NIPES

Journal of Science and Technology Research

Journal homepage: www.nipesjournals.org.ng

Hepatotoxic Responses in Female Wistar Rats Exposed to Geophagic Clay ('Eko') from Ubiaja, in South-South, Nigeria

[1]Edene, O. A & [2]Onoagbe, I. O.

[1]Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

*Corresponding Author's Email: *osemudiamen.anao@uniben.edu*, Phone: +234 7061857637

of Benin, Benin city, Nigeria.

*Email of the corresponding author; egheoyedoh@uniben.edu

Article Info

Keywords:*Hepatotoxicity, geophagia, geophagic clay, oxidative stress, Ubiaja..*

Received 09 May 2023
Revised 15 June 2023
Accepted 19 June 2023
Available online 11 August 2023

https://doi.org/10.5281/zenodo.8229695

Abstract

Geophagia, the practice of consuming geophagic clay, is an uncommon behavior observed in various cultures worldwide. However, extended or excessive exposure to specific clay types can negatively impact health, due to the presence of heavy metals in geophagic clay. Our study focused on examining the effects of geophagic clay consumption on the liver considering its central role in managing and processing the substances absorbed from geophagic clay. To conduct the study, we used thirty-six female albino Wistar rats, dividing them into six groups. The control group received only distilled water, while the other five groups were administered geophagic clay at varying concentrations (250, 500, 1000, 1500, and 2000 mg/kg bw) daily for forty-two days. We assessed oxidative stress parameters such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GPx), reduced glutathione (GSH), and malondialdehyde (MDA) in the liver tissues of the Wistar rats. The results showed increased levels of antioxidant enzymes [SOD (1000-1500 mg/kg bw), CAT, GPx (1000 mg/kg bw)] accompanied by a notable reduction in GSH (1000-1500 mg/kg bw) concentration, and a significant rise in MDA (1000-2000 mg/kg bw) levels, indicating oxidative stress. Additionally, microscopic examination of liver tissue sections stained with haematoxylin and eosin revealed slight changes in hepatocellular architecture, particularly in rats treated with higher concentrations of geophagic clay (1500-2000 mg/kg bw), aligning with our previous results. The study suggests that exposure to Ubiaja geophagic clay may lead to potential liver toxicity through oxidative stress mechanisms. Thus, emphasizing the importance of educating consumers regarding the possible risks linked to geophagic clay consumption.

1.0. Introduction

The consumption of non-nutritive substances usually seen as an aberration to normal behavioral patterns is known as pica [1]. When pica involves the ingestion of clay or other soil materials, it is known as geophagy [2]. Across racial and cultural boundaries, there have been reports on the engagement of humans in this practice. Notably, in African settings, it is usually a habit associated with pregnant women, children, and sometimes men [3, 4]. Various reasons for its consumption, including its perceived benefits in supporting gestation, reducing pregnancy-induced nausea, and aiding placenta delivery have been documented [2, 5]. However, geophagic clays may contain both

beneficial and harmful metals due to geological and anthropogenic influences, raising concerns about potential toxicities associated with their consumption [6, 2].

In our initial investigation, on the elemental composition of the Ubiaja geophagic clay, we identified the presence of cadmium, lead, and chromium in the clay [7]. The identification of these heavy metals in the geophagic clay raises critical concerns regarding potential implications for human health due to the established toxicological implications of theses metals. When ingested, these heavy metals can accumulate in the body over time and lead to a range of health problems [8]. It is relatable, that these metals may be responsible for certain reported toxicities associated with geophagic clay consumption [9]. Of concern is the fact that undue exposure to these metals on consumption of this clay material may pose certain consequences to the health of its consumers [5]. The digestive tract serves as the entry point for the absorption of cadmium and mercury. However, it is within the liver that both of these substances are subsequently metabolized [10]. The liver plays a crucial role in detoxification and protection against xenobiotics, but exposure to high concentrations of toxicants can disrupt liver function and induce oxidative stress, leading to liver diseases and systemic complications [11]. Xenobiotics that can produce ROS in excess will cause an imbalance in the body's homeostatic state [12]. This phenomenon is recognized as a crucial mechanism contributing to the onset and advancement of different liver conditions.

Exposure to substances containing toxicants in high concentrations can be damaging to the liver as the natural mechanisms which protect the liver including the presence of antioxidants may be compromised [13]. When there is an alteration in the normal oxidative state of the human system it causes a stress condition known as oxidative stress [14]. Systemic oxidative stress that may arise concerning the development of liver disease may lead to the damage of extra-hepatic organs, including kidney failure and brain damage [15]. Consequently, it becomes imperative to conduct a biochemical assessment of the antioxidants in the liver of individuals consuming geophagic clay to determine whether exposure to these substances leads to any deviation from the normal antioxidant levels.

Carrying out this assessment will give useful information on the extent of toxicity that could be seen on exposure to this xenobiotic. This can also help in explaining the proposed mechanism of action of the xenobiotic. Many authors have assessed different geophagic clays from different study locations in Nigeria [16, 17, 18, 19], but there is only a scanty information available concerning geophagic clay materials sourced specifically from Ubiaja, Edo State.

Therefore, the primary objective of this research is to investigate the impact of geophagic clay, sourced from Ubiaja, in Edo State, on the liver. The findings from this study will offer essential guidance regarding its consumption as it may be a predisposing factor to the onset of varying diseases. The study involved the evaluation of antioxidant enzymes in female Wistar rats, which were exposed to various concentrations of the geophagic clay. Additionally, comprehensive histopathological examinations were conducted to detect any possible changes in liver structure, providing valuable insights into the effects of Ubiaja geophagic clay on liver health.

