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DISRUPTION OF CELLULAR REDOX EQUILIBRIUM IN *DROSOPHILA MELANOGASTER* EXPOSED TO AN EDIBLE CLAY FROM SOUTHERN NIGERIA

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ABSTRACT

Geophagia, the consumption of clay, is a culturally embedded practice in many regions, including Nigeria, where it is particularly prevalent among pregnant women. Although often linked to nutritional, cultural, or therapeutic beliefs, concerns persist regarding the safety of geophagic materials due to potential toxicological risks. This study investigated the biological effects of Ubiaja-sourced geophagic clay using Drosophila melanogaster, a widely recognized model organism in toxicology. Adult flies were exposed to dietary concentrations of 0.025 g/mL, 0.05 g/mL, and 0.1 g/mL for seven days, after which whole-body homogenates were analyzed for biochemical markers of oxidative stress. Results demonstrated concentration-dependent alterations in antioxidant defense systems, with increased enzymatic activity reflecting physiological attempts to counteract the accumulation of reactive species. Elevated lipid peroxidation products were observed at higher clay concentrations, indicating cellular membrane damage. Although some parameters showed no significant variation relative to control, the overall pattern suggests that geophagic clay may disrupt redox homeostasis. These findings highlight potential biochemical risks associated with clay ingestion and underscore the importance of further molecular investigations to clarify mechanisms of toxicity. Such evidence is critical for informing public health guidance on geophagia, particularly in vulnerable populations. It emphasizes the need for continued research into the safety of naturally occurring substances consumed for cultural or therapeutic purposes.

Keywords: Oxidative stress, Ubiaja geophagic clay, Edible clay, Cellular redox, *Drosophila melanogaster*

1. INTRODUCTION

The practice of consumption of earth, soil, or clay by humans and animals is known as geophagy (Woywodt and Kiss, 2002). Geophagia is a type of pica practiced throughout the world, but especially in Africa, South America, and parts of Asia (Ogidi and Okiemute, 2015). This practice is done for several reasons, including cultural customs, potential health benefits, or personal preferences; people consume clays such as kaolin, bentonite, or chalk (Vignando *et al*., 2020). Traditional beliefs held by some indigenous cultures in Nigeria, such as the Edo, Igbo and Tiv women, encourage them to eat "*eko* or *nzu*" (a local name for geophagic clay among the Edos and Igbos in Nigeria), with a special focus on pregnant women (Edene and Aghedo, 2023). One such theory is that geophagic clay enhances the overall dietary status of expectant mothers (Davies, 2023). Ubiaja geophagic clay is a natural clay harvested in Ubiaja, a town in Edo State, located in the Southern region of Nigeria. It is popular for its unique texture and mineral content, and has been shown to contain minerals such as sodium, magnesium, potassium, aluminum, iron, copper, zinc, manganese, chromium, lead, and cadmium, at various concentrations in which some can be

detrimental to human health (Edene and Aghedo, 2023).

The health effect of consumption or over consumption of geophagic clay may include oxidative stress that may arise due to potential contamination with toxic metals such as lead (Pb), copper (Cu), mercury (Hg), nickel (Ni), cobalt (Co), chromium (Cr), cadmium (Cd), and zinc (Zn) (Orisakwe *et al*., 2020; Bonglaisin *et al*., 2022; Edene and Ogbeide, 2023). Oxidative stress arises when the balance between reactive oxygen species (ROS) and antioxidant defenses is disrupted, leading to cellular injury. This pathological state contributes to the development of several conditions, including cancer, genetic mutations, endocrine disturbances, and cardiovascular diseases (Sies, 2020). Oxidative stress is a valuable biomarker for evaluating the toxicity of chemical substances. This assessment can be performed by measuring stress parameters either in living organisms (*in vivo*) or in isolated tissues and fluids (*in vitro*) (Jomova *et al.,* 2024). This enables early detection of potential disease risks associated with oxidative imbalance and provides important insights that may guide the development of novel therapeutic interventions (Reddy, 2023; Garcia-Llorens *et al.*, 2025).

To safely assess health effects associated with the consumption and/or over-consumption of clay, a model is essential; model organisms play a pivotal role in scientific research, serving as representative systems to understand complex biological processes (Cole *et al*., 2018). While mammals such as mice and rats are commonly used due to their physiological similarity to humans, *Drosophila melanogaster,* commonly known as the fruit fly, offers a compelling alternative, with its genetic tractability, rapid life cycle, and cost-effectiveness. *D. melanogaster* provides a powerful platform for studying developmental biology, genetics, and human diseases (Vidal *et al*., 2024). *D. melanogaster* is widely employed as a model organism across diverse scientific disciplines, ranging from fundamental genetics to studies on tissue and organ development (Irion and Nüsslein-Volhard, 2022). Despite the apparent evolutionary distance between flies and humans, their genomes share approximately 60% similarity, and importantly, more than 75% of human disease-related genes have homologs in *Drosophila*. This genetic conservation underscores the relevance of the fruit fly as a powerful system for investigating mechanisms underlying human health and disease (Yamamoto *et al*., 2024; Victor Atoki *et al*., 2025). Employing *D. melanogaster* allows for rapid, reproducible assessment of the biological consequences of Ubiaja clay consumption, bridging cultural practices with scientific inquiry. This study, therefore, aimed at investigating the biological effects of Ubiaja- sourced geophagic clay on *Drosophila melanogaster as it* addresses a culturally significant behavior with potential health risks, by applying a robust model organism to generate evidence that can inform public health policy, guide awareness campaigns, and promote safer practices among communities where geophagy is prevalent.

