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Research Article

Copper Toxicity and Antioxidant Effect of Chlorogenic Acid in *Drosophila melanogaster*

Adeyemo-Salami O.A. and Akano S.O.

Department of Biochemistry, College of Medicine, University of Ibadan, Oyo State, Nigeria.

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Abstract

Copper is a vital trace element in biological systems. However, toxic levels can precipitate neurodegenerative diseases like Alzheimer's disease which occurs with advancement in age. Chlorogenic acid (CGA) is a phytonutrient widely distributed in the diet. This study serves as a preliminary investigation to studying the preventive or ameliorative role of CGA in the light of Alzheimer's disease with copper toxicity being the model in *Drosophila melanogaster*.

D. melanogaster (Oregon strain) were allotted into four (4) groups of five vials with 50 flies each and exposed to copper sulphate 0, 0.25, 0.5 and 1.0 mM, respectively) for 7 days for survival assay, biochemical indices (H₂O₂, non-protein thiols, total thiol and nitric oxide (NO), and behavioural activities (negative geotaxis and eclosion rate). For the study with CGA, the flies were allotted to six (6) groups of five vials with 50 flies each and treated as follows: 0- control (distilled water), 2% ethanol (vehicle), 15, 30, 45, and 60 mg/kg diet of CGA, respectively for 28 days for the longevity assay. Biochemical (H₂O₂, non-protein thiols, total thiol and nitric oxide (NO) and behavioural (negative geotaxis and eclosion rate) assays were performed in flies treated with CGA for 7 days using similar groups comprising of 2% ethanol (control), and CGA at 15, 30, and 45 mg/kg diet.

Copper reduced the survival of the flies, especially at 1mM, and the non-protein thiol and total thiol levels were significantly ($p < 0.05$) reduced while negative geotaxis and eclosion rate were unaffected. At 15, 30, and 45 mg/kg diet of CGA, the longevity of the flies was improved. The concentrations of hydrogen peroxide and nitric oxide were significantly ($p < 0.05$) reduced in a dose-dependent manner, and levels of total thiol and non-protein thiols increased in a dose-dependent fashion with it being significant at 45 mg/kg diet of CGA. CGA did not affect the negative geotaxis and the eclosion rate of the flies.

Copper demonstrated toxicity at the concentrations tested, and chlorogenic acid gives the presentation that it is a potential antioxidant agent.

Key Words: Chlorogenic acid, copper, survival, non-protein thiols, total thiol, nitric oxide

INTRODUCTION

Copper (Cu) is a micronutrient and an essential trace element found in all the tissues of the body where it plays various vital roles (Ware, 2017). The roles include assisting the body to produce red blood cells in the presence of iron, maintenance of proper nerve and immune function, healthy bones, well constituted blood vessels, synthesis of collagen and elastin, inhibition of cancer cells, a cofactor in the activity of various cellular enzymes, and modulating gene expression. Therefore, low levels have been associated with osteoporosis, as well as cardiovascular diseases (Ware, 2017; Morales-Brown, 2023; Charkiewicz, 2024). Although the half-life in biological systems has been found to be 13-33 days, it is influenced by factors such as life style, age, genes and diet (Charkiewicz, 2024). Toxic levels as a result of excessive ingestion have been shown to result in various neurological and neurodegenerative diseases which includes Alzheimer's disease (Ware, 2017; Lanza et al., 2018; Abolaji et al., 2020). Hence, it can be adopted as a model for the disease condition. Chlorogenic acid (CGA) is a secondary metabolite (a polyphenol) from plant sources including vegetables, herbs, fruits and spices with a significant concentration in green

coffee beans (unroasted coffee). It has been found to mediate various therapeutic manifestations such as in the treatment of diabetes, cancer, cardiovascular diseases, and insults on the kidney and liver (Adeyemo-Salami et al., 2021; Nguyen et al., 2024).

The rising incidence of neurodegenerative diseases, has necessitated the search for preventive or ameliorative solutions from natural sources. This study was designed to investigate the biological effect of copper and chlorogenic acid in *Drosophila melanogaster* as a preliminary study.

MATERIALS AND METHODS

Test compounds and reagents: CGA and copper sulfate were procured from AK Scientific, U.S.A. All the other reagents were Sigma Aldrich, U.S.A. products.