2.0. Materials and Method

Reagents

Chloroform, picric acid, normal saline, distilled water, hydrochloric acid, carbonate buffer, sodium hydroxide, pyrogallol, phosphate buffer, adrenaline, hydrogen peroxide, potassium permanganate, sulphuric acid (H_2SO_4), thiobarbituric acid (TBA), trichloroacetic acid (TCA), formaldehyde and Ellman's reagent, sulfanilic acid, dimethylsulfoxide, sodium nitrite, adrenaline solution, distilled water, 30 mM phosphate buffer, hydrogen peroxide, 0.01 M $KMnO_4$.

2.1. Method

The samples were sourced from Ubiaja, Esan South East Local Government Area of Edo State, South-South, Nigeria, from open-source market stalls. Six representative samples of the geophagic

clay ('eko') were purchased and were carefully sealed in zip-lock bags to ensure complete protection from any potential contamination. They were later pulverized under aseptic conditions before the analysis.

2.2. Experimental design

Thirty-six (36) female rats were used in this study; they were separated according to weight into six (6) different groups. Five (5) of the groups were the treatment groups, while one group was taken as the control. Geophagic clay sample was administered orally to animals using a gavage, at concentrations of 250, 500, 1000, 1500, and 2000 mg/kg bw, and designated as groups I, 2, 3, 4, and 5 respectively. The concentration of stock was 10%. A single dose of the treatment was given every 24 hours for 42 days. Stock concentration was made by dissolving 10 g of geophagic clay in 90 ml of distilled water. Volumes administered were calculated using the formula:

Volume = dose × weight/ Conc. of stock (1)

2.3. The sacrifice of experimental animals

The animals were handled according to the guidelines for the treatment of laboratory animals. After six (6) weeks of administration of the geophagic clay sample, the animals were sacrificed after an overnight fast. This was done to ensure that all the rats were in the same metabolic state. The rats were humanely euthanized through cervical dislocation. Subsequently, their livers were carefully dissected, harvested, and then washed with ice-cold 1.15% KCl buffer.

2.4. Tissue sample preparation

A potter-Elvehgen homogenizer was employed in homogenizing the weighed organ after placing it in a 0.1 M phosphate buffer pH 7.4. Centrifugation was done at 10,000 g for 10 min in a cold centrifuge at a temperature of 4 ^{o}C. The supernatant obtained were subjected to further analytical procedures using standard methods for the different markers of oxidative stress. Standard procedures [20] were also employed in fixing sections of the liver for histopathological examination using the hematoxylin and eosin stain.

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) was estimated using the method described by [21].

Catalase (CAT)

The method of [2] was used.

Glutathione peroxidase (GPx)

The activity of the Glutathione peroxidase enzyme was determined using the method described by (23).

Reduced glutathione (GSH)

Reduced GSH was determined using the method described by [24].

Malondialdehyde (MDA)

This assay was carried out using the method by [25].

2.5. Statistical analysis

The data underwent analysis using the SPSS statistical tool. For presentation, bar charts were utilized, showing the values as mean ± SEM (standard error of the mean). To determine significance, the threshold was set at $P < 0.05$, with 95% confidence intervals.

3.0. Results and Discussion

3.1. Effect of geophagic clay on some oxidative stress parameters in liver of albino Wistar rats.

A major mechanism for the pathogenesis of diseases is oxidative stress. It is a major mechanism of action for most disease conditions [26]. The principal culprit in oxidative stress is the reactive oxygen species which are free radicals that possess the ability to attack cellular molecules [27]. The human system comprises a mechanism responsible for maintaining an oxidative balance, which consists explicitly of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)], and non-enzymatic molecule, reduced glutathione (GSH), capable of mopping up these free radicals [28]. Assessment of the state of these antioxidants provides information on the responses of the different organs, to the introduction of xenobiotics [29]. Geophagic clay which is the xenobiotic used in this study, is composed majorly of metal elements [16, 17, 18, 19]. Some of these elements have been reported to elicit some level of toxicity in exposed organisms [30, 31]. The liver being the first point of detoxification is highly susceptible to possible attacks from components of this clay material [9].