2. MATERIALS AND METHODS

Study area, sampling, and duration of study: The geophagic clay examined in this study was collected from Ubiaja, the administrative headquarters of Esan South East Local Government Area in Edo State, Nigeria. Sampling was carried out at three distinct sites, with coordinates recorded as Latitude: 7°05′01.8″N Longitude:

6°39′02.7″E; Latitude: 6°51′08.8″N Longitude: 6°33′27.4″E; and Latitude: 7°21′12″N Longitude: 6°41′42″. From each location, three (3) replicate clay samples were obtained and securely stored in sterile zip-lock bags to preserve their integrity before laboratory analysis. Using a mortar and pestle, they were ground into a fine powder, and a composite sample was made.

Experimental design, grouping, and treatment of experimental animals: The experiment was conducted using a completely randomized design (CRD) with four (4) treatments (Table 1), each replicated three (3) times. The treatments consisted of three concentrations of Ubiaja geophagic clay (UGC) dissolved in distilled water: 0.025 g/mL, 0.05 g/mL, and 0.1 g/mL alongside a control treatment (0.00 g/mL), in which flies were fed the experimental diet without clay. The flies used in this study were *D. melanogaster* wild-type (Harwich strain), which were obtained from the *Drosophila* insectary in the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. The flies were maintained and reared in Biotoxics *Drosophila* Laboratory, at the University of Benin, Benin City, Edo State, Nigeria.

For each replicate, 9.8 g of prepared *Drosophila* meal was placed in Falcon tubes, to which the respective clay concentrations were added. Each treatment replicate contained thirty (30) adult *Drosophila melanogaster*, ensuring uniform exposure conditions. This design allowed for systematic comparison across treatments, with replication ensuring statistical reliability. The control group provided a baseline for evaluating the effects of clay exposure, while the graded concentrations enabled assessment of dose-dependent responses.

Table 1: Experimental Diets for *Drosophila Melanogaster*

Groups	*Experimental diets*
Group A	Control (0.2 mL of distilled water + 9.8 g of cornmeal)
Group B	200 µL of 0.025 g/mL (UGC) + 9.8 g of cornmeal
Group C	200 µL of 0.05 g/mL (UGC) + 9.8 g of cornmeal
Group D	200 µL of 0.1 g/mL (UGC) + 9.8 g of cornmeal

Thirty (30) flies were transferred into each of the twelve (12) Falcon tubes covered with cotton wool, and were monitored daily, for the duration of the experiment (7 days). UGC- Ubiaja geophagic clay.

Meal preparation: The meal was composed of corn meal (52 g), agar (7.9 g), glucose (3.5 g), nipagin (1 g), ethanol (1.0 mL), yeast (5 g), and distilled water. Cornmeal was mixed with one-quarter (1/4) of the water needed for the preparation (850 mL), while the rest of the water was brought to a boil. Then, yeast, agar, glucose, ethanol, nipagin, and the mixed cornmeal were added to the boiling water with continuous stirring to obtain a creamy consistency.

Homogenization and extraction of supernatant: After seven (7) days of exposure, the flies were transferred into empty Falcon tubes with appropriate labels corresponding to the experimental group and immobilized by freezing for about four minutes. After that, empty Eppendorf tubes were labeled and weighed using a weighing balance, then the immobilized flies from the freezer were added to each tube with appropriate labels, in order to calculate the exact weight of flies in each group. The

homogenates were made for each replicate group. After calculating the exact weight of flies, the flies were crushed inside the Eppendorf tubes, then phosphate buffer (PO_4) in microliters was added at a proportion of ten (10) times the calculated weight of flies in milligrams in the Eppendorf tube. The Eppendorf tubes were placed inside a centrifuge and set to run at 4000 rpm for seven (7) minutes. Then the supernatant was collected from the samples using a micro-pipette and transferred to labeled Eppendorf tubes, and subsequently analyzed.

Oxidative stress assays: Catalase (CAT) activity was measured following the procedure of Cohen *et al*. (1970), which quantifies the enzyme's ability to decompose hydrogen peroxide into water and oxygen. Superoxide dismutase (SOD) activity was determined using the method of Misra and Fridovich (1972), based on the enzyme's capacity to inhibit the auto-oxidation of epinephrine. Glutathione peroxidase (GPx) activity was assessed according to Nyman (1959), where the enzyme reduces hydrogen peroxide and organic peroxides using glutathione as a substrate. Malondialdehyde (MDA), a marker of lipid peroxidation, was estimated using the thiobarbituric acid reactive substances (TBARS) assay described by Buege and Aust (1978), which measures the formation of a pink chromogen detectable spectrophotometrically.