***Drosophila melanogaster* culture:** *Drosophila melanogaster* (fruit fly; Oregon strain) obtained from the National Species Stock Centre, Bowling Green, Oklahoma, U.S.A., were maintained and reared in the *Drosophila* Laboratory, Department of Biochemistry, University of Ibadan, Oyo State, Nigeria on cornmeal medium containing brewer's yeast (1%

w/v), nipagin (preservative, 0.08% v/w), and agar-agar (1% w/v) at constant temperature (23 ± 2 °C). The 12 h dark/light cycle was maintained.

Survival rate analysis and biochemical assay of exposure of *D. melanogaster* to copper: *D. melanogaster* (both genders, 1 to 3 days old) were allocated into four (4) groups of five vials with 50 flies each and administered copper sulphate (0- control (distilled water), 0.25, 0.5 and 1 mM, respectively) for 7 days for survival assay. Daily mortality was recorded, and data were analyzed and plotted as percentage of live flies per day. The effect on antioxidant parameters (total thiol (Ellman, 1959) and non-protein thiol (Jollow et al., 1974) concentrations); oxidative status (nitric oxide (Green et al., 1982) and hydrogen peroxide (Wolff, 1994) levels); and behavioural profile (negative geotaxis and eclosion rate) by adopting the method of Farombi et al. (2018) after 7 days of exposure to copper were also observed.

Effects of exposure of *D. melanogaster* to Chlorogenic acid

i) Longevity analysis: CGA was exposed to six (6) groups of five vials with 50 flies each (0- control (distilled water), 2% ethanol (vehicle), 15, 30, 45, and 60 mg/kg diet respectively) for 28 days. Daily mortality was recorded, and data were analyzed and plotted as percentage of live flies for each day.

ii) Biochemical and behavioural assays: From the results of the longevity assay, 4 groups having 5 vials each with 50 flies/vial were prepared and exposed to 2% ethanol (control), 15, 30 and 45 mg/kg diet of CGA, respectively for 7 days after which the levels of total thiol (Ellman, 1959), non-protein thiol (Jollow et al., 1974), hydrogen peroxide (Wolff, 1994) and nitric oxide (Green et al., 1982) were evaluated; and the behavioural assay (negative geotaxis and eclosion rate) was determined using the method of Farombi et al. (2018).

Preparation of samples for biochemical assays: For the biochemical assays, 50 control and treated flies per group were anaesthetized on ice, weighed, homogenized in 0.1 M potassium phosphate buffer, pH 7.4 (1:10 (flies/volume (μL)),

and centrifuged at 10,000 g for 10 min at 4°C. The supernatants were separated from the pellets into Eppendorf tubes, kept at -20°C freezer and used for the evaluation of the biochemical assays.

Statistical analysis: All the assays were carried out in duplicates for each of the groups of control and treated flies. The data are presented as mean \pm standard error of mean. One-way analysis of variance (ANOVA) was used to assess the significant difference among the groups, and p-values < 0.05 were considered statistically significant. Post-hoc test was carried out using Tukey's posthoc test.

RESULTS

Effects of copper treatment on *Drosophila melanogaster*

The survival assay of Cu after 7 days of exposure shows that it is in this order, control > 0.25 mM Cu > 0.5 mM Cu > 1 mM Cu (Figure 1).

Figure 2 reveals that after 7 days of exposure to Cu, it did not affect hydrogen peroxide level. However, non-protein thiols and total thiol levels were significantly ($p < 0.05$) reduced at the 3 different concentrations of Cu, while nitric oxide concentration was elevated and was significant ($p < 0.05$) at 1 mM of Cu (Figure 2).