3.2. Effects of geophagic clay consumption on superoxide dismutase (SOD) in the Liver of adult female Wistar rats

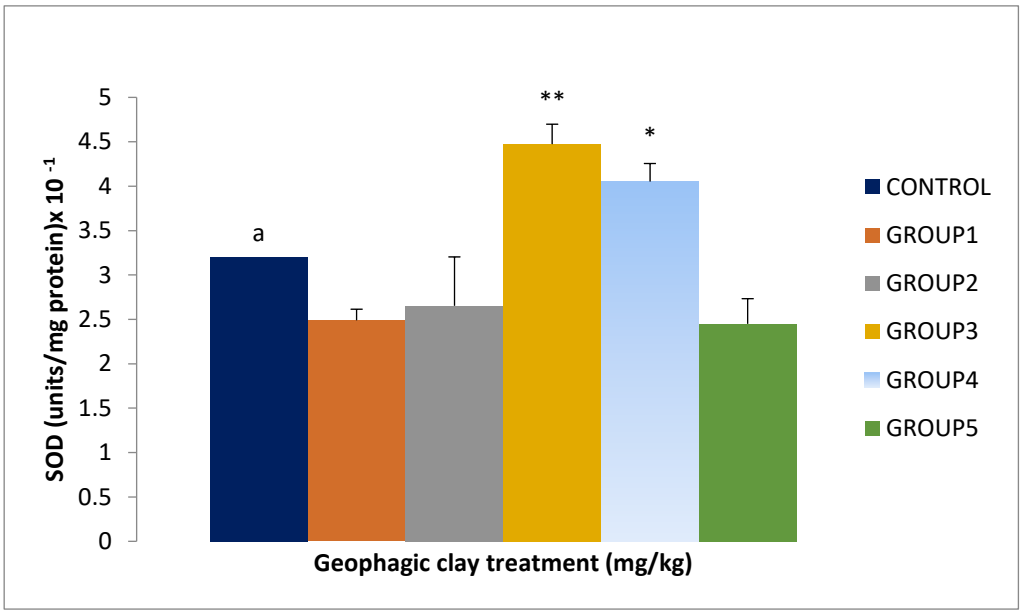

Figure 1: Effects of geophagic clay consumption on SOD activity in Liver of adult female Wistar rats.

Data were presented as Mean ± SEM (n=6); *Significant as compared with control; ($P < 0.05$), **More significant; ($P < 0.05$).

The liver is a major organ susceptible to attack by reactive oxygen species [32]. Increased production of ROS triggers the release of cytokines, which can lead to inflammation and initiate varying disease pathways associated with the liver [33]. SOD is an enzyme that is activated in response to increased production of radicals. It serves as a first line of defence in response to the upsurge in radicals as a result of exposure to xenobiotics [34]. An increase in the activity of SOD enzyme is an indication of oxidative stress. The results shown in figure one revealed that there was an increase in the activity of SOD enzyme in groups 3 and 4 which were administered 1000 and 1500mg/kg of geophagic clay. A decline in the activity of this enzyme in the group administered 2000mg/kg of geophagic clay may be as a result of a depletion in the enzyme concentration as it is being used up in response to increased free radical production. Some other publications have also reported similar patterns in the activity of SOD on exposure to toxicants when treatments were compared with the control group [35, 36]. A contrary result was presented in the study by [37] in

which concentrations of SOD remained unaltered when modified-geophagic clays at concentrations between 250-1000 mg/kg bw were administered to Wistar rats. The differences seen may be attributed to the modifications made in their studied clay. Though in our study we observed a similar trend in the groups administered lower concentrations (250- 500 mg/kg bw) of the Ubiaja geophagic clay.

3.3.Effects of geophagic clay consumption on catalase (CAT) activity in Liver of adult female Wistar rats.

The activity of catalase enzyme can be described as one that acts as a second line of defense against the production of free radicals. Its activity is somewhat supplementary to that of the glutathione peroxidase enzyme [38]. In this study catalase activity was elevated significantly in the group administered 1000 mg/kg bw of geophagic clay. This may be an adaptive response to the generation of free radicals which may be produced by the enzyme SOD in acting as a first line of defense. The presence of some heavy metals in the clay may be responsible for the free radical generation [30]. Although there was a slight elevation in catalase activity in group 4 (1500 mg/kg), it was not significant when the treatment group was compared with the control. The decrease in catalase activity seen in the treatment group administered the highest dose may be the reason for increased oxidative damage as seen in the liver histology (Plate 1), as reduction in the activity of catalase enzyme will cause an accumulation of H_2O_2 in the system accounting for more oxidative stress and lipid peroxidation. This result is similar to results of exposure to heavy metals in which a decrease in catalase and superoxide dismutase activity at higher exposure to heavy metals was reported [39]. The significant increase seen in the group administered 1000 mg/kg of geophagic clay is similar to the results of [31] who reported significant increase in catalase activity in rats fed 1.0% and 8.0% clay compared to control (0 clay), thus signifying an oxidative response.

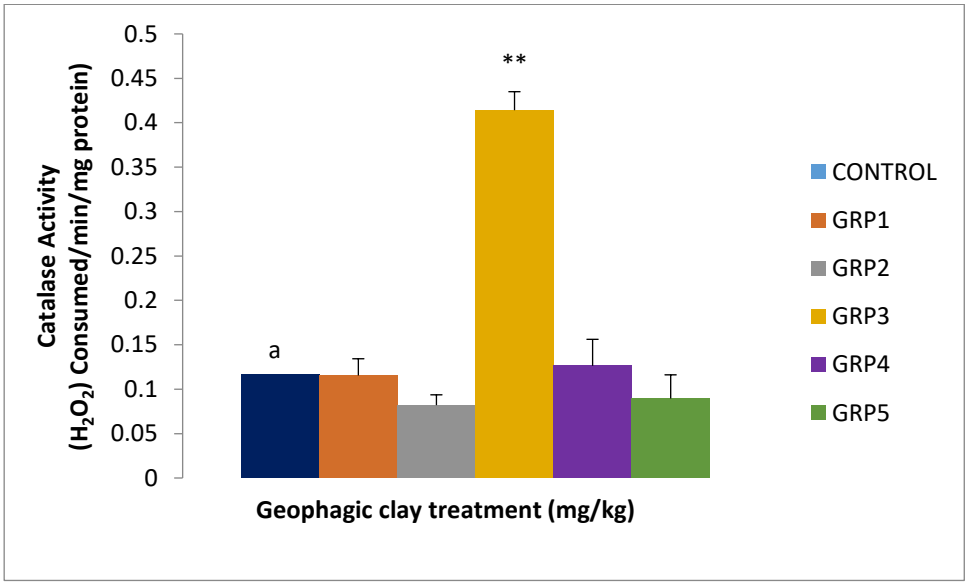

Figure 2: Effects of geophagic clay consumption on CAT activity in Liver of adult female Wistar rats.

