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# Chrysin Rescinds Paraquat-Elicited Spleen Oxidative Stress in Male Wistar Albino Rats

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**ABSTRACT**

**Acute paraquat (PQ) overdose disrupts the spleen's biochemical profile, causing oxidative stress and organ damage. Chrysin (CHR), a natural flavone with potent antioxidant properties, was investigated for its protective effect against PQ-induced spleen toxicity in rats. Twenty-eight male rats were divided into four groups (n = 7): control, PQ, CHR, and CHR+PQ. CHR and CHR+PQ groups received oral CHR (100 mg/kg) for seven days, while the PQ and control groups received olive oil (2 mL/kg b.wt. per day). The PQ and CHR+PQ groups received a single PQ dose (35 mg/kg) on day seven. Twenty-four hours later, spleens were collected. PQ exposure significantly declined reduced glutathione (GSH) levels and activities of glutathione-related antioxidant enzymes [glutathione S-transferase (GSH-ST), glutathione peroxidase (GSH-Px), and glutathione reductase (GR)], and other antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT)]. Additionally, PQ elevated malondialdehyde (MDA) and nitric oxide (NO) levels, indicating oxidative stress. Conversely, chrysin pretreatment significantly ($p < 0.05$) attenuated paraquat-induced changes, restoring GSH levels and enzyme activities and reducing MDA and NO to significant ($p < 0.05$) degrees. Chrysin alone did not alter ($p > 0.05$) the levels of nitric oxide, GSH, GSH-ST, and MDA in healthy rats. These findings reveal that CHR efficiently protects the spleen against PQ-mediated oxidative stress and damage, suggesting its potential as a therapeutic agent for managing paraquat poisoning.**

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## 1. INTRODUCTION

Pesticides are critical in agriculture for managing weeds and insects for enhanced food production, including fruit, vegetable, and cereal output (Gelaye & Negash, 2024). Nevertheless, paraquat (PQ), a commonly used herbicide to control weeds in many agricultural and non-agricultural settings, poses remarkable health risks due to its highly toxic impacts, especially on accidental ingestion, which could cause severe morbidity and mortality (Gelaye & Negash, 2024). It has been outlawed in many advanced countries. Still, PQ poisoning remains a remarkable public health concern worldwide in agricultural communities and non-agricultural milieus, especially in low- and middle-income countries (Gelaye & Negash, 2024).

Acute paraquat poisoning chiefly impairs vital organs, including the lungs, kidneys, liver, and spleen (Souza et al., 2023). The spleen is an influential organ of the hematologic and reticuloendothelial systems. It filters young erythrocytes and platelets, stores erythrocytes and platelets, removes/traps old and damaged erythrocytes via phagocytization by splenic macrophages, stores and transports iron, and maintains fluid balance (Lewis et al., 2019). Due to the above-stated roles, the spleen is highly susceptible to oxidative injury (Li et al., 2021). PQ-instigated spleen damage can compromise all the above functions, weaken immunity, and enhance infection vulnerability.

Oxidative stress (OS) and inflammation are well-established hallmarks of PQ intoxication (Beigoli et al., 2024). OS occurs when the production of free radicals overwhelms the body's antioxidant defenses. Free radicals, such as superoxide radicals ($O_2^{*-}$) and hydrogen peroxide ($H_2O_2$), generated during PQ metabolism, can trigger lipid peroxidation, protein oxidation, and DNA damage, ultimately leading to cellular dysfunction and tissue injury (Yao et al., 2021). Inflammation is characterized by increased pro-inflammatory molecules and decreased anti-inflammatory molecules (Jiang et al., 2014).

Chrysin (CHR), a flavonoid (5,7-dihydroxyflavone), is found in various plants, with *Passiflora incarnata* being a well-known source. It has been shown that some flavonoids, including chrysin, exhibit various potential biological, prophylactic, and therapeutic properties in both laboratory and animal studies (Faheem et al., 2023). These properties include scavenging free radicals, enhancing the body's natural antioxidant defenses, inhibiting lipid peroxidation, and exerting anti-atherogenic and anti-diabetic effects. Additionally, chrysin possesses anti-inflammatory and immunomodulatory properties by regulating immune system cells (T helpers, cytotoxic T lymphocytes, B cells, and natural killer cells), acting as an NF-κB antagonist and peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist or activator (Yao et al., 2021). This interaction leads to the downregulation of critical pro-inflammatory enzymes (myeloperoxidase, cyclooxygenase-2, and phospholipase A2) and the suppression of crucial inflammatory cytokine production, ultimately reducing inflammation (Jiang et al., 2014).

