with chrysin and rutin could rescind TCE-provoked erythrocytotoxicity and hence may help to preserve the membrane of RBCs against TCE induced free radical damage. This study investigated the therapeutic efficacy of chrysin and rutin on structural dynamics, endothelial-derived relaxing factor (NO), antioxidant status, lipid peroxidation, triacylglycerol, cholesterol, and LDH activity in erythrocyte of TCE-exposed rats.

MATERIALS AND METHODS

Chemicals

The 5,7-dihydroxy-2-phenylchromen-4-one ($C_{15}H_{10}O_4$, CODE: C 2294), quercetin-3-rhamnoglucoside ($C_{27}H_{30}O_{16}$, CODE: R 1380), 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), haemoglobin kit (Hb, Anamol, Product Code: LD01), disodium hydrogen phosphate anhydrous (Na_2HPO_4, CAS No. 7758-11-4), sodium dihydrogen phosphate anhydrous (NaH_2PO_4, CAS No. 7778-77-0), dihydrogen dioxide (H_2O_2, CAS No. 7722-84-1), 2-Methoxyphenol ($C_7H_8O_2$, CAS No. 90-05-1), trichloroethanoic acid (TCA, CCl_3COOH, CAS No. 76-03-9), p-aminobenzenesulfonic acid ($C_6H_7NO_3S$, CAS No. 121-57-3), orthophosphoric acid (H_3PO_4, CAS No. 7664-38-2), 1,2-Ethanediamine, N-1-naphthalenyl-, dihydrochloride ($C_{10}H_7NHCH_2CH_2NH_2$•2HCl, CAS No. 1465-25-4), N-(N-L-gamma-Glutamyl-L-cysteinyl)glycine (GSH, $C_{10}H_{17}N_3O_6S$, CAS No. 70-18-8), 5,5'-dithiobis(2-nitrobenzoic acid (DTNB, $C_{14}H_8N_2O_8S_2$, CAS No. 69-78-3), 2,4-Dinitrochlorobenzene (DNCB, $C_6H_3Cl(NO_2)_2$, CAS No. 97-00-7), 2-Amino-2-(hydroxymethyl)propane-1,3-diol ($C_4H_{11}NO_3$, CAS No. 77-86-1), trihydroxybenzene ($C_6H_6O_3$, CAS No. 87-66-1), ethylenediaminetetraacetic acid [{$(HOOCCH_2)_2NCH_2$}$_2$, CAS No. 60-00-4], diammonium dioxidomolybdenum [$(NH_4)_2MoO_4$, CAS No. 13106-76-8], sodium trinitride (NaN_3, CAS No. 26628-22-8), dihydronicotinamide adenine dinucleotide phosphate (DHNADP, $C_{21}H_{29}N_7O_{17}P_3$, CAS No. 100929-71-3), thiobarbituric acid (TBA, $C_4H_4N_2O_2S$, CAS No. 504-17-6), and 2-(Acetylsulfanyl)-N,N,N-trimethylethanaminium iodide ($C_7H_{16}INOS$, CAS No. 1866-15-5). Otto Chemie Private Limited (Maharashtra, India) was contacted and contracted to supply chrysin. Anamol kits were secured from Anamol Laboratories Pvt. Ltd (Kolgaon, Maharashtra 401404, India). Sigma-Aldrich (St. Louis, MO, 63118, US) produced the other chemicals. This research employed pure, analytical-grade substances.

Animal handling

Forty-eight male Wistar rats (13 weeks old, 177–199 g weight) obtained from the College of Biosciences, Federal University of Agriculture, Abeokuta (FUNAAB), were lined up for this current examination. The rats were placed in acceptable ventilated standard plastic rat cages with the pertinent aspen chippings for bedding in a temperature-regulated (29 ± 2°C) and humidity-checked (49 ± 3%) vivarium, with a characteristic 12:12 light-dark cycle. The laboratory animals were fed standard rat feed and had access to a clean drinking water source without impediment. Before the commencement of the exploration, all animals spent seven days adapting to the new surroundings. The animals got compassionate welfare treatment as recommended by the FUNAAB Ethical Committee and in accordance with the guidelines provided by the United States of America National Academy of Science (NRC, 2010). The institution acknowledged the study protocol and allotted researcher with the number FUNAAB/COLBIOS/PG/18/1/0656.

Experimental schedule

Forty-eight acclimatized rats were randomly allotted into eight groups:

- Control,
- Chrysin (100 $mg.kg^{-1}day^{-1}$),
- Rutin (100 $mg.kg^{-1}day^{-1}$),
- Chrysin (100 $mg.kg^{-1}day^{-1}$) + rutin (100 $mg.kg^{-1}day^{-1}$),

- TCE only (1,000 $mg.kg^{-1}day^{-1}$),
- Chrysin (100 $mg.kg^{-1}day^{-1}$) + TCE (1,000 $mg.kg^{-1}day^{-1}$),
- Rutin (100 $mg.kg^{-1}day^{-1}$) + TCE (1,000 $mg.kg^{-1}day^{-1}$),
- Chrysin (100 $mg.kg^{-1}day^{-1}$) + rutin (100 $mg.kg^{-1}day^{-1}$) + TCE (1,000 $mg.kg^{-1}day^{-1}$), chrysin and/or rutin (100 $mg.kg^{-1}day^{-1}$).

The rats were pre-treated orally by gavage with chrysin and rutin every day for fourteen (14) days before administering a single dosage of TCE. Twenty-four hours following TCE administration, the rats were euthanized, and their blood was collected. All administrations were completed between 8.00 a.m. and 9.00 a.m. The motive for opting for a 100 mg/kg dosage of chrysin/ rutin pretreatment, a healthy dose (El-Marasy *et al.*, 2019, Bilgin *et al.*, 2020), and TCE (1,000 mg/kg) was grounded in the previous studies (Heydari *et al.*, 2017, Luo, *et al.*, 2018). Sub-acute pretreatment (14 days for chrysin and rutin) and acute (24 hrs) for TCE were likewise selected based on the previous studies (El-Marasy *et al.*, 2019, Bilgin *et al.*, 2020, Heydari *et al.*, 2017, Luo, *et al.*, 2018).