Data were presented as Mean ± SEM (n=6); *Significant as compared with control; P<0.05, **More significant; p<0.05.

3.4. Effects of geophagic clay consumption on glutathione peroxidase (GPx) activity in Liver of adult female Wistar rats.

Glutathione peroxidase-1 (GPx-1) is an essential intracellular antioxidant enzyme responsible for enzymatically converting hydrogen peroxide into its less harmful form, water, thereby maintaining

oxidative balance [13]. The study results indicate a significant increase (P<0.05) in GPx enzyme activity in group 3 (1500 mg/kg bw) compared to the control group. However, in the groups exposed to higher doses of geophagic clay, a decrease in GPx activity was observed, but this decrease was not statistically significant (P>0.05) when compared to the control group.
The reduction in GPx activity among the groups exposed to higher doses of geophagic clay suggests potential risk, as there might be an inability to effectively break down hydrogen peroxide into its less toxic form (H_2O), leading to its accumulation in the system. Consequently, this accumulation could initiate damage to macromolecular structures within the liver cells. Previous studies have reported increased GPx activity in organisms exposed to high concentrations of toxic substances, but a reduction in the enzyme activity was observed at much higher doses [40]. In a similar study GPx activity was said to be unaltered on administration of nanoclay materials to Wister rats [41]. This finding is similar to that seen in our study in the groups administered lower concentrations of geophagic clay (250-500 mg/kg bw).

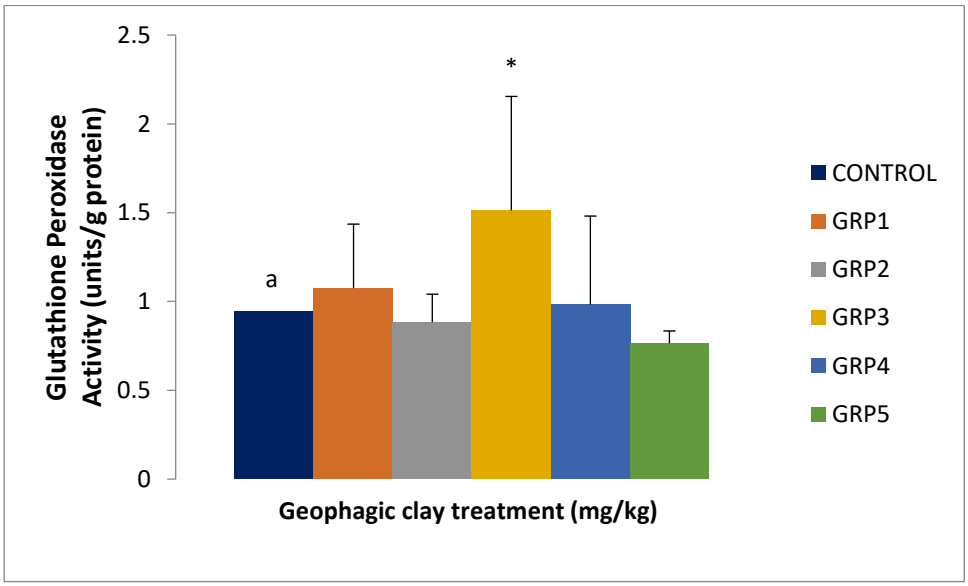

Figure 3: Effects of geophagic clay consumption on GPx activity in Liver of adult female Wistar rats. Data are presented as Mean ± SEM (n=6); *Significant as compared with control; p<0.05, **More significant; p<0.05.

3.5. Effects of geophagic clay consumption on Reduced Glutathione (GSH) levels in Liver of adult female Wistar rats.

Reactive oxygen species which can be generated on exposure to foreign substances need to be mopped up from the system [42]. One of the most powerful antioxidant present in the human system for this task is the reduced glutathione (GSH) [43]. In its reduced form, it has the ability to donate reducing equivalents which emanates from the thiol groups present in the cysteine moiety of its structure, this donation confers stability on the reactivity of generated free radicals such as H_2O_2 [44]. This it does with the help of the antioxidant enzyme GPx, leading to the formation of oxidized glutathione (GSSG). A depletion in the concentration of GSH with increased formation of GSSG is indicative of oxidative stress [45]. Results from this study indicated a reduction in the concentration of GSH, in a dose dependent manner. The observed depletion was significant (P<0.05) in groups 3 (1000 mg/kg bw) and 4 (1500 mg/kg bw). This result indicated that GSH content of the liver was being used up in response to the free radical generation on exposure to geophagic clay. Similarly, a depletion in the concentration of GSH in testis of rats exposed to cadmium was reported by [46] in their study. The implication of this outcome suggests that exposure to geophagic clay may illicit the generation of free radicals which has the ability to deplete GSH concentrations in liver cells.