Current treatment options for paraquat poisoning are limited and often ineffective. With its promising antioxidant and anti-inflammatory properties, chrysin has emerged as a potential prophylactic and therapeutic agent against PQ-induced damage. While research has explored chrysin's benefits in various *in vitro* and *in vivo* models of oxidative stress-related disorders, its potential to alleviate PQ-

provoked spleen intoxication remains comparatively uncharted. Elucidating the mechanisms by which chrysin protects against PQ-mediated toxicosis could have remarkable implications for managing PQ poisoning and pave the way for developing novel prophylactic and therapeutic schemes. Therefore, this study aimed to assess the chemopreventive influence of chrysin against paraquat-elicited oxidative stress and spleen intoxication in male Wistar albino rats.

## 2. MATERIALS AND METHODS

### *2.1 Chemicals*

Paraquat dichloride (PQ; $C_{12}H_{14}Cl_2N_2$, CAS No. 1910-42-5), ketamine hydrochloride ($C_{13}H_{17}Cl_2NO$, CAS No. 1867-66-9), and xylazine hydrochloride ($C_{12}H_{17}ClN_2S$, CAS No. 23076-35-9) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Chrysin (5,7-dihydroxyflavone; $C_{15}H_{10}O_4$, CAS No. 480-40-0) was acquired from Solarbio Science & Technology, located in Beijing, China. All chemicals used in this study were of analytical grade and were product of Sigma-Aldrich.

*Experiential design*

Twenty-eight adult male Wistar rats (*Rattus norvegicus*) weighing 200-250 g (aged 10-12 weeks) were obtained from the animal facility of the Department of Biochemistry, Federal University of Agriculture Abeokuta, Nigeria. The animals were housed under standard laboratory conditions (temperature: 26±1°C, relative humidity: 45±5%, and 12-hour light/dark cycle) and had ad libitum access to standard rodent chow and water. The animals were adapted for a week before the commencement of the intubation and they were given with human treatment as per the regulations authorized by the FUNAAB Ethical Committee and were conducted according to the ARRIVE guidelines (Percie du Sert et al., 2020). In addition, the institution authorized the study protocol with the number FUNAAB/COLBIOS/BCH/PG/17/0544

The 28 rats were randomly divided into four groups of seven animals each:

- Control group (NC): Rats received olive oil (2 mL/kg body weight) by oral gavage for 7 days distilled water on day 8.
- CHR group: Rats received CHR (100 mg/kg body weight) daily for 7 days followed by distilled water on day 8.
- PQ group: Rats received olive oil for 7 days followed by a single dose of PQ (35 mg/kg body weight) on day 8.
- CHR+PQ group: Rats received CHR (100 mg/kg body weight) daily for 7 days followed by PQ (35 mg/kg) on day 8.

The CHR and PQ intubation were carried out between 8:00 a.m. and 9:00 a.m. daily.

### *2.2 Spleen homogenate preparation*

Twenty-four hours following paraquat administration, rats were euthanized, and spleen were collected bilaterally. To eliminate blood contamination, tissues were rinsed with ice-cold saline (0.9% NaCl). A 10% (w/v) homogenate was prepared by pulverizing 0.4 g of spleen in 3.6 mL of 0.1 M phosphate buffer (pH 7.4) using a Teflon homogenizer. The homogenate was then centrifuged at 4000 rpm for 10 minutes. The resulting supernatants were aliquoted and stored appropriately at -20°C for subsequent analysis of oxidative stress and antioxidant biomarkers.

### *2.3 Oxidative stress and antioxidant biomarkers assessment*

Enzyme activities (U/L) of glutathione-S-transferase (GSH-ST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione

reductase (GSH-GR) were measured using standard spectrophotometric methods as described by (Akamo et al., 2021). Specific activities (U/g protein) were determined by normalizing enzyme activity to protein concentration (g/L). Also, the reduced glutathione (GSH), nitric oxide (NO), and malondialdehyde (MDA) levels were also quantified using standard spectrophotometric methods as described by Akamo et al. (2021) and expressed in g/g tissue.

*2.4 Statistical analysis*

All data are expressed as the mean ± SEM. Differences between experimental groups were evaluated using one-way 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 IBM SPSS Statistics version 26. Graphical representations of the data were constructed using GraphPad Prism version 9.0 software.