Blood collection and processing procedures

Following 14 days of chrysin/rutin treatment and a single dose of TCE on day 15, rats were submitted to ketamine/xylazine (100-/10 mg kg-1, IP) administration 24 hours after the last TCE dosage (day 16), as described by Wellington *et al* (2013). The blood was collected from the retro-orbital sinus and placed into plain 10 mL vials. After allowing the blood to clot, serum was separated by centrifugation at 3,000 g for 15 minutes at room temperature (30 ± 2°C). The serum (the clear supernatant) was first collected. Thereafter, suction was used to remove the buffy coat and retrieve the erythrocytes (RBCs). RBCs were mixed and washed in sodium phosphate-buffered saline (8.1 mM, pH 7.4, 2℃) before being centrifuged at 5000 rpm for 10 minutes at 4 degrees Celsius. Once more, suction was employed to remove the supernatant, which was disposed of properly. More RBCs were skipped to get rid of any lingering buffy covering. The washing operation was performed twice. The adoption of this method resulted in a final RBC preparation with nearly no white blood cells. After washing, the RBCs were lysed by suspending them in an isotonic Tris-HCl solution at pH 7.6. Until further biochemical testing could be done, the samples were frozen at -5 degrees Celsius.

Biochemical investigation

Each assay was spectrophotometrically determined by observing the rise or fall in absorbance of a specific chromophoric molecule at a particular wavelength.

Haemoglobin assay

Cyanmethemoglobin reagent (5,800 μL) interacts with hemolysate (200 μL). Potassium ferricyanide transforms hemoglobin into methemoglobin, which is then converted to a stable bright-red cyanmethemoglobin chromogenic complex by potassium cyanide [Hb + K3Fe(CN)6+ KCN → Cyanmethemoglobin + K4Fe(CN)6]. This complex's intensity is comparable to the hemoglobin concentration designated in the Anamol kit (Tietz, 1995). Whole blood in the heparinized capillary tube was centrifuged using a micro hematocrit centrifuge at 10,000 rpm for 3 minutes to separate the erythrocytes from plasma. The microhematocrit reader was used to read the ratio of the erythrocyte layer to the total blood volume provided by the hematocrit. The hemocytometer was used to

determine the Leukocyte count as described by (Tietz, 1995).

Lipid appraisal

Erythrocytes triacylglycerol, and cholesterol analysis was performed using a quinone imine-based enzymatic assay described in a commercially available enzymatic assay kit called LABKIT (Tietz, 1995).

Triacylglycerol (TAG) assay: is converted to glycerol (G) and fatty acids (FAs) via lipoprotein lipase: TAG + 3H2O → G + FAs. Dihydroxylacetone phosphate (DHAP) and hydrogen peroxide (H2O2) are made when G3P (glyceraldehyde-3-phosphate) is oxidized by G3P oxidase (G3P + O2 → DHAP + H2O2). The H2O2, 4-amino antipyrine, 4-chlorophenol, and H2O2 eventually combine in the presence of peroxidase to form a red quinone imine (QI): H2O2 + 4-AAP + 4-CPh → QI + HCl + 4H2O. The intensity of QI dye at 546 nm represents the TAG level.

Cholesterol esterase catalyzes the hydrolysis of cholesterol ester, forming cholesterol (chol) and free fatty acids (chol-ester + H2O → chol + FAs). Cholesterol oxidase catalyzes the oxidation of unbound cholesterol into cholest-4-en-3-one and hydrogen peroxide (chol + O2 → cholest-4-en-3-one + H2O2). H2O2 interacts with phenol and 4-amino antipyrine (4-AAP) in the presence of peroxidase to produce a red-colored quinone imine (QI) dye (H2O2 + Ph + 4-AAP → QI + 4H2O). The dye's optical density is consistently compared to the cholesterol contents at 500 nm.

Oxidative stress appraisal

Free radical and ROS generating enzyme (myeloperoxidase) appraisal

Myeloperoxidase, an enzyme involved in the generation of reactive oxygen species, oxidizes guaiacol to a brownish chromophore tetraguaiacol product: (4Guaiacol + 4H2O2 → Tetraguaiacol + 8H2O). The intensity of this colored product, measured at 470 nm, reflects myeloperoxidase activity in the red blood cells. (Klebanoff *et al.*, 1984).

Reactive oxygen/nitrogen specie (nitric oxide) appraisal

Nitric oxide synthase converts arginine to citrulline and nitric oxide [2L-arginine + 6O2 + 3NADPH + 3H+ → 2L-citrulline + 6NO + 6NADP+ + 9H2O]. NO3− and NO2− are products of the oxygen oxidation of nitric oxide (NO). In the presence of NADPH, nitrate reductase converts NO3− to NO2−. A combination of NO2− with Griess reagents generates a red-violet dye [NO2− + sulfanilamide/sulfanilic acid → diazonium salt, + N-(1-naphthyl) ethylenediamine → azo dye]. The dye intensity decides NO content at 520 nm (Sreejayan and Rao, 1997).

Antioxidants appraisal

We used conventional chemical techniques to evaluate total reduced thiols (TRSH), glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR).

Total reduced thiols (TRSH) appraisal: TRSH positioned in cysteine, homocysteine, cysteinylglycine, and glutathione were evaluated. The reduced form of thiols is oxidized by DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] to produce 2-nitro-5-thiobenzoic acid (TNB), a yellow chromophore [R-SH + DTNB → R-SS-R + TNB]. The increased yellow intensity is directly proportional to

the TRSH level at 420 nm, as demonstrated by (Prakash *et al*., 2009).

Glutathione-S-transferase (GST) appraisal: GST conjugates GSH and 1-chloro -2, 4-dinitrobenzene (CDNB) to glutathione conjugate, a yellow chromophore (CDNB + GSH → CDNB-SG conjugate + HCl). The upsurge in optical density at 340 nm determines CDNB-SG conjugate, delivering a measure of GST enzymatic efficacy (Habig *et al*., 1974).

Superoxide dismutase (SOD) appraisal: Pyrogallol is autoxidized by superoxide anion radical to purpurogallin, a brown chromophoric compound [pyrogallol + O2*- → purpurogallin], enhancing the absorbance. SOD sequesters the superoxide anion (2O2*- → H2O2 + O2), consequentially preventing pyrogallol autoxidation. The decreased brown intensity is directly proportional to the degree of pyrogallol autoxidation inhibition and SOD reactivity at 420 nm (Marklund and Marklund, 1974).