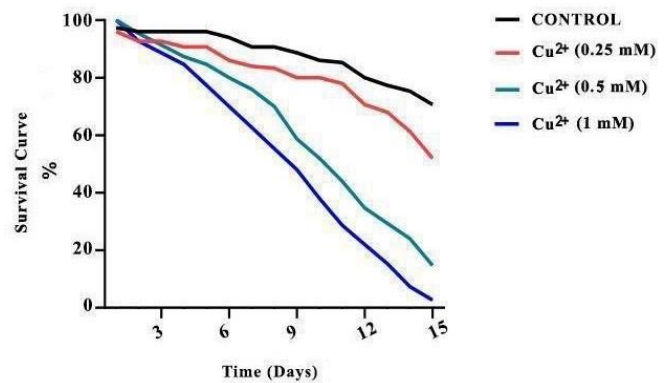


Figure 1: Survival assay of copper in *D. melanogaster* after 7 days

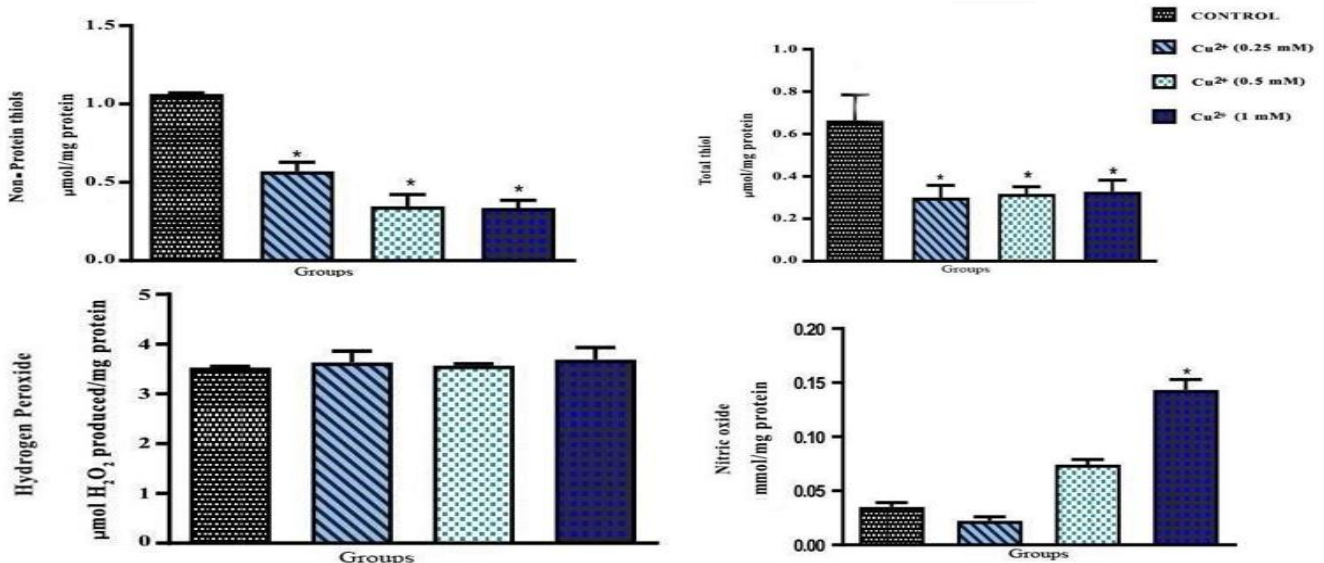


Figure 2: Influence of exposure to copper on biochemical parameters after 7 days in *D. melanogaster*. *-significantly different from control at $p < 0.05$

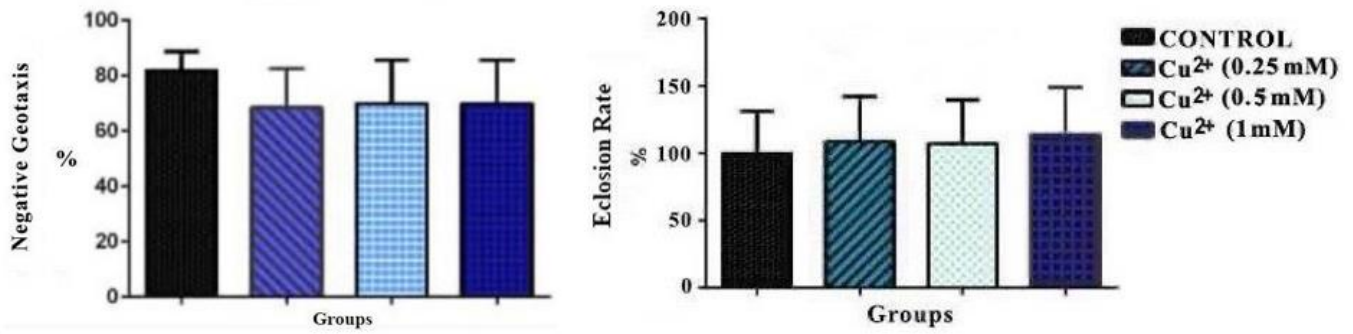


Figure 3: Effect of exposure to copper on the behavioural profile after 7 days in *D. melanogaster*

The negative geotaxis was decreased, though not significantly in the groups exposed to Cu after 7 days while eclosion rate was not affected (Figure 3).

Longevity and effect on biochemical indices in *D. melanogaster* exposed to chlorogenic acid: Figure 4 displays that at 15, 30 and 45 mg/kg diet of CGA, the flies survived for over 28 days, but this was not observed at 60 mg/kg diet. Therefore further investigations was carried out with 15, 30 and 45 mg/kg diet of CGA.