## 3. RESULTS AND DISCUSSION

*3.1 Chrysin chemotherapy annulled the paraquat-triggered increases in spleen nitric oxide (NO) and malondialdehyde (MDA) levels.*

In Figure 1, the graphical representation characterizes the effects of chrysin and paraquat on NO and MDA levels in the spleen. The administration of paraquat led to a significant ($p < 0.05$) upsurge in spleen NO and MDA levels by 46.15% and 88%, respectively compared to the control group. However, pretreatment with chrysin effectively normalized the elevated NO levels and attenuated PQ-induced accumulated MDA by 21%. Notably, chrysin alone did not alter ($p > 0.05$) spleen NO and MDA contents compared to the standard control group.

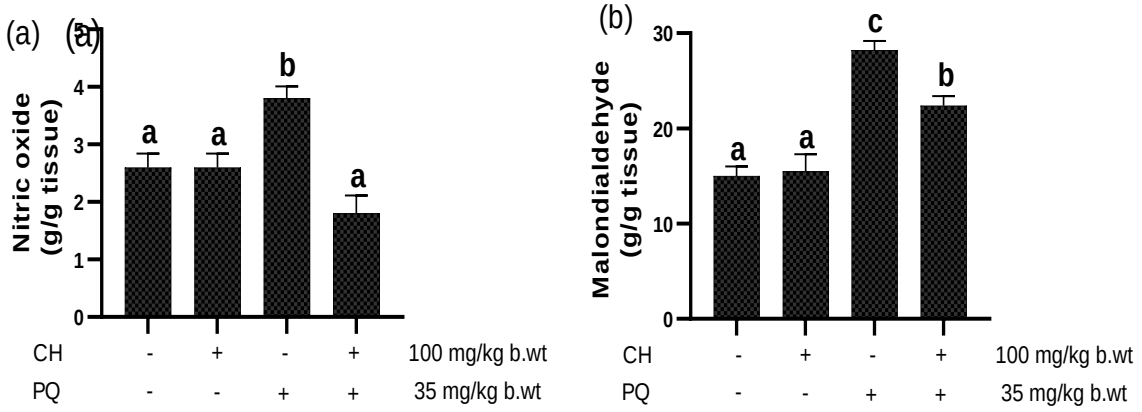


**Figure 1: Impact of chrysin (CH) pretreatment on paraquat (PQ)-induced increase in spleen (a) nitric oxide and (b) malondialdehyde levels in rats. Bars with different letters indicate significant differences ($p < 0.05$).**

*3.2 Chrysin intervention abated the paraquat-**engendered** decreases in the spleen's antioxidant peptide and proteins*

Figure 2 depicts the impact of chrysin on reduced glutathione levels in the spleen of rats treated with paraquat. Paraquat exposure resulted in a significant ($p < 0.05$) decrease in

spleen reduced glutathione(GSH) levels as well as the activities of glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-R) enzymatic activities by 7.75%, 88.38%, 76.30%, 73.03%, 65.54%, and 73.37%, respectively, compared to the control cluster. Notwithstanding, pretreatment with chrysin mitigated this decline in GSH, GST, SOD, CAT, GSH-Px, and GSH-R by 4.06%, 87.10%, 117.78%, 245.83%, 70.59, and 103.73%, respectively compared to PQ administered group.

Additionally, the chrysin-alone group has similar ($p > 0.05$) spleen GSH levels, GST activity compared to healthy rats. Interestingly, chrysin alone significantly ($p < 0.05$) inhibited spleen SOD, CAT, GSH-Px, and GSH-R activities by 40.64%, 50%, 45.95% and 26.13%, respectively, compared to normal control rats.