Data analysis: Data are expressed as Mean ± Standard Error of the Mean (SEM; n = 3). Differences among treatment groups were assessed by one-way analysis of variance (ANOVA), followed by post hoc Tukey's test, using GraphPad Prism 8. Statistical significance was set at $p < 0.05$.

Results and Discussion

Excessive consumption of geophagic clay has been associated with adverse health outcomes, one of which is oxidative stress (Edene and Ogbeide, 2023). Oxidative stress occurs when the balance between reactive oxygen species and antioxidant defenses is disrupted, leading to cellular damage. The primary antioxidant enzymes (glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD)) constitute the first line of defense against free radical-induced injury, playing a critical role in maintaining redox homeostasis and protecting tissues from oxidative damage. Furthermore, thioredoxin (Trx-Red or Trx-SH) and glutathione (GSH), which are low molecular weight antioxidants, function as co-factors for some enzymes (Ighodaro and Akinloye, 2018). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) often exhibit increased activity as an adaptive response to oxidative stress. This upregulation helps neutralize excess reactive oxygen species; however, a decline in their activity may occur when the enzymes are consumed at higher rates due to sustained oxidative pressure (Jelic *et al*., 2021). In addition to these primary defenses, glutathione-S-transferase (GST) functions as another important enzymatic antioxidant. GST plays a critical role in cellular metabolism and detoxification by conjugating reduced glutathione to electrophilic compounds, thereby facilitating their removal and reducing oxidative damage (Gusti *et al*., 2021).

Superoxide dismutase (SOD) represents a crucial intracellular defense mechanism against oxidative damage by catalyzing the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen (Wang *et al*., 2018). As shown in Table 2, statistically significant differences in SOD activity were observed among the treatment groups ($p = 0.0301$). Figure 1 further

illustrates that exposure to 0.05 g/mL UGC resulted in a marked increase in SOD activity compared with the control group ($p < 0.05$). Although SOD activity also rose in the group exposed to 0.1 g/mL UGC, this increase did not reach statistical significance. Collectively, these findings suggest that UGC exposure enhances SOD activity in a dose- dependent manner. Comparable results were reported by Fernandes *et al*. (2023), who observed elevated SOD activity in *D. melanogaster* following exposure to free curcumin and curcumin- loaded nanocapsules.

Table 2: Results of oxidative stress assay in *Drosophila melanogaster* exposed to Ubiaja geophagic clay.

Parameters	Control	0.025 g/mL UGC	0.05 g/mL UGC	0.1 g/mL UGC	P-value
SOD (µ/g Prot.)	3.84 ± 0.31	3.30 ± 0.38	5.46 ± 0.65	4.76 ± 0.26	0.0301
CAT (µ/g Prot.)	1.93 ± 0.14	1.69 ± 0.16	2.60 ± 0.26	2.38 ± 0.11	0.0232
GPx (µ/g Prot.)	3.93 ± 0.27	4.14 ± 0.46	7.10 ± 0.71	5.85 ± 0.14	0.0031
MDA (mol/g Prot.)	0.19 ± 0.02	0.28 ± 0.04	0.40 ± 0.09	0.42 ± 0.08	0.1195
GSH (µg/mL)	24.64 ± 1.17	38.45 ± 7.54	37.29 ± 4.59	37.58 ± 3.29	0.2038
GST Activity (µmol/min/g Prot.)	3.02 ± 0.35	1.76 ± 0.25	2.57 ± 0.38	2.22 ± 0.15	0.0805
H_2O_2 (µg/mL)	10.79 ± 1.64	29.99 ± 6.92	14.77 ± 1.35	25.37 ± 3.67	0.0326
Nitric Oxide (µg/mL)	37.88 ± 6.11	48.39 ± 8.35	47.06 ± 4.96	47.69 ± 8.06	0.6944

Data are presented as mean ± standard error of the mean (SEM), with n = 3. Statistical significance was considered at p < 0.05.

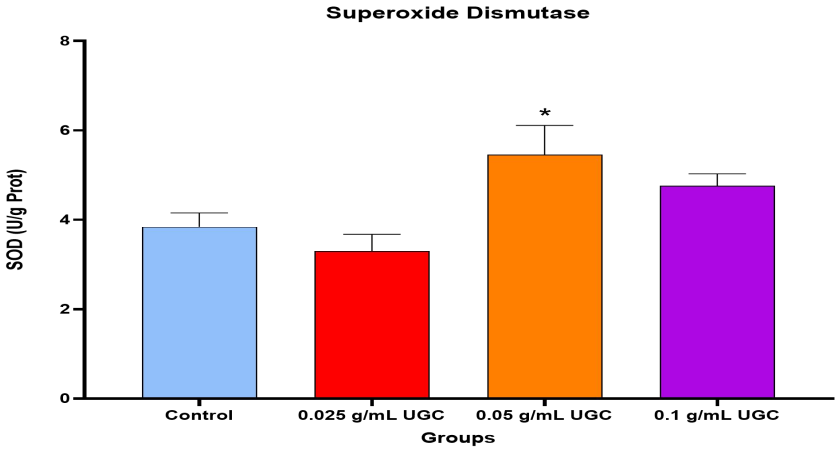

Figure 1: Superoxide dismutase (SOD) activity in *Drosophila melanogaster* on exposure to UGC. *Data are presented as mean ± standard error of the mean (SEM), with n = 3. Statistical significance was considered at p < 0.05.* UGC- Ubiaja geophagic clay.