Catalase appraisal: Catalase transforms hydrogen peroxide into water and oxygen (2H2O2 → 2H2O + O2). The leftover H2O2 reacts with ammonium molybdate to create a yellow chromogenic complex (H2O2 + ammonium molybdate → molybdenum complex). At 405 nm, the complex-evoked reduction in absorbance is inversely proportionate to leftover H2O2 but directly proportionate to catalase bio-catalytic efficacy (Hadwan and Abed, 2016).

Glutathione peroxidase (GPx) appraisal: GPx converts hydrogen (organic) peroxide, employing GSH as a substrate, to produce alcohol and GSSG (H2O2 + 2GSH → 2H2O + GSSG). The reaming GSH reacts with DTNB to produce oxidized glutathione and 2-nitro-5-thiobenzoic acid (TNB), a yellow chromogenic molecule [2GSH + DTNB → GSSG + TNB]. At 420 nm, the chromogen-evoked reduction in absorbance is directly relative to unreacted GSH but inversely relative to GPx enzymatic proficiency (Rotruck *et al*., 1973).

Glutathione reductase (GR) appraisal: GR reduces oxidized glutathione to reduced glutathione (GSSG + NADPH → 2GSH + NADP+). The NADPH depletion-induced decrease in absorbance at 340 nm is unswervingly proportionate to the GR enzymatic kinetics (Sahreem *et al*., 2011).

Malondialdehyde (MDA) appraisal

MDA, a lipid peroxidation indicator and thiobarbituric acid reactive substance (TBARS) prototype, interacts with thiobarbituric acid (TBA) to create a pink chromophoric product (MDA + 2TBA → MDA-TBA adduct). The chromophoric-evoked optical density increases proportionately to the MDA level and lipid oxidative disturbance at 523 nm (Buege and Aust, 1978).

Statistical analysis

Quantitative variables were expressed using the mean plus or minus the standard error of the mean (S.E.M.). The group differences were examined using the Duncan Multiple Range Test and one-way ANOVA, with significance indicated at $p < 0.05$. All analyses were conducted using the Statistical Package for the Social Sciences 20.0. GraphPad Prism 8.0 was used to generate the graphs.

RESULTS

Exposure to TCE led to statistically consequential decreases in hematocrit, hemoglobin, and leukocytes contents by 14.79%, 14.83%, and 10.05%, respectively, compared to the control group (Fig. 1). However, pre-

treatment with chrysin or rutin, alone or combined restored the observed perturbation to normalcy. The hematocrit, hemoglobin, and leukocytes levels of normal rats treated with chrysin and/or rutin were statistically similar to those of the standard control (Fig. 1).

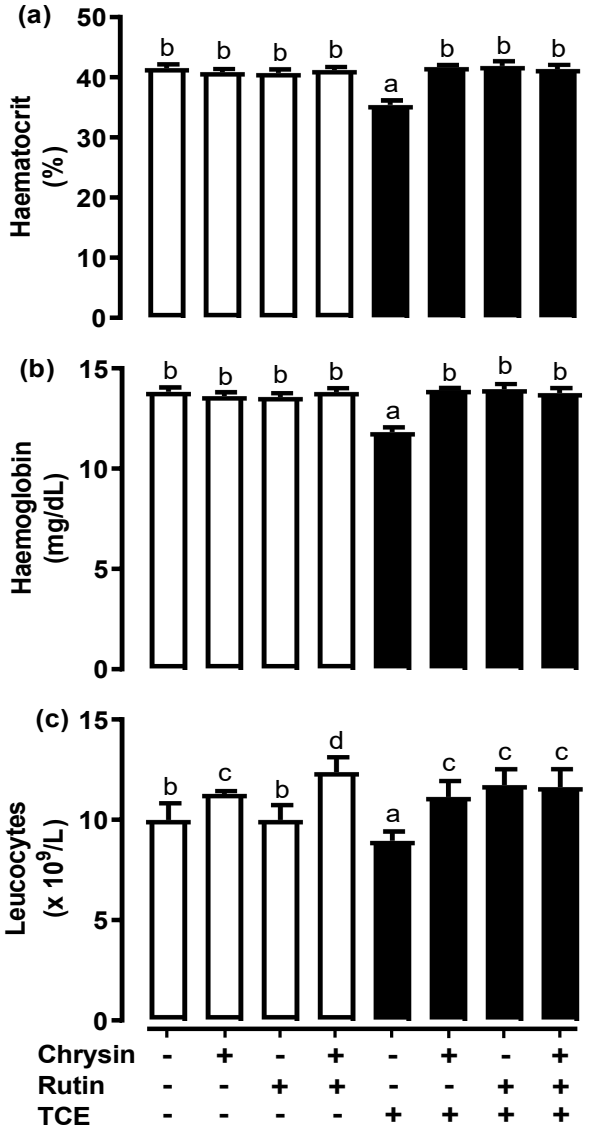

Fig. 1: Effect of chrysin and/or rutin 14 days sub-acute chemotherapy on haematocrit (graph a), haemoglobin contents (graph b), and leukocytes (graph c) contents in trichloroethylene (TCE)-treated rats. The data are shown as mean plus or minus the standard error of the mean (n = 6 rats). Bars with different alphabets are significantly different.

TCE-treated rats evoked a significant decrease in erythrocyte lactate dehydrogenase by 59.73% (Fig. 2). Significant increases of 2157.02% and 1062.92% in myeloperoxidase activity and nitric acid content were recorded compared to the standard control group. Nevertheless, However, pretreatment with chrysin or rutin, alone or combined attenuated the TCE-induced decrease in NO by 102.89%, 71.63%, and 111.74%, while also diminishing the TCE-mediated increase by 60.45%, 40.94%, and 70.25 for MPO and 58.55%, 45.09%, and 74.18 % for NO. The LDH, MPO, and NO in standard rats given chrysin and rutin showed no statistically significant difference compared to the standard group (Fig. 2).