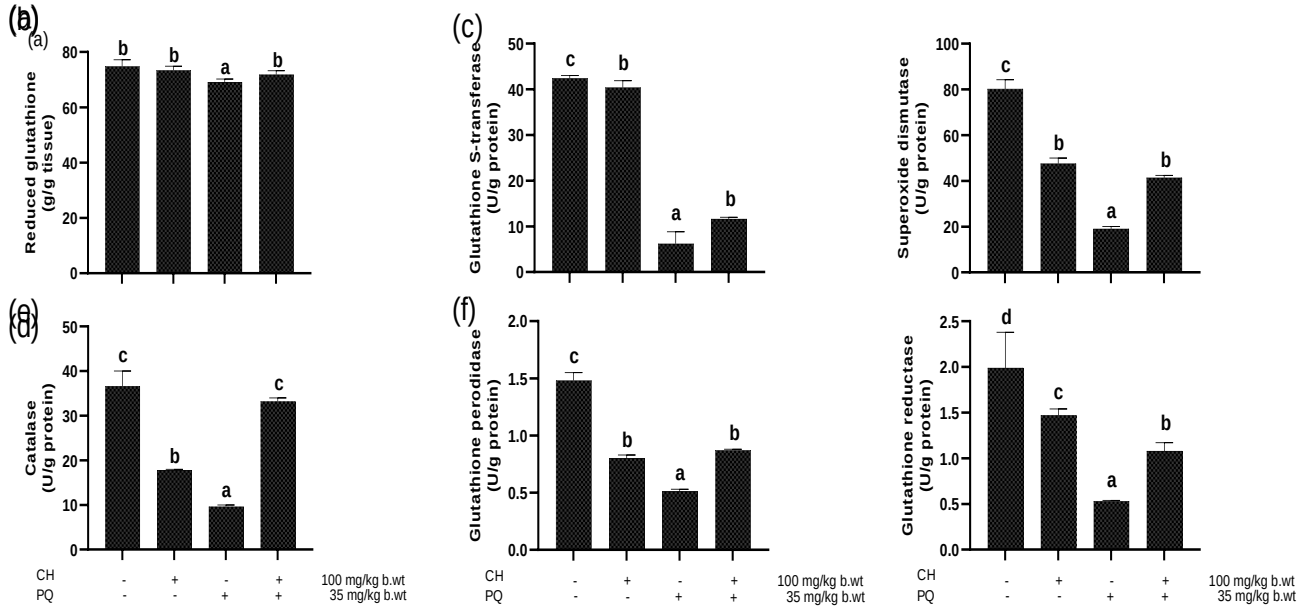


**Figure 2: Impact of chrysin (CH) pretreatment on paraquat (PQ)-induced decreases in spleen (a) reduced glutathione (b) glutathione S-transferase (c) superoxide dismutase (d) catalase (e) glutathione peroxidase (f) glutathione reductase enzymatic activities in rats. Bars with different letters (a or b) indicate significant differences ($P < 0.05$).**

*3.3. Discussion of the Results*

Paraquat (PQ) poisoning constitutes a remarkable problem for public health worldwide because of its increased perniciousness and the lack of treatment alternatives. Acute PQ poisoning principally affects multiple viscera, such as the spleen, leading to oxidative stress and visceral injury. The spleen, the biggest peripheral lymphatic organ, plays a vital role in innate and adaptive immune reactions and acts as a first line of defense against xenobiotics, including chemical, physical, or biological agents.

This current research probed the chemopreventive outcomes of chrysin (CHR), a natural flavone with vigorous antioxidant assets, against PQ-incited spleen toxicity in male Wistar albino rats. We discovered that CHR pretreatment efficiently revered PQ-triggered oxidative stress and reinstated antioxidant defenses in the spleen,

underscoring its propensity as a chemoprophylactic agent for managing acute PQ poisoning.

The detected upsurge in nitric oxide (NO) contents following PQ exposure is consistent with results from previous experiment (Cho et al., 2004), symbolizing the role of PQ in engendering nitrosative stress. NO is a small, labile, lipid-permeable free radical molecule synthesized by NO synthase (NOS), which diffuses to neighboring cells. It involves various physiological and pathological processes in the spleen (an essential organ in the immune system), including immune regulation via gasoneurotransmission; vasodilation and blood flow; antimicrobial defense by inhibiting the growth and replication of pathogen; anti-inflammation and splenic hematopoiesis (Giovinazzo et al., 2014; Lewis et al., 2019). NO's biological effects are mediated via covalent and noncovalent bonds with protein and nonprotein substrates. (Lee et al., 2014). So, NO acts like a double-edged sword with cytoprotective and cytotoxic roles in different settings (Giovinazzo et al., 2014). The notable reduction in NO contents following CHR pretreatment advocates that CHR may abate PQ-activated nitrosative stress, probably via inhibition of NOS or its receptor soluble guanylyl cyclase receptor, its intrinsic antioxidant and anti-inflammatory potentials (Cho et al., 2004).