The antioxidant enzyme catalase (CAT) regulates the amount of hydrogen peroxide (H_2O_2), a crucial physiological signaling molecule, by catalyzing its breakdown (Baker *et al*., 2023). Catalase (CAT) is a key antioxidant enzyme that protects cells by catalyzing the decomposition of hydrogen peroxide into water and oxygen, thereby mitigating oxidative damage. Table 2 shows statistically significant differences in CAT activity among the treatment groups ($p = 0.0232$). As illustrated in Figure 2, exposure to 0.05 g/mL UGC resulted in a marked increase in CAT activity compared with the control group ($p < 0.05$). Although CAT activity also increased in the group exposed to 0.1 g/mL UGC, this elevation did not reach statistical significance. These findings suggest that CAT activity rises in response to higher concentrations of UGC exposure, indicating a concentration-dependent response to oxidative stress. Elevated CAT activity reflects an adaptive cellular response aimed at neutralizing hydrogen peroxide generated during oxidative stress Shrivastav *et al*., 2026). However, the lack of statistical significance at higher concentrations may suggest either enzyme saturation or compensatory involvement of other antioxidant systems. Comparable results have been reported by Saraiva *et al*. (2018), who observed significant increases in CAT activity in *D. melanogaster* exposed to varying concentrations of Mancozeb, a broad-spectrum fungicide. This highlights the conserved nature of antioxidant responses across species and different xenobiotic exposures. Together, these findings reinforce the importance of CAT as a biomarker of oxidative stress and demonstrate that UGC exposure can modulate antioxidant enzyme activity in a dose-responsive manner.

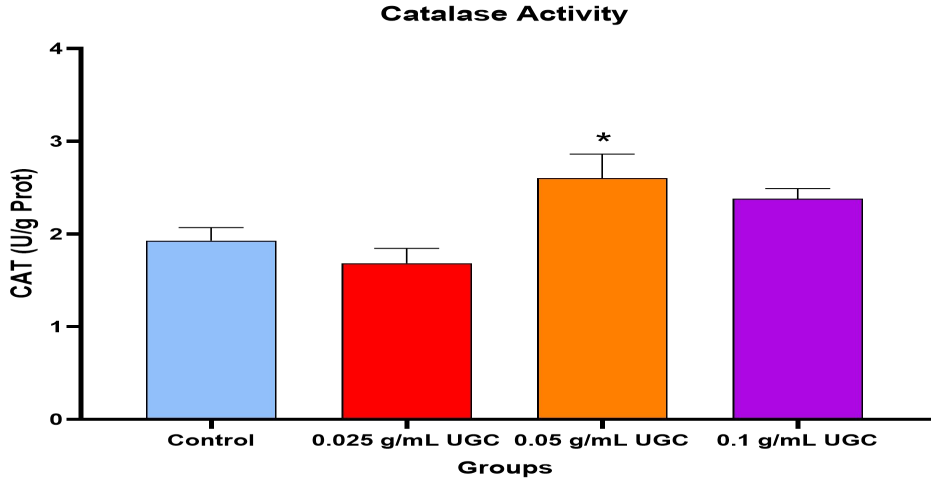

Figure 2: Catalase (CAT) activity in *Drosophila melanogaster* on exposure to UGC. *Data are presented as mean ± standard error of the mean (SEM), with n = 3. *Statistical significance was considered at p < 0.05.* UGC- Ubiaja geophagic clay.

In order to prevent oxidative stress and preserve redox balance, glutathione peroxidase (GPx) is essential (Pei *et al*., 2023). Glutathione peroxidase (GPx) is a central antioxidant enzyme that protects cells by reducing hydrogen peroxide and

lipid peroxides through the oxidation of glutathione. Table 2 revealed statistically significant differences in GPx activity across treatment groups ($p = 0.0031$). As shown in Figure 3, exposure to 0.05 g/mL and 0.1 g/mL UGC resulted in significantly higher GPx activity compared with the control group ($p < 0.05$), with the 0.05 g/mL group exhibiting the greatest increase. Although GPx activity was slightly elevated in the 0.025 g/mL group, this difference was not statistically significant. The marked elevation in GPx activity at moderate and high UGC concentrations is indicative of oxidative stress. Increased enzyme activity reflects an adaptive cellular response to neutralize excess reactive oxygen species generated under these conditions. At higher concentrations, the sustained upregulation of GPx suggests that cells are under considerable oxidative pressure, requiring enhanced detoxification capacity to maintain redox balance. This pattern underscores the role of GPx as a sensitive biomarker of oxidative stress, where elevated activity signals the presence of oxidative damage rather than improved cellular health. Similar findings have been reported in other experimental studies. For example, Oluwayemi *et al*. (2022) observed increased GPx activity in *D. melanogaster* exposed to varying doses of imidazole, further supporting the interpretation that heightened GPx activity is a compensatory response to oxidative stress. Collectively, these results demonstrate that UGC exposure induces oxidative stress in a dose-dependent manner, with GPx activity serving as a reliable indicator of the cellular burden imposed by reactive oxygen species.