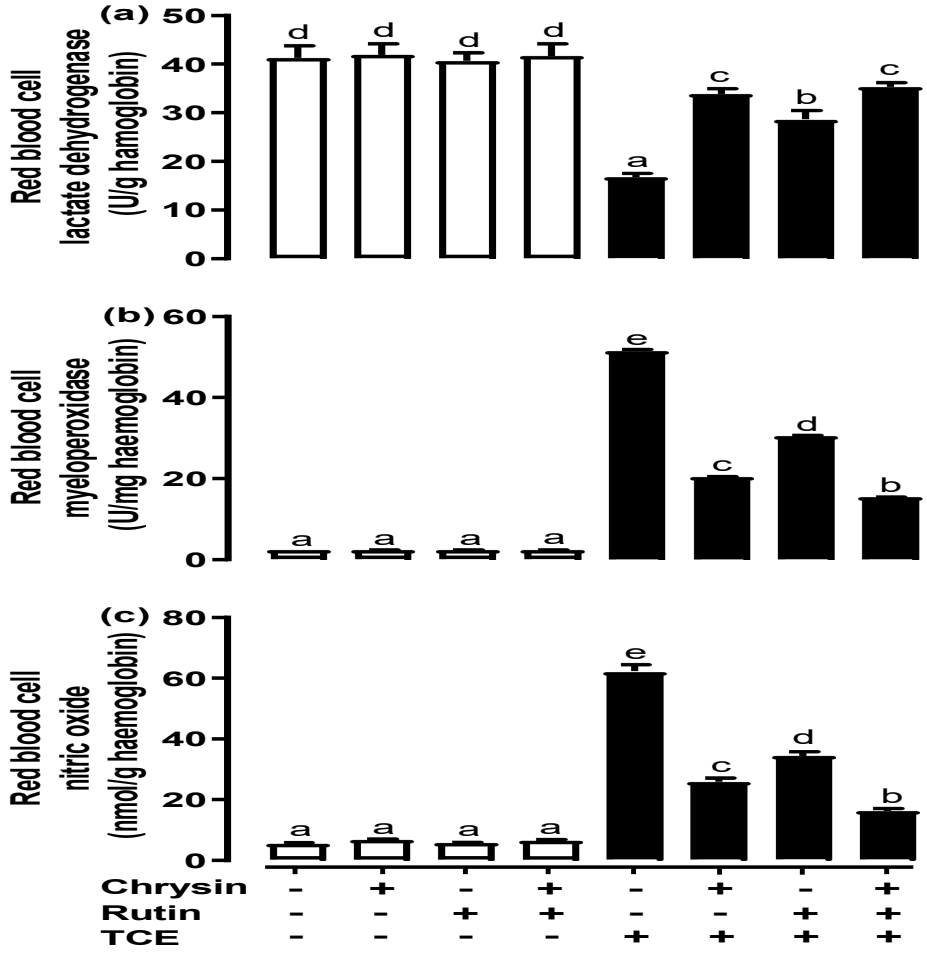

Fig. 2: Effect of chrysin and/or rutin 14 days sub-acute chemotherapy on RBCs LDH activity (graph a), RBCs MPO activity (graph b), and RBCs NO content (graph c) in trichloroethylene (TCE)-treated rats. The data are shown as mean plus or minus the standard error of the mean (n = 6 rats). Bars with different alphabets are significantly different.

The study scrutinized the effects (Figure 3) of chrysin and rutin (at a dose of 100 mg/kg/day each) on red blood cell major lipids (triacylglycerol and cholesterol) in rats following TCE administration. The erythrocyte triacylglycerol (Fig. 3a) and cholesterol (Fig. 3b) levels were significantly increased by 1.36 times each in TCE-treated rats over baseline control. Nonetheless, prior administration of chrysin or rutin (100 mg/kg each) alone or combined for seven days mitigated the reduction in triacylglycerols and cholesterol by TCE by 20.59%, 15.50%, and 22.37% for TAG and 12.54%, 16.11%, and 232.22% for cholesterol. Moreover, the triacylglycerol and cholesterol contents of typical baseline rats administered chrysin and rutin were comparable to those of the baseline control group.

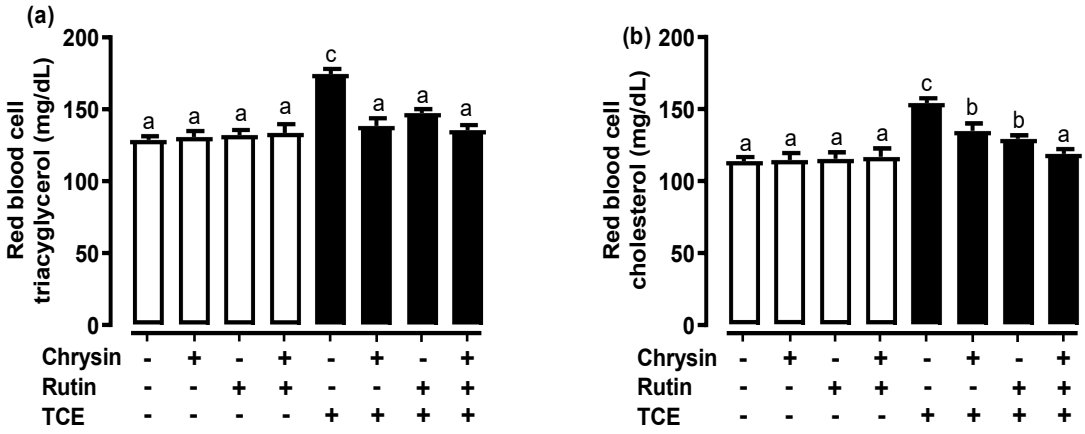

Fig. 3: Effect of chrysin and/or rutin 14 days subacute chemotherapy on RBCs triacylglycerol level (graph a) and RBCs cholesterol level (graph b) in trichloroethylene (TCE)-treated rats. The data are shown as mean plus or minus the standard error of the mean (n = 6 rats). Bars with different alphabets are significantly different.