After the liver, the spleen in the second organ with the maximum concentration of reduced glutathione (Akamo et al., 2021), one of crucial antioxidant peptides whose function in the spleen includes detoxification of xenobiotics and endogenous toxins via conjugation, neutralizing free radical generated by immune cells thereby protecting the spleen from free radical-occasioned spleen damage; regulates the proliferation, activation, and differentiation of immune cells (lymphocytes, macrophages, and dendritic cells) and some cytokines signaling substances; iron metabolism; and erythrocyte recycling (Giustarini et al., 2023)

Our data revealed that PQ-treated rats exhibited significantly decreased GSH concentrations in the spleen, consistent with prior investigations expressing PQ-prompted GSH depletion (Chen et al., 2021). Nevertheless, pretreating rats with CHR before the paraquat challenge remarkably reversed PQ-propagated reduction in the concentration and function of this peptide antioxidant in the spleen, indicating its capability to enhance cellular antioxidant defenses and protect against GSH depletion invoked by PQ could be via scavenging free radical-elicited by PQ and PQ reactive metabolites, stimulating glutamate cysteine ligase (GCL) the rate-limiting enzyme in GSH biosynthesis or GCL receptor.

Our observed striking decrease in functions and activities of GSH-related antioxidant enzymes, including GSH S-transferase, GSH peroxidase, and GSH reductase, following the PQ challenge further corroborates the significance of PQ in impairing antioxidant defense mechanisms in the spleen (Rudyk et al., 2017; Chen et al., 2021). While non-enzymatic GSH conjugation is possible, GSH S-transferase catalyzes most GSH conjugation events.

GSH S-transferase metabolizes, detoxifies, and stimulates cellular oxidation of xenobiotics absorbed from the bloodstream or within the spleen itself via electrophilic conjugation, making this exotoxin and endotoxins less toxic, more water-soluble, and easily transported to the kidney for excretion (Tripathi et al., 2022). Immune cells produce free radicals during normal metabolism in response to infection and inflammation. The spleen GSH peroxidase

scavenges $H_2O_2$, protects the spleen from free radical-induced oxidative damage, and maintains the cellular integrity and function of the immune cells (Pei et al., 2023). GSH reductase safeguards adequate supply and recycling of GSH, which is key for GSH S-transferase and GSH peroxidase activities. Thus, it sustains the detoxification and cellular redox homeostasis system for the spleen's optimal performance (Zhu et al., 2022). Pretreating rats with CHR outstandingly abrogated the PQ-motivated reduction in the GSH-ST, GSH-Px, and GSH-R enzymatic activities, specifying its capability to augment antioxidant enzyme activity and protect against PQ-triggered oxidative stress. Other mechanisms by which CHR protects against the toxic effects could be by regulating the Nrf-2 transcription factors pathway (Akamo et al., 2021).

Superoxide dismutase (SOD) and catalase are crucial antioxidant enzymes implicated in the quenching of superoxide radicals and hydrogen peroxide, respectively (Akamo et al., 2021). Our findings emphasized that exposure to PQ appreciably reduces spleen SOD and catalase activity, agreeing with earlier investigations displaying PQ-elicited inhibition of antioxidant enzyme activity (Beigoli et al., 2024). Still, pretreating rats with CHR noticeably transposed PQ-depleted spleen SOD and catalase activity, suggesting CHR's capability to augment antioxidant enzymatic activity and protect against PQ-instigated oxidative stress.

Malondialdehyde (MDA) is a biomarker of lipid peroxidation and oxidative damage, showing the degree of oxidative stress in tissues (Akamo et al., 2021). Our data show that PQ exposure ensued in pronouncedly enhanced spleen MDA contents and is interterm with earlier experiments by Ijaz et al., 2024, suggesting increased lipid peroxidation and oxidative damage. Conversely, pretreating rats with CHR markedly annulled the PQ-provoked MDA content elevation, advocating its capacity to safeguard against spleen lipid peroxidation and oxidative damage mediated by PQ.

The link between oxidative stress and immunity is well-known. PQ or its active metabolite can induce free radical generation, which modifies self-antigen and oxidative stress that instigates autoimmune disorders, such as systemic lupus erythematous and diabetes mellitus (Sotler et al., 2018). While a plant-rich diet, including fruits, vegetables, and phytochemicals, has convincing and compelling evidence via their antioxidant properties to prevent or treat health-threatening diseases, including cardiovascular diseases, cancer, aging, and autoimmune disorders, they should be consumed with caution (Hu et al., 2023). Fruits and vegetables have been reported to elicit both prooxidant and antioxidant effects in humans (Hu et al., 2023). Interestingly, in this study, we observe that normal healthy rats administered chrysin only at a dose of 100 mg/kg body weight developed decreased activities in SOD, catalase, GSH peroxidase, and GSH reductase. This exceptional trend could be attributed to proposed mechanisms, including availability of transition metal ions (iron, copper), matrix content, and redox potential (Sotler et al., 2018).