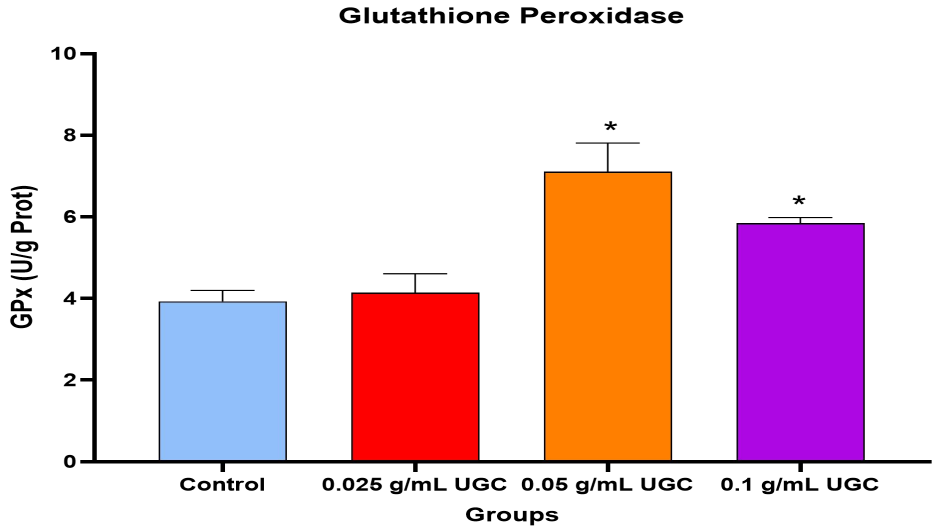

Figure 3: Glutathione peroxide (GPx) activity in *Drosophila melanogaster* on exposure to UGC. *Data are presented as mean ± standard error of the mean (SEM), with n = 3. *Statistical significance was considered at p < 0.05.* UGC- Ubiaja geophagic clay.

The enzyme family known as glutathione S-transferase (GST) is essential for metabolism and detoxification. GST plays a crucial function in antioxidant defense by detoxifying endogenously produced electrophilic chemicals (Gusti *et al*., 2021). Glutathione-S-transferase (GST) is an important phase II

detoxification enzyme that catalyzes the conjugation of reduced glutathione (GSH) to electrophilic compounds, thereby facilitating their removal and protecting cells from oxidative damage. Table 2 shows no statistically significant differences in GST activity across treatment groups ($p = 0.0805$). However, Figure 4 reveals that the group exposed to 0.025 g/mL UGC exhibited significantly lower GST activity compared with the control ($p < 0.05$). Although not statistically significant, GST activity also declined in the 0.05 g/mL and 0.1 g/mL UGC-exposed groups relative to the control. These findings suggest that GST activity decreases as UGC concentration increases, pointing to impaired detoxification capacity under conditions of oxidative stress.

The reduction in GST activity is consistent with the notion that prolonged oxidative stress can deplete enzymatic antioxidants. Since GST requires GSH as a cofactor to catalyze conjugation reactions (Kennedy *et al*., 2020), diminished GST activity may reflect either enzyme inhibition or substrate depletion. Interestingly, the decline in GST activity observed in Figure 4 corresponds with the accumulation of GSH reported in Figure 5, suggesting that reduced GST utilization may contribute to glutathione buildup in the system. This imbalance highlights a disruption in redox homeostasis, where antioxidant defenses are compromised despite elevated oxidative pressure.

Comparable results have been documented in other experimental models. Abolaji *et al*. (2014) reported decreased GST activity in *D. melanogaster* exposed to varying concentrations of 4-vinylcyclohexene, further supporting the interpretation that xenobiotic exposure can suppress GST activity. Taken together, these findings indicate that UGC exposure impairs GST function, thereby weakening the detoxification pathway and reinforcing the evidence that UGC induces oxidative stress in a dose-dependent manner. Non-enzymatic oxidative stress parameters include glutathione (GSH), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and nitric oxide (NO) (Marinho *et al*., 2014; Modun *et al*., 2014; Jové *et al*., 2020; Aoyama, 2021).