Trichloroethylene (TCE) exposure caused a statistically relevant drop in erythrocytes, reduced glutathione level, glutathione S-transferase, superoxide dismutase, catalase, and glutathione peroxidase by 54.18%, 55.85%, 72.14%, 72.31%, and 25.31%, respectively, but elicited a significant rise in malondialdehyde by 647.72% when compared with the regular control group (Fig. 4). Regardless, chrysin or rutin administered beforehand, alone or combined countered the TCE-mediated decline in reduced glutathione level by 72.78%, 43.74%, and 106.38%, glutathione S-transferase activity by 37.85%, 44.33%, and 97.25%, superoxide dismutase activity by 91.95%, 41.79%, and 233.01%, catalase activity by 133.59%, 80.77%, and 195.41%, glutathione peroxi-

dase activity by 29.83%, 15.78%, and 38.20%, simultaneously decreasing the TCE -induced rises by 27.73%, 58.66%, and 70.98% for malondialdehyde. There were no significant differences in all the analyzed oxidative stress biomarkers between regular rats treated with chrysin and rutin and the regular control group (Fig. 4).

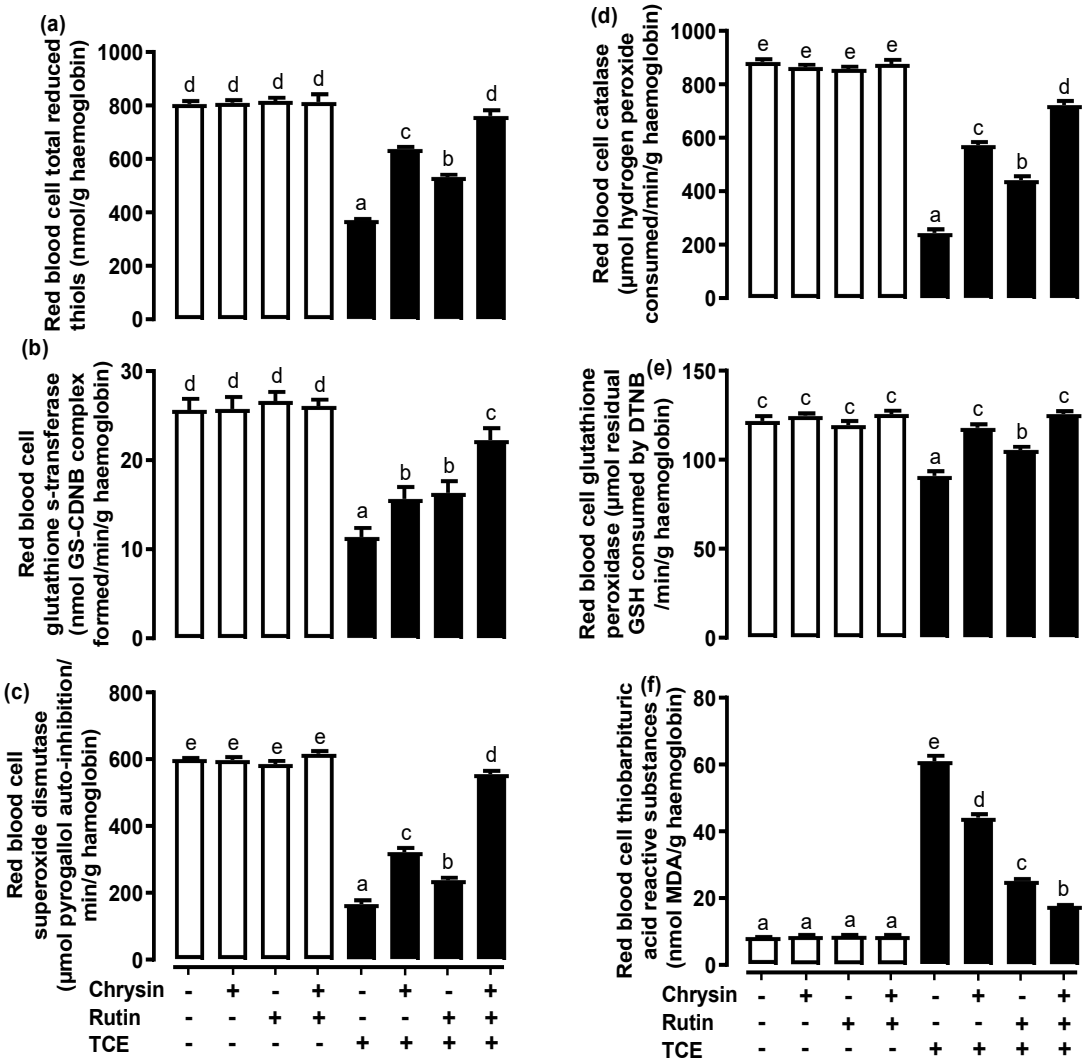

Fig. 4: Effect of chrysin and/or rutin 14 days subacute chemotherapy on the GSH concentration (graph a), GST activity (graph b), SOD activity (graph c), CAT activity (graph d), GPx activity (graph e), and GR activity (graph f) in trichloroethylene (TCE)-treated rats. The data are shown as mean plus or minus the standard error of the mean (n = 6 rats). Bars with different alphabets are significantly different.

Pre-treatment with chrysin and/or rutin remarkably reduced TCE-induced elevations in triacylglycerol, cholesterol, nitric oxide, malondialdehyde, and myeloperoxidase activity. Both flavonoids counteracted the TCE-induced decreases in hemoglobin, hematocrit, total thiol levels, and the activities of antioxidant enzymes (glutathione-S-transferase, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) as well as lactate dehydrogenase (LDH) - Fig. 5.