Several prominent antioxidants (vitamin C, α-tocopherol, flavonoids) could be transformed from being antioxidants to harmful prooxidants via metal ions (iron, copper), matrix content, and redox potential (Sotler et al., 2018). Vitamin C reduces Fe3+ to Fe2+ or Cu2+ to Cu+ at high doses. The reduced transition metals, in turn, reduce hydrogen peroxide in the presence of oxygen to hydroxyl radicals via the Fenton reaction. Also, vitamin C impairs the absorption of iron, copper, or vitamin B12 (Kaźmierczak-

Barańska et al., 2020). Alpha-tocopherol reacts with ROS at high amounts and becomes a radical itself. It overwhelms vitamin C, which regenerate alpha-tocopherol to an unreactive state. Flavonoids (quercetin and kaempferol) at high doses have been documented to act as prooxidants in systems that contain transition metals, leading to phenolic radical formation and inducing DNA damage and lipid peroxidation (Sotler et al., 2018).

In the words of Paracelsus, the father of Toxicology, the dose determines toxicity. So, in recommending a dietary substance, any plant-based phytochemical or nutraceutical, as a potential antioxidant, it is necessary to establish its antioxidant and/or prooxidant properties in vivo, as well as its general safety because these substances can act as both antioxidant (especially at low dose) and/or prooxidant (at high dose) thus drawing caution on the use of anti-oxidative agents generally.

## 5. Conclusion

Taking together, our findings express that pretreating rats with a CHR regimen as low as 7 days efficaciously contracted PQ-instigated oxidative stress and reinstated spleen antioxidant defenses, thereby shielding against PQ-elicited spleen damage.

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## References

Akamo, A.J., Rotimi, S.O., Akinloye, D.I., Ugbaja, R.N., Adeleye, O.O., Dosumu, O.A., Eteng, O.E., Amah, G., Obijeku, A. and Cole, O.E. (2021). Naringin prevents cyclophosphamide-induced hepatotoxicity in rats by attenuating oxidative stress, fibrosis, and inflammation. *Food and chemical toxicology*, *153*, 112266.

Beigoli, S., Hajizadeh, A. A., Yazdi, M. E. T., Khosravi, R., Vafaee, F. and Boskabady, M. H. (2024). Improvement of inhaled paraquat induced lung and systemic inflammation, oxidative stress and memory changes by safranal. *Toxicon*, 107687.

Chen, J., Su, Y., Lin, F., Iqbal, M., Mehmood, K., Zhang, H. and Shi, D. (2021). Effect of paraquat on cytotoxicity involved in oxidative stress and inflammatory reaction: A review of mechanisms and ecological implications. *Ecotoxicology and Environmental Safety*, *224*, 112711.

Cho, H., Yun, C.W., Park, W.K., Kong, J.Y., Kim, K.S., Park, Y., Lee, S. and Kim, B.K. (2004). Modulation of the activity of pro-inflammatory enzymes, COX-2 and iNOS, by chrysin derivatives. *Pharmacological Research*, *49*(1), 37-43.

Faheem, M.A., Akhtar, T., Naseem, N., Aftab, U., Zafar, M.S., Hussain, S., Shahzad, M. and Gobe, G.C., (2023). Chrysin is immunomodulatory and anti-inflammatory against complete Freund's adjuvant-induced arthritis in a pre-clinical rodent model. *Pharmaceutics*, *15*(4), 1225.

Gelaye, Y. and Negash, B. (2024). Residue of Pesticides in Fruits, Vegetables, and

Their Management in Ethiopia. *Journal of Chemistry*, *2024*.

Giovinazzo, D., Dawson, V. L. and Dawson, T. M. (2014). Nitric oxide. In *Encyclopedia of the Neurological Sciences* (pp. 597-600). Elsevier Inc.

Giustarini, D., Milzani, A., Dalle-Donne, I. and Rossi, R. (2023). How to increase cellular glutathione. *Antioxidants*, *12*(5), 1094.