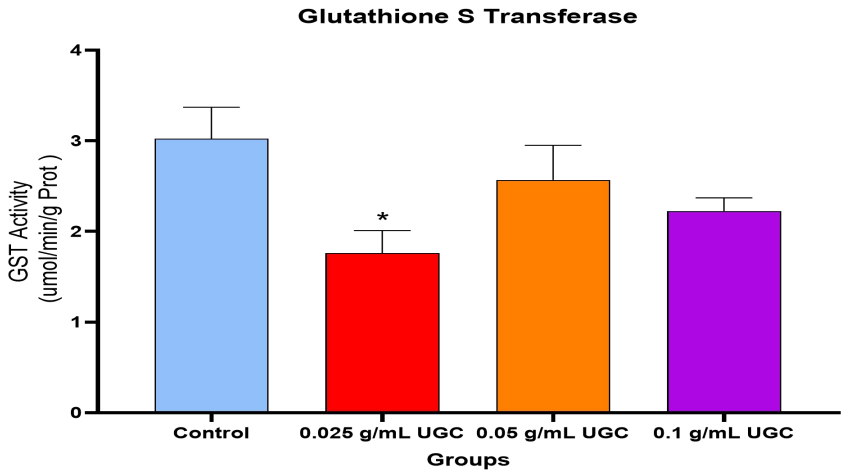

Figure 4: Glutathione-S-Transferase (GST) activity in *Drosophila melanogaster* on exposure to UGC.

*Data are presented as mean ± standard error of the mean (SEM), with n = 3. *Statistical significance was considered at p < 0.05.* UGC- Ubiaja geophagic clay.

One important antioxidant that keeps cells' redox balance and sustains physiological processes in vivo is glutathione (GSH) (Aoyama, 2021). Reduced glutathione (GSH) is a major non-enzymatic antioxidant that plays a central role in maintaining cellular redox homeostasis by directly scavenging reactive oxygen species and serving as a cofactor for detoxification enzymes. Table 2 indicates that there were no statistically significant differences in GSH levels among the treatment groups ($p = 0.2038$). However, Figure 5 shows a trend toward elevated GSH concentrations in the groups exposed to 0.025 g/mL, 0.05 g/mL, and 0.1 g/mL UGC compared with the control, although these increases did not reach statistical significance. The observed elevation in GSH levels, despite the lack of statistical significance, is biologically relevant as it suggests an adaptive response to oxidative stress. Under conditions of increased reactive oxygen species, cells often upregulate GSH synthesis to counteract oxidative damage and maintain redox balance. The accumulation of GSH may also reflect reduced utilization by glutathione-dependent enzymes such as GST, which showed decreased activity in UGC-exposed groups. This imbalance between GSH availability and enzyme activity highlights a disruption in antioxidant defense pathways, consistent with oxidative stress induction at higher UGC concentrations.

Taken together, these findings suggest that UGC exposure alters glutathione metabolism, leading to elevated GSH levels as a compensatory mechanism against oxidative stress. Although not statistically significant, the upward trend in GSH concentrations supports the interpretation that UGC may impose oxidative pressure on cells, requiring enhanced non-enzymatic antioxidant defenses to mitigate damage.

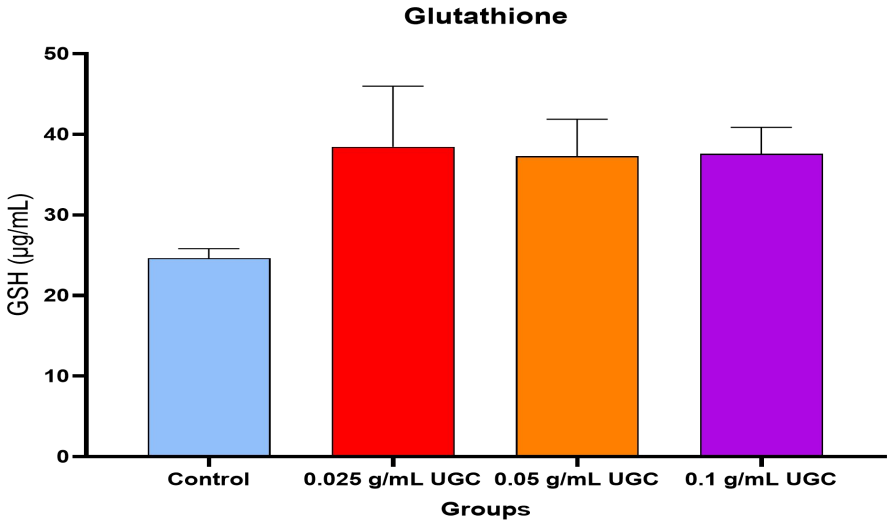

Figure 5: Glutathione (GSH) concentration in *Drosophila melanogaster* on exposure to UGC.
*Data are presented as mean ± standard error of the mean (SEM), with n = 3. *Statistical significance was considered at p < 0.05.* UGC- Ubiaja geophagic clay.

Malondialdehyde (MDA) is a well-established biomarker of oxidative stress, formed as a by-product of lipid peroxidation and widely used to assess cellular damage (Tsikas, 2017). Table 2 indicates that there were no statistically significant differences in MDA levels across the treatment groups ($p = 0.1195$). However, Figure 6 shows that MDA concentrations were significantly higher in the groups exposed to 0.05 g/mL and 0.1 g/mL UGC compared with the control ($p < 0.05$).