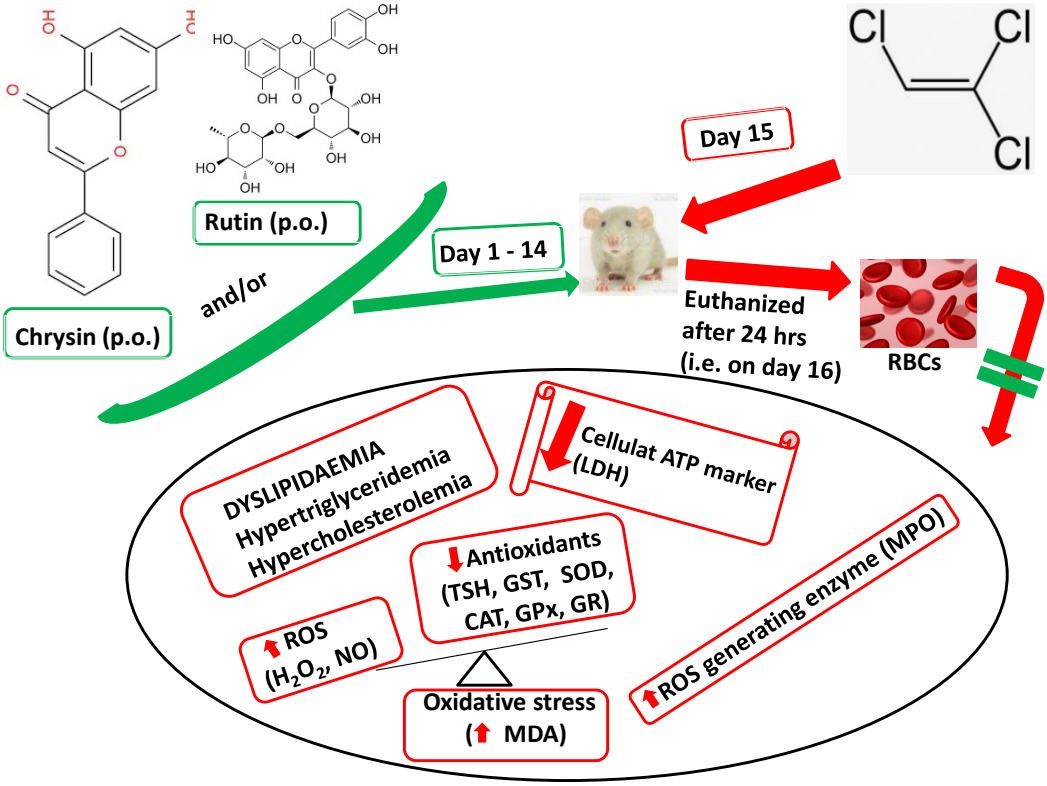

Figure 5. Possible mechanism of how chrysin and/or rutin prevent erythrocytotoxicity via their intrinsic anti-inflammatory and anti-oxidative capacities in the trichloroethylene-treated Wistar rat model.
Red arrows indicate the reaction pathways of the detrimental impact of trichloroethylene.
Green symbol epitomized the inhibition by chrysin and rutin chemotherapy.

DISCUSSION

The current examination explored the conceivable chemoprophylactic impacts of two phytonutrients, chrysin and rutin, on TCE-evoked erythrocytotoxicity in the Wistar albino rat model. The data of the current investigation express that TCE exposure spurred remarkable dynamics in the scrutinized parameters related to red blood cell function, lipid metabolism, oxidative stress,

and endothelial-derived relaxing factor, abrogated by pre-treatment with chrysin and rutin, either single or combined.

One of the striking findings of this research is that chrysin and rutin pre-treatment therapy, either as single or combined interventions, considerably abated TCE-incited decreases in red blood cell parameters, namely hematocrit, hemoglobin, and leukocyte count. Prior studies have shown that rats exposed to TCE have enhanced decreased erythrocyte count, hemoglobin contents, and leukocytes (Lan *et al*., 2010; Cichocki *et al*., 2016; Ordaz *et al*., 2017; Nwaogwugwu *et al*., 2023), resulting in diminished oxygen-transporting capability and potential tissue hypoxia (Dumas *et al*., 2018). Researchers have also noted that TCE depletes the body's immune system, leading to susceptibility to infection and autoimmune diseases, including scleroderma (Winans *et al*., 2011). The findings of this current study suggest that chrysin and rutin mitigate these detrimental outcomes by preserving erythrocyte and leukocyte integrity and role, probably via their antioxidant and anti-inflammatory proclivities.

Results of the study also illustrate that chrysin and rutin pre-treatment, either singly or combined, remarkably ricocheted to varying degrees the TCE-occasioned decrease in erythrocyte lactate dehydrogenase enzymatic activity and TCE-triggered increases in myeloperoxidase activity, and nitric oxide contents. Erythrocyte LDH activity appraisal provides perspicuity into their membranes' integrity, as erythrocytes rely exclusively on glucose derived from the glycolytic pathway for intracellular ATP production (Kato *et al*., 2006). Previous studies have documented that xenobiotics can induce the depletion of erythrocytes' LDH and ATP levels (Makni *et al*., 2012; Ramdani *et al*., 2014). Depleted ATP is responsible for the fragmentation, distortion, vesiculation, vacuolization, and eryptosis of erythrocyte membranes (Akamo *et al*., 2021). Nevertheless, chrysin and rutin significantly rescinded "TCE-engendered decline" in LDH activity, and therefore possibly maintained erythrocyte plasma membrane architecture and integrity.

Myeloperoxidase (MPO), a heme-containing peroxidase, is abundantly expressed in immune cells, including neutrophils, lymphocytes, monocytes, and macrophages (Khan *et al*., 2018). MPO is the sole peroxidase that employs H_2O_2 to oxidize halides and pseudohalides to hypohalous acids, a ROS with antibacterial properties. Controlled MPO release at the site of infection is crucial for its practical actions (Klebanoff, 2005). Uncontrolled degranulation worsens inflammation and damages tissue even without inflammation. MPO-derived oxidants are connected to tissue damage and the etiology of critical chronic illnesses, including rheumatoid arthritis, cardiovascular disease, liver disease, diabetes, and cancer (Klebanoff, 2005; Lacy, 2006). Thus, elevated MPO activity is a promising biomarker for inflammatory and oxidative damage in these prevalent illnesses (Khan *et al*., 2018). In this context, TCE exposure acts as an inflammatory stimulus that triggers an immune reaction. Regardless, chrysin and rutin remarkably annulled the TCE-elicited increase in MPO activity, thereby reducing oxidative injury and inflammation.

Elevated nitric oxide (NO) has been connected to oxidative stress, endothelial dysfunction, and hypotension (Wong *et al*., 2015). The observed dynamics in NO indicate that chrysin and rutin may exert chemo-

protective influences against TCE-mediated oxidative stress and inflammation, thereby preserving erythrocyte function and viability and reversing hypotension.