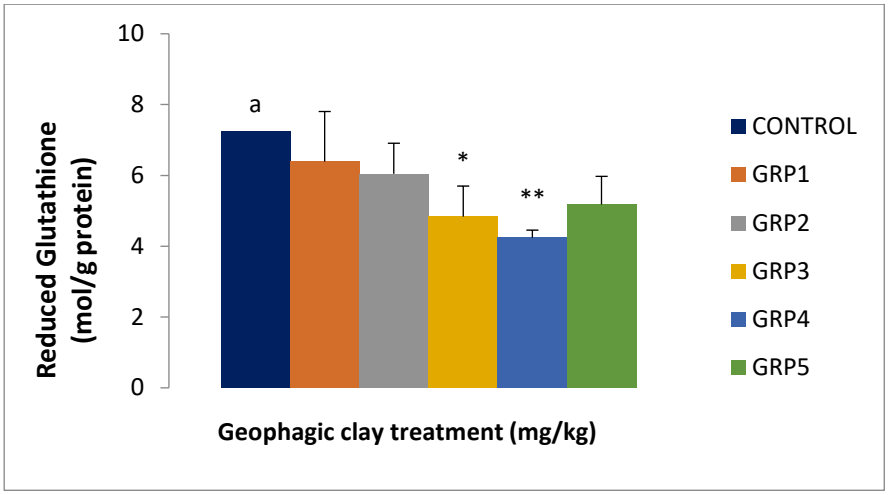

Figure 4: Effects of geophagic clay consumption on GSH activity in Liver of adult female wistar rats.
Data are presented as Mean ± SEM (n=6); *Significant as compared with control; $p<0.05$, **More significant; $p<0.05$.

3.6. Effects of geophagic clay consumption on Malondialdehyde levels in Liver of adult female Wistar rats.

The substance Malondialdehyde (MDA) is a result of lipid peroxidation and is known to contribute to cellular toxicity, commonly serving as a biomarker for oxidative stress [47]. In this study, there was an increase in the levels of MDA with higher concentrations of geophagic clay exposure. MDA is associated with lipid peroxidation, thus, a rise in MDA levels indicates an attack on the liver's lipid membranes, leading to peroxidation of the cellular lipid membrane. The increase in MDA concentration was found to be statistically significant ($p<0.05$) in groups 3 (1000 mg/kg bw), 4 (1500 mg/kg bw), and 5 (2000 mg/kg bw). Similarly, Agomuo *et al*. 2019 in their study reported, slight elevations in MDA levels during pregnancy in Wistar rats which were greatly elevated with clay beverage intake [50]. Similar trends of increased MDA levels have been observed in other studies involving exposure to specific toxicants [48, 46, 49]. Based on these findings, the present study suggests that exposure to geophagic clay may trigger oxidative responses in the liver, which could have detrimental effects on its health.

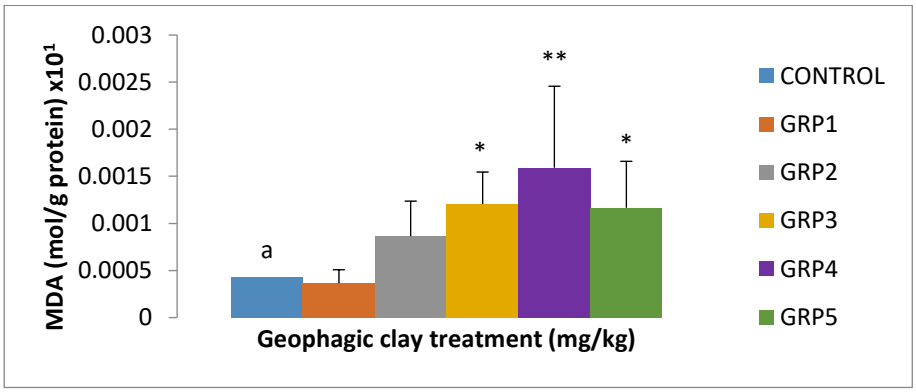

Figure 5: Effects of geophagic clay consumption on MDA levels in liver of adult female Wistar rats. Data are presented as Mean ± SEM (n=6); *Significant as compared with control; $p<0.05$, **More significant; $p<0.05$.

3.7. Photomicrographs of Haematoxylin and Eosin stained Liver sections exposed to geophagic clay at different concentrations