The elevation of MDA levels in UGC-exposed groups strongly suggests enhanced lipid peroxidation, reflecting oxidative stress at higher concentrations of UGC. This outcome highlights that while statistical variation across all groups was not evident, the significant increases at moderate and high doses provide clear evidence of oxidative damage to membrane lipids. Elevated MDA levels are therefore indicative of compromised cellular integrity and confirm that UGC exposure may impose oxidative pressure on biological systems. These findings are consistent with previous research. Saraiva *et al*. (2018) reported significantly increased MDA levels in *D. melanogaster* exposed to Mancozeb, a broad-spectrum fungicide, further supporting the interpretation that xenobiotic exposure enhances lipid peroxidation. Taken together, the results of this study demonstrate that UGC exposure leads to oxidative stress, with MDA serving as a reliable biomarker of lipid peroxidation and cellular damage.

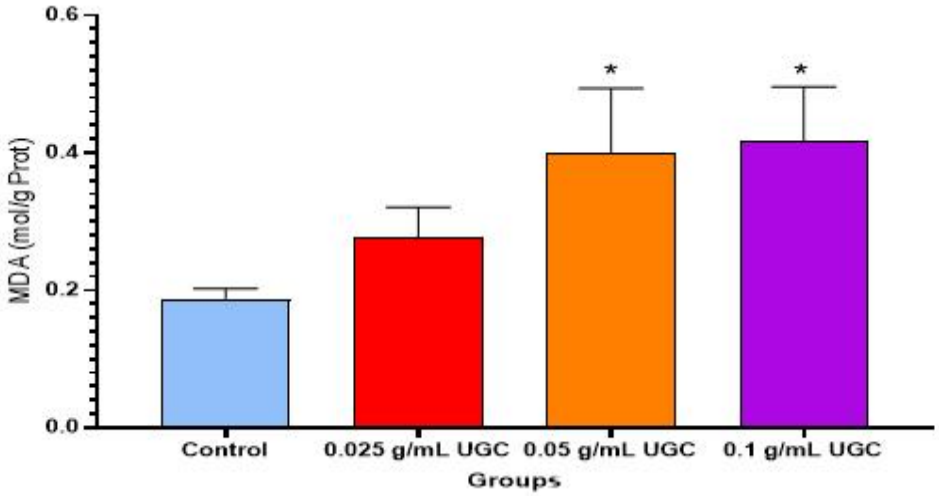

Figure 6: Malondialdehyde (MDA) activity in *Drosophila melanogaster* on exposure to UGC. *Data are presented as mean ± standard error of the mean (SEM), with n = 3. *Statistical significance was considered at p < 0.05.* UGC- Ubiaja geophagic clay.

One important redox metabolite involved in redox signaling and control is hydrogen peroxide (H_2O_2) (Marinho *et al*., 2014). The study found that the H_2O_2 levels in the treated groups differed significantly (p-value = 0.0326). Hydrogen peroxide (H_2O_2) is a reactive oxygen species that plays dual roles in cellular physiology, functioning as a signaling molecule at low concentrations but contributing to oxidative damage when excessively accumulated. As

illustrated in Figure 7, H_2O_2 levels were significantly higher in the groups exposed to 0.025 g/mL and 0.1 g/mL UGC compared with the control ($p < 0.05$). This outcome indicates that UGC exposure promotes the generation of H_2O_2, with concentrations rising in a dose- dependent manner.

The elevation of H_2O_2 levels is a clear indicator of oxidative stress, as excessive accumulation can overwhelm antioxidant defenses such as catalase and glutathione peroxidase, leading to lipid peroxidation, protein oxidation, and DNA damage. The significant increases observed at both low and high UGC concentrations suggest that even minimal exposure may disrupt redox balance, while higher doses exacerbate oxidative pressure. Comparable findings have been reported in other experimental studies. Osunbor and Orobor (2023), documented similar outcomes in *D. melanogaster* exposed to lead and treated with *Picralima nitida*, where elevated H_2O_2 levels were observed. This consistency across studies reinforces the interpretation that xenobiotic exposure, including geophagic clay, induces oxidative stress by promoting reactive oxygen species accumulation. Taken together, these results highlight H_2O_2 as a sensitive biomarker of oxidative stress in UGC- exposed organisms, underscoring the potential of geophagic clay to disrupt redox homeostasis and compromise cellular integrity.