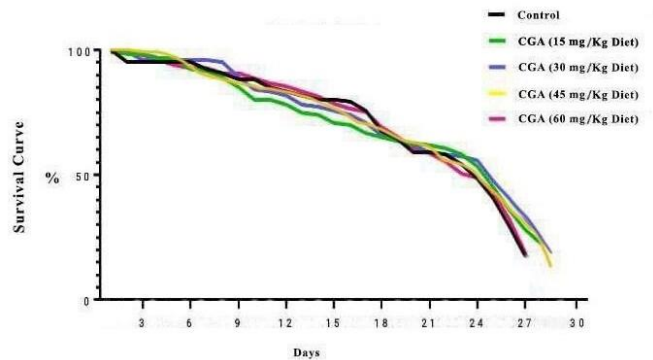


Figure 4: Longevity assay of chlorogenic acid in *D. melanogaster* after 28 days

Figure 5 shows that hydrogen peroxide concentration was significantly ($p < 0.05$) decreased at the 45 mg/kg diet of CGA. This was similarly observed for nitric oxide concentration while the levels of non-protein thiols and total thiol were significantly ($p < 0.05$) increased at 30 and 45 mg/kg diet of CGA (Figure 5).

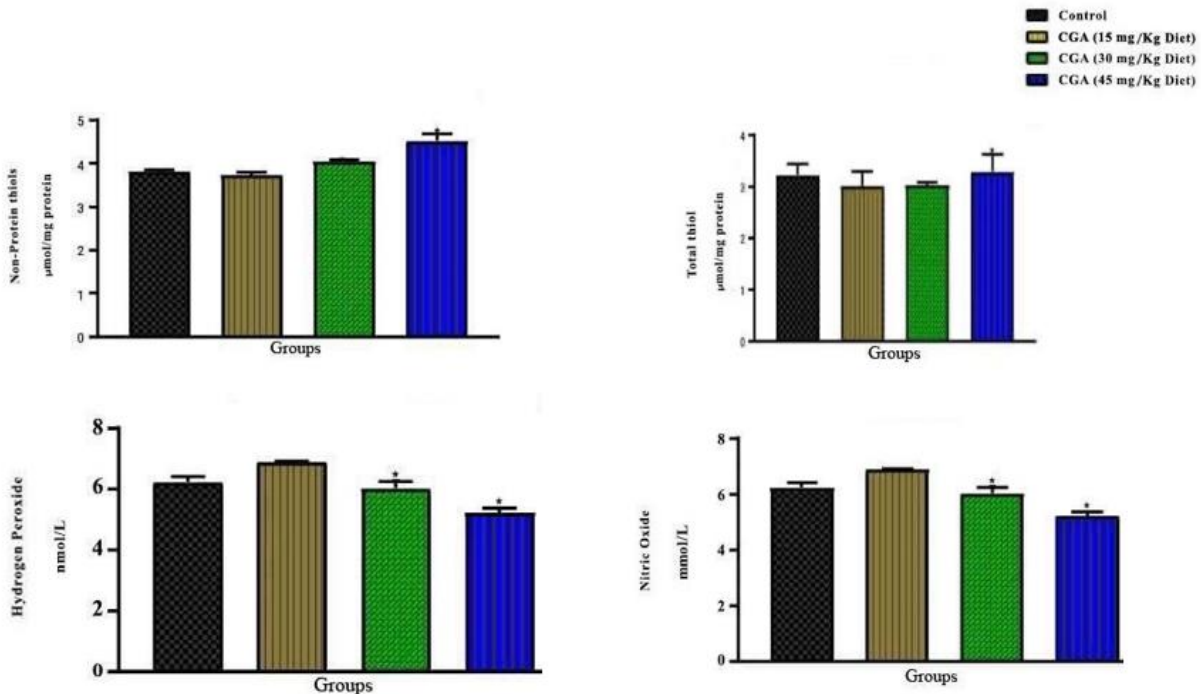


Figure 5: Influence of exposure to chlorogenic acid on biochemical parameters after 7 days in *D. melanogaster*. *-significantly different from control at $p < 0.05$