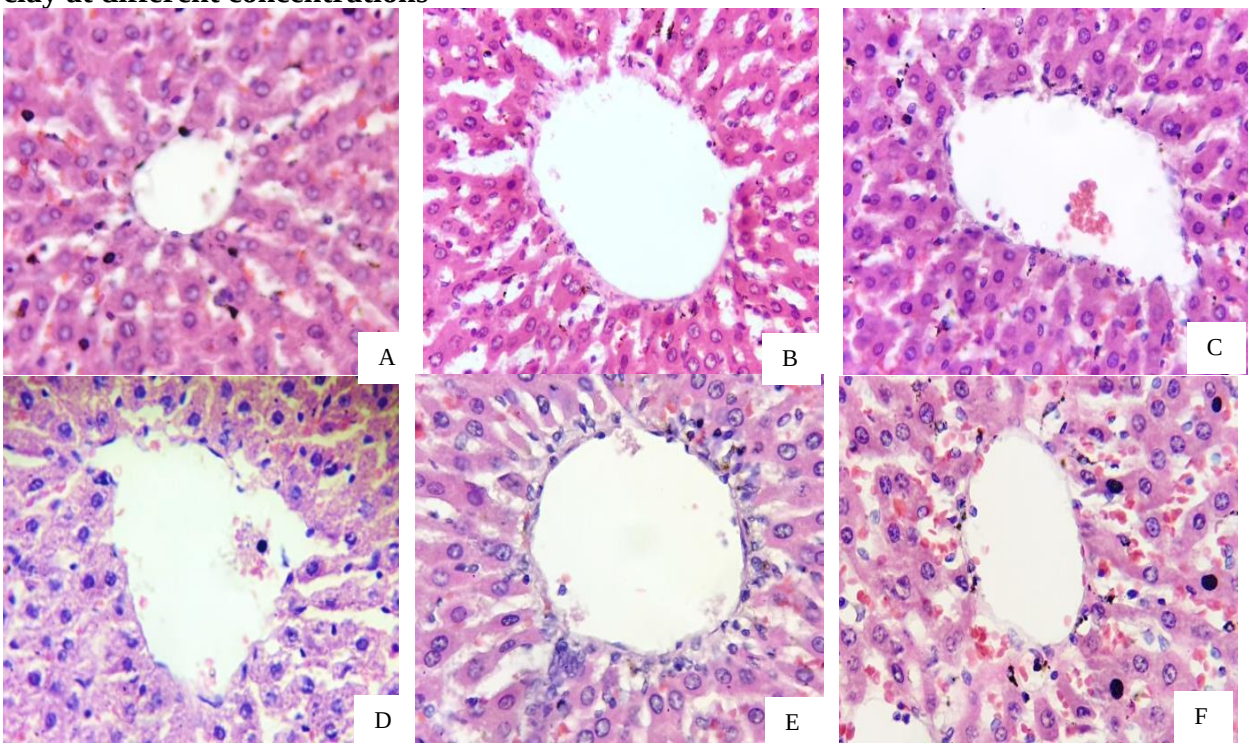

Plate 1 (A-F): Photomicrographs of H&E stained histological slides of the liver exposed to different concentrations of geophagic clay. **A) CONTROL**: Normal histological appearance of liver in the control group with a well fenestrated sinusoid. **B) 250 mg/kg**: Destructed portal trial and disarranged hepatocytes of liver parenchyma**. C) 500 mg/kg**: destructed portal trial, distorted sinusoids, slight congestion in portal triad. **D) 1000 mg/kg**: distorted portal trial and disarranged hepatocytes of liver parenchyma **E) 1500 mg/kg**: Wall of portal triad thickened and enlarged, with mild steatosis **F) 2000 mg/kg**: Appearance of focal cellular degeneration, distorted and congested sinusoids, and steatosis.

Accompanying elevated MDA levels, photomicrographs of the hematoxylin and eosin stained liver sections revealed cellular degeneration and steatosis in the groups administered higher concentrations of the geophagic clay, D-F (1000-2000 mg/kg bw). In groups B and C (250-500 mg/kg bw), the changes seen in the liver architecture were mild when compared with the control (A) group. This is indicative of damage to the cells of the liver as there were visible alterations in the architectural make-up of the liver tissue, different from the normal liver architecture in the control organisms. Nwauche *et al*. (2021) had similar results as they reported mild periportal and intraparenchymal inflammation in their treated rats fed edible clay at the highest dose administered (8.0%) (31). Similarly, Mahajan *et al*. (2018), reported alterations in the hepatic cells in imidacloprid and arsenic exposed Wistar rats [51]. This is a clear indication of the damage done on exposure to their administered toxicant. Putting our findings together, the results suggests that exposure to Ubiaja geophagic clay may illicit possible toxicity in the liver via the oxidative stress mechanism. Consequently the effects seen in this study can be traceable to the elemental makeup of the geophagic clay [52].

4.0. Conclusion

Overall, this study highlighted that exposure to geophagic clay from Ubiaja in Edo, State, may illicit some toxic responses in an oxidative inducing manner, by increasing concentrations of MDA and depletion in GSH concentration. These are causative factors for oxidative stress and puts strain on

the enzymatic antioxidants especially on exposure to the geophagic clay at high concentrations. Some of the responses seen are an adaptive response, on exposure to this geophagic material. In view of this, attention should be drawn to undue consumption of this clay material as it may have debilitating effects on the liver of exposed individuals.

Acknowledgements
The contributions of Mr Akpeh, Chidinma, Judith, Jemilat, Esther, all of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, is well appreciated.