Further, findings from the study display the role of chrysin and rutin pre-treatment on red blood cell lipid levels, specifically triacylglycerol and cholesterol. TCE exposure has been linked to disruption of lipid homeostasis, characterized by elevated lipid levels (Zamanian *et al*., 2018), which in turn has been implicated in cardiovascular disease and other metabolic disorders (Iritas *et al*., 2021). The data from the present study indicate that chrysin and rutin may mitigate TCE-induced dyslipidemia by reducing lipid accumulation in erythrocytes, perhaps via their ability to modify lipid metabolism and transport.

The chemopreventive outcome of chrysin and rutin against TCE-instigated erythrocytotoxicity further aligned with results on red blood cell antioxidant status (Nwaogwugwu *et al*., 2023). TCE-treated rats have been recorded to upset redox status, exacerbating oxidative stress and cellular injury. In this research, chrysin and rutin pre-treatment abrogated TCE-invoked decline in antioxidant enzyme activities, namely lutathione S-transferase, superoxide dismutase, catalase, and glutathione peroxidase. Attenuation of these antioxidant enzymes could be a result of their amplified functional activities to either inhibit, quench, neutralize, or scavenge TCE-generated reactive metabolites and TCE-provoked ROS or RNS (Cichocki *et al*., 2016; Nwaogwugwu *et al*., 2023). TCE also concomitantly enhanced malondialdehyde levels (Lin *et al*., 2022), a marker of lipid peroxidation.The data suggest that chrysin and rutin may augment antioxidant defenses, enhance antioxidant action, and mitigate oxidative stress, thereby protecting against TCE-mediated red blood cell dysfunction.

The chemopreventive machinery of chrysin and rutin against TCE-activated dysfunction encompasses their antioxidant, anti-inflammatory, and lipid-lowering properties. Chrysin and rutin have been shown to scavenge free radicals, inhibit inflammatory pathways, and modulate lipid metabolism in various experimental models (Brunori *et al*., 2018; Frutos *et al*., 2019; Mohammed *et al*., 2023; Rao *et al*., 2023)). Also, their capability to augment antioxidant enzyme activities and reduce oxidative stress biomarkers may contribute to their preventive role against TCE-mediated erythrocytotoxicity.

Conclusively, this exploration highlighted the chemoprophylactic and therapeutic intervention outcomes of chrysin and rutin against TCE-elicited erythrocytotoxicity in rats via free radical scavenging, improved antioxidant protein, anti-dyslipidemia, and membrane stabilizing potential demonstrated by positive changes in the levels of antioxidant enzymes, lipid profile, hematology profile and LDH activities in the rats.

REFERENCES

Adamson, A., Ilieva, N., Stone, W.J. and De Miranda, B.R. 2023. Low-dose inhalation exposure to trichloroethylene induces dopaminergic neurodegeneration in rodents. *Toxicological Sciences* 196(2): 218 - 228.

Akamo, A.J., Akinloye, D.I., Ugbaja, R.N., Adeleye, O.O., Dosumu, O.A., Eteng, O.E., Antiya, M.C., Amah, G., Ajayi, O.A. and Faseun, S.O. 2021. Naringin prevents cyclophosphamide-induced erythrocytotoxicity in rats by abrogating oxidative stress. *Toxicology Reports* 8: 1803 - 1813.

Bilgin, A. O., Mammadov, R., Suleyman, B., Unver, E., Ozcicek, F., Soyturk, M. and Suleyman, H. 2020. Effect of rutin on cytarabine-associated pulmonary oedema and oxidative stress in rats. *Anais da Academia Brasileira de Ciências* 92(1): e20190261.

Brunori, A., Baviello, G., Nobili, C., Palumbo, D. and Végvári, G. 2018. Correlation Between Grain Yield and Rutin Content in Common Buckwheat Germplasm Cultivated in Southern Italy. In: Buckwheat Germplasm in the World. Academic Press, London. Pp. 215 – 224.

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. In: Methods in Enzymology. Academic Press, London. Pp. 302 – 310.

Chiu, W.A., Jinot, J., Scott, C.S., Makris, S.L., Cooper, G.S., Dzubow, R.C. and Caldwell, J.C. 2013. Human health effects of trichloroethylene: key findings and scientific issues. *Environmental Health Perspectives* 121(3): 303 - 311.

Cichocki, J.A., Guyton, K.Z., Guha, N., Chiu, W.A., Rusyn, I. and Lash, L.H. 2016. Target organ metabolism, toxicity, and mechanisms of trichloroethylene and perchloroethylene: key similarities, differences, and data gaps. *Journal of Pharmacology and Experimental Therapeutics* 359(1): 110 - 123.

Dorsey, E., Zafar, M., Lettenberger, S.E., Pawlik, M.E., Kinel, D., Frissen, M., Schneider, R.B., Kieburtz, K., Tanner, C.M., De Miranda, B.R. and Goldman, S.M. 2023. Trichloroethylene: An invisible cause of Parkinson's disease? *Journal of Parkinson's Disease* 13(2): 203 - 218.

Dumas, O., Despreaux, T., Perros, F., Lau, E., Andujar, P., Humbert, M., Montani, D. and Descatha, A. 2018. Respiratory effects of trichloroethylene. *Respiratory Medicine* 134: 47 - 53.

El-Marasy, S.A., El Awdan, S.A. and Abd -Elsalam, R.M. 2019. Protective role of chrysin on thioacetamide-induced hepatic encephalopathy in rats. *Chemico-Biological Interactions* 299: 111 - 119.

Frutos, M.J., Rincón-Frutos, L. and Valero-Cases, E. 2019. Rutin. In *Nonvitamin and Nonmineral Nutritional Supplements,* 111 - 117.

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* 71(10): 3879 – 3882.

Hadwan, M.H. and Abed, H.N. 2016. Data supporting the spectrophotometric method for the estimation of catalase activity. *Data in Brief* 6:194 – 199.

Haruna, A. and Yahaya, S.M. 2021. Recent advances in the chemistry of bioactive compounds from plants and soil microbes: A review. *Chemistry Africa* 4(2): 231 - 248.

Heydari, M., Ahmadizadeh, M. and Angali, K. A. 2017. Ameliorative effect of vitamin E on trichloroethylene-induced nephrotoxicity in rats. *Journal of Nephropathology* 6(3): 168.