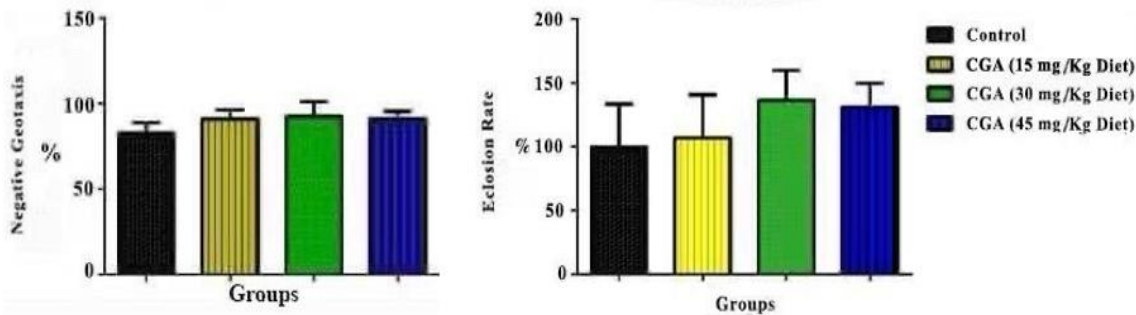


Figure 6:

Effect of exposure to chlorogenic acid on the behavioural profile after 7 days in *D. melanogaster*

Figure 6 reveals that the negative geotaxis was not affected in the flies exposed to CGA, however, the eclosion rate increased at 30 and 45 mg/kg diet, though not significantly.

DISCUSSION

The study shows that there were aberrations in the antioxidant parameters, and therefore the oxidative status upon exposure to copper and the survival of the flies was affected. Exposure to chlorogenic acid improved the longevity, biochemical and behavioural profile of the flies at 30 and 45 mg/kg diet.

Exposure of the flies to copper showed that the least-survival rate was observed at 1 mM concentration. This resonates with the previous observation of least mortality at this concentration (Abolaji et al., 2020) and in consonance with the observations of Halmenschelager and Rocha (2018). Longevity experiment with CGA showed that among the concentrations tested, 30 and 45 mg/kg of CGA were the most effective. Therefore, with regards to the ingestion of CGA, moderate levels would be more beneficial to increase longevity.

Hydrogen peroxide is a powerful oxidant which has both beneficial and deleterious effects in a biological system. The beneficial effects include membrane signal transduction and are involved in gene expression, while the converse is observed when there is compromise of the antioxidant defence system thus resulting in damage to the cell at varying degrees (Sies, 2014; Lennicke et al., 2015; Di Marzo et al., 2018; Pravda, 2020). A reduction of the H_2O_2 level in the groups fed with 45 mg/kg diet of CGA reflects the antioxidant property of chlorogenic acid. This is akin to the observations of Adeyemo-Salami et al. (2021) with the Harwich strain of the flies when exposed to different doses of CGA.

A thiol, is an organic compound having a mercapto or sulphhydryl group (-SH) (Ulrich and Ursula, 2019). They are highly versatile in biological systems where they mediate several cellular processes which are vital for homeostasis and survival. They play a vital role in the antioxidant defence mechanisms where they have been shown to quench free radicals such as reactive oxygen species (Ulrich and Ursula, 2019). The thiol component of a biological system is made up of that which is associated with proteins and the non-protein thiols (Yang and Guan, 2015). In the treatment with copper, the total thiol and non-protein thiol levels reduced significantly while that exposed to CGA increased at the 30 and 45 mg/kg diets. This implies that copper exposure compromised the antioxidant system in the flies while CGA enhanced it. This is similar to the findings of Abolaji et al. (2020) where copper reduced the concentration of total thiol.

Nitric oxide plays a central role in several biological processes including immune responses, reproduction, mRNA translation, and apoptosis. However, high concentrations culminate in oxidative stress leading to cytotoxicity (Andrabi et al., 2023). Again, the nitric oxide concentration was reduced in the flies exposed to CGA, especially at 45 mg/kg diet while it was elevated when exposed to copper, especially at 1 mM. This demonstrates the capacity for CGA to regulate NO concentration in the flies and thus promote the beneficial roles, while the reverse is the case with copper. The observation with copper is in consonance with the findings of Abolaji et al. (2020).

Eclosion is determined by circadian clock gating and neuro-endocrine mechanisms, and is therefore an indication of behavioural pattern (Varma et al., 2019). This increased, though not significantly in the flies treated with 30 and 45 mg/kg diet, while there was insignificant reduction in the negative geotaxis at all the concentrations of the copper treated groups.