References

[1] Schnitzler, E (2022). The neurology and psychopathology of pica. *Current Neurology and Neuroscience Reports*. 22(8): 531-536.
[2] Kambunga, SN; Candeias, C; Hasheela, I; Mouri, H (2019). Review of the nature of some geophagic materials and their potential health effects on pregnant women: some examples from Africa. *Environmental geochemistry and health*. 41: 2949-2975.
[3] Bonglaisin, JN; Kunsoan, NB; Bonny, P; Matchawe, C; Tata, BN; Nkeunen, G; Mbofung, CM (2022). Geophagia: Benefits and potential toxicity to human—A review. *Frontiers in Public Health*. 10(893831): 1-9.
[4] Malepe, RE; Candeias, C; Mouri, H (2023). Geophagy and its potential human health implications-A review of some cases from South Africa. *Journal of African Earth Sciences*. 1: 104848- 104858.
[5] Kortei, NK; Koryo-Dabrah, A; Akonor, PT; Manaphraim, NYB; Ayim-Akonor, M; Boadi, NO; Essuman, EK; Tettey, C (2020). Potential health risk assessment of toxic metals contamination in clay eaten as pica (geophagia) among pregnant women of Ho in the Volta Region of Ghana. *BMC pregnancy and childbirth*. 20(1): 1-7.
[6] Huebl, L; Leick, S; Guettl, L; Akello, G; Kutalek, R (2016). Geophagy in Northern Uganda: Perspectives from consumers and clinicians. *The American Journal of Tropical Medicine and Hygiene*. 95(6): 1440-1450.
[7] Edene, OA; Aghedo, ON (2023). Elemental composition and physicochemical properties of geophagic clay (‘eko’), from Ubiaja in Edo State, Nigeria. *Open Journal of Environmental Research*, 4(1), 1-12. doi.org/10.52417/ojer.v4i1.483.
[8] Liu, S; Xi, B; Chen, L; Zhang, M; Sun, J (2020). Heavy metal exposure and metabolic syndrome: A systematic review and meta-analysis of prospective studies. *Frontiers in Endocrinology,* 11(1): 65-74. doi: 10.3389/fendo.2020.00065.
[9] Bonglaisin, JN; Kunsoan, NB; Bonny, P; Matchawe, C; Tata, BN; Nkeunen. G; Mbofung, CM (2022). Geophagia: Benefits and potential toxicity to human—A review. *Frontiers in Public Health*. 10: 893831.
[10] Nsawir, BJ (2015). Induced geophagy with local kaolin from Cameroon market and heavy metals (lead, cadmium and mercury) profile of rat blood, liver, placentas and litters. *Journal of Medical Sciences*. 15(1): 10.
[11] Anvarjonovich, KM; Anvarovna, YI; Azamatovna, AF (2023). Liver biochemistry; Neutralization of toxic substances in the body. The role of the liver in metabolism. *Science and Innovation*. 2(6): 97-100.
[12] He, L; He, T; Farrar, S; Ji, L; Liu, T; Ma, X (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cellular Physiology and Biochemistry*. 44(2): 532-553.
[13] Li, S; Tan, HY; Wang, N; Zhang, ZJ; Lao, L; Wong, CW; Feng, Y (2015). The role of oxidative stress and antioxidants in liver diseases. *International journal of molecular sciences*. 16(11): 26087-26124.
[14] Sharifi-Rad, M; Anil Kumar, NV; Zucca, P; Varoni, EM; Dini, L; Panzarini, E; Rajkovic, J; Tsouh Fokou, PV; Azzini, E; Peluso, I; Prakash Mishra, A (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*. 11: 694.
[15] Targher, G; Byrne, CD (2017). Non-alcoholic fatty liver disease: an emerging driving force in chronic kidney disease. *Nature Reviews Nephrology*. 13(5): 297-310.
[16] Lar, UA; Agene, JI; Umar, AI (2015). Geophagic clay materials from Nigeria: a potential source of heavy metals and human health implications in mostly women and children who practice it. *Environmental geochemistry and health*. 37: 363-375.
[17] Oyebanjo, O; Ekosse, GI; Odiyo, J. (2020). Health risk evaluation of trace elements in geophagic kaolinitic clays within Eastern Dahomey and Niger Delta Basins, Nigeria. *International Journal of Environmental Research and Public Health*. 17(13): 4813.
[18] Eigbike, CO; Imasuen, IO; Obomese, F; Omoruyi, DI (2022). Assessing the bioavailability of some trace and major elements in geophagical clays of south-western and eastern Nigeria: An invitro study. *International Journal of Earth Sciences Knowledge and Applications*. 4(1): 106-115.
[19] Olajide-Kayode, JO; Kolawole, TO; Oyaniran, OO; Mustapha, SO; Olatunji, AS (2023). Potentially Harmful Element toxicity in Geophagic clays consumed in parts of southeastern Nigeria. *Journal of Trace Elements and Minerals*. 4: 100050.