Iritas, S.B., Dip, A., Gunduzoz, M., Tutkun, L., Turksoy, V.A., Deniz, S., Tekin, G., Oztan, O. and Unlu, A. 2021. Assessment of potential cardiovascular risk in trichloroethylene exposure by serum

methylated arginine levels. *International Journal of Environmental Health Research* 31(1): 63 - 74.

Kato, G.J., McGowan, V., Machado, R.F., Little, J.A., Taylor, J., Morris, C.R., Nichols, J.S., Wang, X., Poljakovic, M., Morris, S.M. and Gladwin, M.T. 2006. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood* 107(6): 2279 – 2285.

Khan, A.A., Alsahli, M.A. and Rahmani, A.H. 2018. Myeloperoxidase as an active disease biomarker: recent biochemical and pathological perspectives. *Medical Sciences* 6 (2): 33.

Klebanoff, S. J. 2005. Myeloperoxidase: friend and foe. *Journal of leukocyte biology*, *77* (5): 598 - 625.

Klebanoff, S. J., Waltersdorph, A. M. and Rosen, H. 1984. Antimicrobial activity of myeloperoxidase. In: Methods in Enzymology. Academic Press, London. Pp. 399 – 403.

Lacy, P. 2006. Mechanisms of degranulation in neutrophils. *Allergy, Asthma & Clinical Immunology 2*: 1 - 11.

Lan, Q., Zhang, L., Tang, X., Shen, M., Smith, M.T., Qiu, C. and Huang, H. 2010. Occupational exposure to trichloroethylene is associated with a decline in lymphocyte subsets and soluble CD27 and CD30 markers. *Carcinogenesis* 31(9): 1592 - 1596.

Lin, W.Y., Tu, C.P., Kuo, H.H. and Kuo, H.W. 2022. Urinary Malondialdehyde (MDA) and N-Acetyl-β-D-Glucosaminidase (NAG) Associated with Exposure to Trichloroethylene (TCE) in Underground Water. *Toxics* 10(6): 293.

Luo, Y.S., Hsieh, N.H., Soldatow, V.Y., Chiu, W.A. and Rusyn, I. 2018. Comparative analysis of metabolism of trichloroethylene and tetrachloroethylene among mouse tissues and strains. *Toxicology* 409: 33 - 43.

Makni, M., Chtourou, Y., Fetoui, H., Garoui, E.M., Barkallah, M., Marouani, C., Kallel, C. and Zeghal, N., 2012. Erythrocyte oxidative damage in rat treated with CCl4: protective role of vanillin. *Toxicology and industrial health 28*(10): 908 - 916.

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* 47(3): 469 - 474.

Mohammed, H.A., Sulaiman, G.M., Albukhaty, S., Al-Saffar, A.Z., Elshibani, F.A. and Ragab, E.A. 2023. Chrysin, the flavonoid molecule of antioxidant interest. *ChemistrySelect* 8(48): e202303306.

Najmi, A., Javed, S.A., Al Bratty, M. and Alhazmi, H.A. 2022. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules* 27 (2): 349.

National Research Council. 2010. Guide for the Care and Use of Laboratory Animals. National Academies Press, London.

Njokunwogbu, A., Amarachukwu, O., Ene, F. C., Iyioku, M. U. and Enyi, C. N. 2022. Separation and infrared (FT-IR) char-

acterization of aromatic hydrocarbons from high boiling fractions (> 200°C) of Nigeria crude oil (using trichloroethylene as the eluant). *International Research Journal of Applied Sciences, Engineering and Technology* 8(8): 1 - 9.

Nwaogwugwu, C.J., Nwankwo, V.C., Chidi, N.I., Nwaogwugwu, C., Ariwodo, U.G., Chukwuemeka, I. and Nwokoma, F. 2023. Effects of alcoholic leaf extract of Hunteria umbellata on various parameters in Wistar female rats exposed to trichloroethylene (TCE). *Journal of Namibian Studies: History Politics Culture* 34: 2521 - 2537.

Ordaz, J.D., Damayanti, N.P. and Irudayaraj, J.M. 2017. Toxicological effects of trichloroethylene exposure on immune disorders. *Immunopharmacology and Immunotoxicology* 39(6): 305 - 317.

Prakash, M., Shetty, M.S., Tilak, P. and Anwar, N. 2009. Total thiols: biomedical importance and their alteration in various disorders. *Online Journal of Health and Allied Sciences* 8(2): 2.

Ramdani, G. and Langsley, G. 2014. ATP, an extracellular signaling molecule in red blood cells: a messenger for malaria? *Biomedical Journal* 37(5): 284 – 292.

Rao, U.M., Dudekula, J.B., Bhatt, S., Kumar, M S., Shah, K., Chauhan, N.S. and Shilpi, S. 2023. Role of phytopharmaceuticals in inflammatory disorders. *Phytopharmaceuticals and Herbal Drugs* 5: 433 - 451.

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* 179 (4073): 588 - 590.

Sahreen, S. Khan, M. R. and Khan, R. A. 2011. Hepatoprotective effects of methanol extract of Carissa opaca leaves on CCl 4-induced damage in rat. *BMC Complementary and Alternative Medicine* 11: 1 - 9.

Sreejayan, X.X. and Rao, M.N.A. 1997. Nitric oxide scavenging by curcuminoids. *Journal of Pharmacy and Pharmacology* 49(1): 105 - 107.

Tietz, N. W. 1995. Clinical guide to laboratory tests. In *Clinical guide to laboratory tests,* pp. 1096 - 1996.

Winans, B., Humble, M.C., and Lawrence, B.P. 2011. Environmental toxicants and the developing immune system: a missing link in the global battle against infectious disease? *Reproductive Toxicology* 31(3): 327 - 336.

Wong, V. and Lerner, E. 2015. Nitric oxide inhibition strategies. *Future Science OA* 1(1): 1 – 5.

Zamanian, Z., Yousefinejad, S., Khoshnoud, M.J., Golbabaie, F., Farhang Dehghan, S., Modaresi, A., Amanat, S., Reza Zare, M. and Rahmani, A. 2018. Toxic effects of subacute inhalation exposure to trichloroethylene on serum lipid profile, glucose and biochemical parameters in Sprague–Dawley rats. *Inhalation Toxicology* 30 (9-10): 354 - 360.

(Manuscript received: 4th April, 2024; accepted: 20th June, 2024).