CONCLUSION

Deducibly, exposure to copper can be toxic, and chlorogenic acid has antioxidative capacity.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

- Abolaji A., Fasae K., Iwezor C., Aschner M. and Farombi E.O. 2020. Curcumin attenuates copper-induced oxidative stress and neurotoxicity in *Drosophila melanogaster*. *Toxicol Rep.* 7: 261-268.
- Adeyemo-Salami O.A., Afonja O.J., Adeleke O.F., Adedara A.O. and Abolaji A. O. 2021. Ameliorative potential of chlorogenic acid on rotenone-induced neurotoxicity in *Drosophila melanogaster* model. *J Public Health Int.* 4 (3): 55-66.
- Andrabi S.M., Sharma N. S., Karan A., Shahriar S.M.S., Cordon B., Ma B., and Xie J. 2023. Nitric oxide: Physiological functions, delivery, and biomedical applications. *Adv Sci (Weinh).* 10(30): 2303259.
- Charkiewicz A.E. 2024. Is copper still safe for us? What do we know and what are the latest literature statements? *Curr Issues Mol Biol.* 46: 8441-8463.
- Di Marzo N., Chisci E. and Giovannoni R. 2018. The role of hydrogen peroxide in redox-dependent signaling: homeostatic and pathological responses in mammalian cells. *Cells* 7:156.

- Ellman G. 1959. Tissue sulphhydryl groups. *Arch Biochem Biophys.* 82: 70–77.
- Farombi E.O., Abolaji A.O., Farombi T.H., Oropo A.S., Owoje O.A. and Awunah M.T. 2018. Garcinia kola seed biflavonoid fraction (Kolaviron), increases longevity and attenuates rotenone induced toxicity in *Drosophila melanogaster*. *Pestic Biochem Physiol.* 145: 39-45.
- Green L.C., Wagner D.A., Glogowski J., Skipper P.L., Wishnok J.S. and Tannenbaum S.R. 1982. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. *Anal Biochem.* 126 131–138.
- Halmenschelager P.T and Rocha J.B.T. 2018. Biochemical CuSO₄Toxicity in *Drosophila melanogaster* depends on sex and developmental stage of exposure. *Biol Trace Elem Res.* 189 (2):574–585.
- Jollow D.J, Mitchell J.R, Zampaglione N and Gillette J.R. 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology.* 11 (3):151-69.
- Lanza V., Milardi D., Di Natale G. and Pappalardo G. 2018. Repurposing of Copper (II)-chelating drugs for the treatment of neurodegenerative diseases. *Curr Med Chem.* 25:525-539.
- Lennicke C., Rahn J., Lichtenfels R., Wessjohann L.A. and Seliger B. 2015. Hydrogen peroxide–production, fate and role in redox signaling of tumor cells. *Cell Commun Signal.* 13:39.
- Morales-Brown P. 2023. Copper toxicity Symptoms and treatment. *Medical News Today.* <https://www.medicalnewstoday.com/articles/copper-toxicity> (Accessed 5th Jan 2025).
- Nguyen V., Taine E.G., Meng D., Cui T. and Tan W. 2024. Chlorogenic acid: A systematic review on the biological functions, mechanistic actions, and therapeutic potentials. *Nutrients* 16: 924.
- Pravda J. 2020. Hydrogen peroxide and disease: towards a unified system of pathogenesis and therapeutics. *Mol Med.* 26: 41.
- Sies H. 2014. Role of metabolic H₂O₂ generation redox signaling and oxidative stress. *J Biol Chem.* 289: 8735–8741.
- Ulrich K. and Ursula J. 2019. The role of thiols in antioxidant systems. *Free Rad Biol Med.* 140: 14-27.
- Varma V., Krishna S., Srivastava M., Sharma V.K. and Sheeba V. 2019. Accuracy of fruit-fly eclosion rhythms evolves by strengthening circadian gating rather than developmental fine tuning. *Biol Open.* 8(8):bio042176.
- Ware M. (2017). Health benefits and risks of copper. *Medical News Today.* <https://www.medicalnewstoday.com/articles/288165> (Accessed 5th Jan 2025).
- Wolff, S.P. 1994. Ferrous ion Oxidation in the Presence of Ferric Ion Indicator Xylenol Orange for Measurement of Hydroperoxides. *Methods in Enzymol.* 233: 182-189.
- Yang Y. and Guan X. 2015. Rapid and thiol-specific high-throughput assay for simultaneous relative quantification of total thiol, protein thiols and non-protein thiols in cells. *Anal Chem.* 87(1): 649-655.