[20] Bancroft, JD; Gamble, M (2008). *Theory and practice of histological techniques*, 6th edn. Churchill Livingstone, London.
[21] Misra, HP; Fridovich, I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*. 247(10): 3170-3175.
[22] Cohen, G; Dembiec, D; and Marcus, J (1970). Measurement of catalase activity in tissue extracts. *Analytical biochemistry*. 34(1): 30-38.
[23] Rotruck, JT; Pope, AL; Ganther, HE; Swanson, AB; Hafeman, DG; Hoekstra, W (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science*. 179(4073): 588-590.
[24] Ellman, GL (1959). Tissue sulfhydryl groups. *Archives of biochemistry and biophysics*. 82(1): 70-77.
[25] Buege, JA; Aust, SD (1978). Microsomal lipid peroxidation. In *Methods in enzymology*, *Academic press.* 52: 302-310.
[26] Barnes, PJ (2020). Oxidative stress-based therapeutics in COPD. *Redox biology*. 33(101544): 1-8.
[27] Sachdev, S; Ansari, SA; Ansari, MI; Fujita, M; Hasanuzzaman, M (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. *Antioxidants*. 10(2): 277.
[28] Eddaikra, A; Eddaikra, N (2021). Endogenous enzymatic antioxidant defense and pathologies. Antioxidants-benefits, sources, mechanisms of action. *IntechOpen*.1-17.
[29] Sies, H; Belousov, VV; Chandel, NS; Davies, M.J; Jones, DP; Mann, GE; Murphy, MP; Yamamoto, M; Winterbourn, C (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature Reviews Molecular Cell Biology*, *23*(7): 499-515.
[30] Nabuuma, J (2021). *Health risks associated with heavy metal exposure in geophagic products (Emumbwa) consumed during pregnancy* (Doctoral dissertation, Kyambogo University).
[31] Nwauche, KT; Okwudike, AE; Dokubo, A (2021). Changes in Antioxidant Markers and Histopathology of Wistar Rats Fed with Edible Clay (Nzu). *Journal of Applied Life Sciences International*, 24(1): 16-20.
[32] Gómez, X; Sanon, S; Zambrano, K; Asquel, S; Bassantes, M; Morales, JE; Caicedo, A (2021). Key points for the development of antioxidant cocktails to prevent cellular stress and damage caused by reactive oxygen species (ROS) during manned space missions. *npj Microgravity*. *7*(1): 35.
[33] Chen, L; Deng, H; Cui, H; Fang, J; Zuo, Z; Deng, J; Li, Y; Wang, X; Zhao, L (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 9(6): 7204.
[34] Parlak, V. (2018). Evaluation of apoptosis, oxidative stress responses, AChE activity and body malformations in zebrafish (Danio rerio) embryos exposed to deltamethrin. *Chemosphere*. 207: 397-403.
[35] da Silva, BP; Toledo, RCL; Mishima, MDV; de Castro Moreira, ME; Vasconcelos, CM; Pereira, CER; Favarato, LSC; Costa, NMB; Martino, HSD (2019). Effects of chia (Salvia hispanica L.) on oxidative stress and inflammation in ovariectomized adult female Wistar rats. *Food & Function*. 10(7): 4036-4045.
[36] Ogueji, E; Nwani, C; Mbah, C; Iheanacho, S; Nweke, F (2020). Oxidative stress, biochemical, lipid peroxidation, and antioxidant responses in Clarias gariepinus exposed to acute concentrations of ivermectin. *Environmental Science and Pollution Research*. 27(14): 16806-16815.
[37] Maisanaba, S; Puerto, M; Gutierrez-Praena, D; Llana-Ruiz-Cabello, M; Pichardo, S; Mate, A; Jorda-Beneyto, M; Camean, AM; Aucejo, S; Jos, A (2014). In vivo evaluation of activities and expression of antioxidant enzymes in Wistar rats exposed for 90 days to a modified clay. *Journal of Toxicology and Environmental Health, Part A*. 77(8): 456-466.
[38] Tehrani, HS; Moosavi-Movahedi, AA (2018). Catalase and its mysteries. *Progress in Biophysics and Molecular Biology*. 140: 5-12.
[39] Rehman, T; Naz, S; Hussain, R; Manan Mustafa Chatha, A; Ahmad, F; Yamin, A; Akram, R; Naz, H; Shaheen, A (2021). Exposure to heavy metals causes histopathological changes and alters antioxidant enzymes in fresh water fish (Oreochromis niloticus). *Asian journal of agriculture and biology*. 1(3): 23-32.
[40] Sobeková A; Holovská K; Lenártová V; Legáth J; Javorskỳ P (2009). The alteration of glutathione peroxidase activity in rat organs after lead exposure. *Acta Physiol Hung*. 96(1): 37-44.
[41] Gmoshinski, IV; Bagryantseva, OV; Arnautov, OV; Khotimchenko, SA (2020). Nanoclays in food products: Benefits and possible risks (literature review). *Health Risk Analysis*. 2(1): 142-164.
[42] Bhattacharya, S. (2015). Reactive oxygen species and cellular defense system. *Free radicals in human health and disease*. 1: 17-29.
[43] Braidy, N; Zarka, M; Jugder, BE; Welch, J; Jayasena, T; Chan, DK; Sachdev, P; Bridge, W (2019). The precursor to glutathione (GSH), γ-Glutamylcysteine (GGC), can ameliorate oxidative damage and neuroinflammation induced by Aβ40 oligomers in human astrocytes. *Frontiers in aging neuroscience.*11:177-185.
[44] Shah, AA, Gupta, A. (2020). Antioxidants in health and disease with their capability to defend pathogens that attack apple species of Kashmir. *Plant Antioxidants and Health*. 3(2): 1-26.
[45] Zhu, Y; Wu, J; Wang, K; Xu, H; Qu, M; Gao, Z; Guo, L; Xie, J (2021). Facile and sensitive measurement of GSH/GSSG in cells by surface-enhanced Raman spectroscopy. *Talanta*. 224: 121852.

[46] Bhardwaj, JK; Panchal, H; Saraf, P (2021). Cadmium as a testicular toxicant: A Review. *Journal of Applied Toxicology*. 41(1): 105-117.
[47] Harliansyah, H; Rahmah, NA; Kuslestari, K (2021). α-Mangosteen as An Oxidative Inhibitor in Hepatocellular Carcinoma. *Indonesian Journal of Cancer Chemoprevention*. 12(2): 106-113.
[48] Ogunka-Nnoka, C; Assor, K; Onuoha, S; Amadi, P (2018). A study of the toxicants and biomarkers of oxidative stress in samples from ebubu and elele-alimini communities in rivers state. *Ovidius university Annals of chemistry*. 29(1):1-7.
[49] Saka, WA; Akhigbe, RE; Abidoye, AO; Dare, OS; Adekunle, AO (2021). Suppression of uric acid generation and blockade of glutathione dysregulation by L-arginine ameliorates dichlorvos-induced oxidative hepatorenal damage in rats. *Biomedicine & Pharmacotherapy*. 138: 111443.
[50] Agomuo, EN; Amadi, PU; Adumekwe, C (2019). Gestational geophagia affects nephrocardiac integrity, ATP-driven proton pumps, the renin–angiotensin–aldosterone system, and F2-isoprostane status. *Medical Sciences*. 7(2): 13-20.
[51] Mahajan, L; Verma, PK; Raina, R; Sood, S (2018). Toxic effects of imidacloprid combined with arsenic: Oxidative stress in rat liver. *Toxicology and industrial health*. 34(10): 726-735.
[52] Okunlola, OA; Owoyemi, KA (2015). Compositional characteristics of geophagic clays in parts of Southern Nigeria. *Earth Science Research*. 4(2): 1-12.