Hu, F. B. (2003). Plant-based foods and prevention of cardiovascular disease: an overview. The American journal of clinical nutrition, 78(3), 544S-551S.

Ijaz, M. U., Alvi, K., Hamza, A., Anwar, H., Al-Ghanim, K. A. and Riaz, M. N. (2024). Curative effects of tectochrysin on paraquat-instigated testicular toxicity in rats: A biochemical and histopathological based study. *Heliyon*, *10*(3).

Jiang, Y., Gong, F. L., Zhao, G. B. and Li, J. (2014). Chrysin suppressed inflammatory responses and the inducible nitric oxide synthase pathway after spinal cord injury in rats. *International journal of molecular sciences*, *15*(7), 12270-12279.

Kaźmierczak-Barańska, J., Boguszewska, K., Adamus-Grabicka, A. and Karwowski, B. T. (2020). Two faces of vitamin C—antioxidative and pro-oxidative agent. Nutrients, 12(5), 1501.

Lee, Y.I., Giovinazzo, D., Kang, H.C., Lee, Y., Jeong, J.S., Doulias, P.T., Xie, Z., Hu, J., Ghasemi, M., Ischiropoulos, H. and Qian, J. (2014). Protein microarray characterization of the S-nitrosoproteome. *Molecular & Cellular Proteomics*, *13*(1), 63-72.

Lewis, S. M., Williams, A. and Eisenbarth, S. C. (2019). Structure and function of the immune system in the spleen. *Science immunology*, *4*(33), eaau6085.

Li, N., Zhao, Y., Shen, Y., Cheng, Y., Qiao, M., Song, L. and Huang, X. (2021). Protective effects of folic acid on oxidative damage of rat spleen induced by lead acetate. *Ecotoxicology and environmental safety*, *211*, 111917.

Pei, J., Pan, X., Wei, G. and Hua, Y. (2023). Research progress of glutathione peroxidase family (GPX) in redoxidation. *Frontiers in pharmacology*, *14*, 1147414.

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U. and Emerson, M.. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *Journal of Cerebral Blood Flow & Metabolism*, *40*(9), 1769-1777.

Rudyk, C.A., McNeill, J., Prowse, N., Dwyer, Z., Farmer, K., Litteljohn, D., Caldwell, W. and Hayley, S. (2017). Age and chronicity of administration dramatically influenced the impact of low dose paraquat exposure on behavior and hypothalamic-pituitary-adrenal activity. *Frontiers in Aging Neuroscience*, *9*, 222.

Sotler, R., Poljšak, B., Dahmane, R., Jukić, T., Pavan Jukić, D., Rotim, C., Trebše, P. and Starc, A., (2019). Prooxidant activities of antioxidants and their impact on health. *Acta Clinica Croatica*, *58*(4.), 726-736.

Souza, M.C.O., Cruz, J.C., Cesila, C.A., Gonzalez, N., Rocha, B.A., Adeyemi, J.A., Nadal, M., Domingo, J.L. and Barbosa, F. (2023). Recent

trends in pesticides in crops: A critical review of the duality of risks-benefits and the Brazilian legislation issue. *Environmental Research*, 115811.

Tripathi, P., Agarwal, S., Tewari, S. and Mandal, K. (2022). Status of catalase, glutathione peroxidase, glutathione S-transferase, and myeloperoxidase gene polymorphisms in beta-thalassemia major patients to assess oxidative injury and its association with enzyme activities. *Journal of Pediatric Genetics*, *11*(03), 198-212.

World Health Organization. 2007. Pesticide Residues in Food 2007: Joint FAO-WHO Meeting on Pesticide Residues; Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pestice Residues, Geneva, Switzerland, 18-27 September 2007 (Vol. 191). *Food & Agriculture Org*.

Yao, W., Cheng, J., Kandhare, A. D., Mukherjee-Kandhare, A. A., Bodhankar, S. L. and Lu, G. (2021). Toxicological evaluation of a flavonoid, chrysin: morphological, behavioral, biochemical and histopathological assessments in rats. *Drug and Chemical Toxicology*, *44*(6), 601-612.

Zhu, W., Ge, M., Li, X., Wang, J., Wang, P., Tai, T., Wang, Y., Sun, J. and Shi, G. (2022). Hyperoside Attenuates Zearalenone-induced spleen injury by suppressing oxidative stress and inhibiting apoptosis in mice. *International Immunopharmacology*, *102*, 108408.