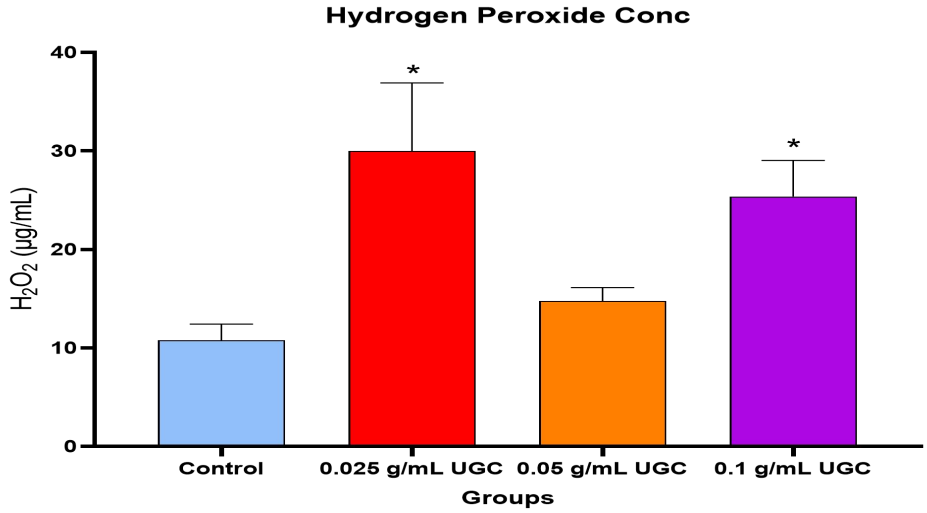

Figure 7: Hydrogen peroxide (H_2O_2) concentration in *Drosophila melanogaster* on exposure to UGC. *Data are presented as mean ± standard error of the mean (SEM), with n = 3. *Statistical significance was considered at $p < 0.05$.* UGC- Ubiaja geophagic clay.

Nitric oxide (NO) is a versatile signaling molecule that regulates numerous physiological processes, including vascular tone, neurotransmission, and immune responses (Modun *et al*., 2014). Results from this study show that there were no statistically significant differences in nitric oxide (NO) levels among the treatment groups ($p = 0.6944$). As shown in Figure 8, although the UGC- exposed groups exhibited a trend toward elevated NO concentrations compared with the control, these increases did not reach statistical significance. The slight elevation in NO levels observed in UGC- exposed groups

may reflect an adaptive response to oxidative stress, as NO production is often upregulated under conditions of cellular stress and inflammation. However, the absence of statistical significance suggests that UGC exposure did not markedly disrupt NO homeostasis within the tested concentrations.

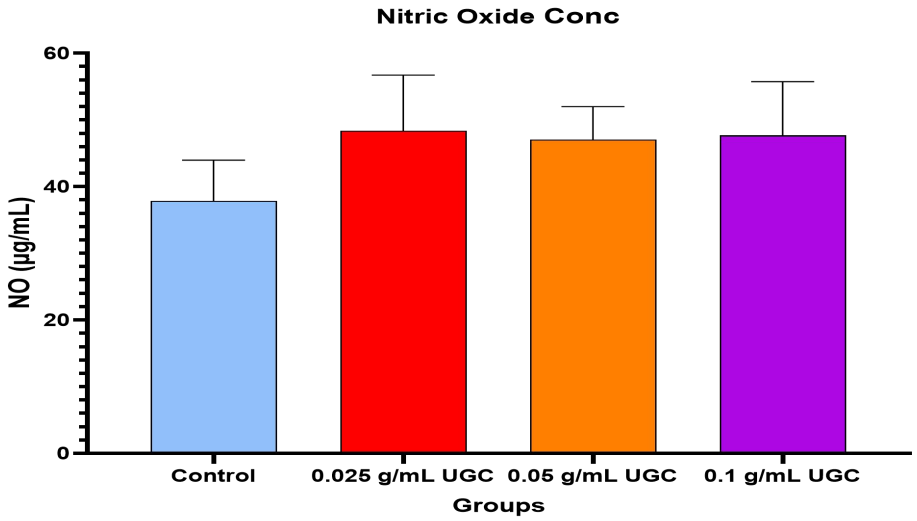

Figure 8: Nitric oxide (NO) concentration in *Drosophila melanogaster* on exposure to UGC. *Data are presented as mean ± standard error of the mean (SEM), with n = 3. *Statistical significance was considered at p < 0.05* UGC- Ubiaja geophagic clay.

This outcome indicates that while UGC may influence redox signaling pathways, its effect on NO production is relatively modest compared with other oxidative stress biomarkers such as H_2O_2. Comparable findings have been reported in other experimental models. Adedara *et al*. (2023) observed no significant differences in NO concentrations between exposed and control groups of *D. melanogaster* co- exposed to rotenone and iron, which aligns with the present data. Taken together, the results from this study suggest that NO levels may not be a sensitive indicator of oxidative stress under UGC exposure, reinforcing the importance of evaluating multiple biomarkers to characterize redox imbalance fully.

Conclusion: Conclusively, results from this study indicated that UGC exposure may result in oxidative stress, with dose-dependent changes observed in key antioxidant and oxidative stress markers, particularly SOD, CAT, GPx, and H_2O_2 levels. Although some parameters exhibited different trends, these findings provide an initial toxicological indication for toxicity and require further studies to evaluate the effects at the genetic level for a more comprehensive understanding of *D. melanogaster*'s response to UGC exposure. Histopathological as well as mammalian studies are also recommended.

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