



Changes in Antioxidant Markers and Histopathology of Wistar Rats Fed with Edible Clay (Nzu)

A. Dokubo^{1*}, A. E. Okwudike¹ and K. T. Nwauche²

¹*Department of Biochemistry, Rivers State University, Nkpolu, Nigeria.*

²*Department of Biochemistry, Rhema University, Abia State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author AD designed the work and developed the first draft. Authors AEO and KTN managed the analysis of data from the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2021/v24i130216

Editor(s):

(1) Dr. Palanisamy Arulselvan, Institute of Bioscience, Universiti Putra Malaysia, Malaysia.

Reviewers:

(1) Hassan Abdulsalam, University of Maiduguri, Nigeria.

(2) Maha Zaki Rizk, National Research Center, Egypt.

(3) Evelyn Sharon Sukumaran, SRM College of Pharmacy, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/52617>

Original Research Article

Received 15 September 2019

Accepted 19 November 2019

Published 23 February 2021

ABSTRACT

The changes in the antioxidant markers and histopathology in both adult male and female wistar rats fed with Calabash Chalk (nzu) was investigated. Twenty (20) wistar rats weighing between (120-150 g) were used for this study. They were randomly divided into four (4) Groups containing five (5) animals each (n=5) A-D. Groups (B-D) were fed with 1.0%, 2.0% and 8.0% of clay. Group A, not fed with clay served as control. After 21 days of continuous feeding, the animals were sacrificed and their liver organs excised for the following antioxidant markers (Catalase CAT, Reduced Glutathione GSH, Superoxide dismutase, SOD) and histopathology. The results showed significant ($p < 0.05$) increase in catalase activity in rats fed with 1.0% and 8.0% clay compared to control (0 clay) and group fed with 2.0%. However, no significant ($p > 0.05$) difference was obtained for the group fed with 2.0% when compared to control. The results obtained for GSH and SOD also showed no significant ($p > 0.05$) difference in the fed groups when compared to control. Histopathological changes indicated mild periportal and intraparenchymal inflammation in group fed with 8.0%. From the study it can be deduced that clay consumption has the potential to elicit the activities of antioxidant markers and subsequent depletion providing weak defenses against reactive oxygen species (ROS) and liver damage in the rats.

*Corresponding author: E-mail: dokubo.awolayefori@ust.edu.ng;

Keywords: Calabash chalk; nzu, edible clay; antioxidant markers; activity; histopathology.

1. INTRODUCTION

The consumption of earth material also known as geophagia is common practice in many parts of the world [1]. In most African countries including Nigeria, calabash chalk popularly known as Nzu is one of the most commonly consumed geophagia material. It is heated and molded into blocks or pellets of different sizes and sold in many markets in Nigeria [2]. Multi elemental analysis of this product using Energy Dispersive, X ray and fluorescence spectroscopy has shown the presence of heavy metals like Al, Pb, and Ni [3,4]. Persistent organic pollutants include alpha Lindane, endrin, endosulphan II, dichloro diphenyl ethane [5]. Many people believed that consumption of clay aid in weight reduction, act as anti-diarrhea agent, soothing agent and remedy for morning sickness [6]. This study seeks to investigate changes in antioxidant markers and histopathology in wistar rats fed with varying percentages of edible clay (nzu)with feeds.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Dry edible clay (nzu) pellets were purchased from traders in Mile 1 market, Port Harcourt, Rivers State, Nigeria.

2.2 Experimental Animals

Wistar rats weighing between (120-150 g) were used for this study. They were obtained from the animal housed of University of Port Harcourt and allowed to acclimatize under laboratory conditions prior to conduct of experiment for two weeks. The rats were randomly assigned into different cages with five (5) animals in each (n=5). Food and water were given *ad libitum*.

2.3 Feeding Dose Formulation

Dry nzu pellets were turned into powder form using mortar and pestle and were fed to the animals. The average daily intake of feeds was maintained at 15 g/ day [7]. An adult human weighing 60 kg consume 70 g, 120 g and 500 g as the minimum, modal and maximum intake of clay per day. Then the minimum, medium and maximum equivalent amount by the average weight of rats (0.128 kg) was determined by using the following formula:

$$\frac{\text{Minimum, Medium and Maximum Intake of Clay by Rat}}{\text{Average rat weight}} = \frac{\text{Average human weight}}{\text{minimum, medium and maximum intake of clay}}$$

The percentage of clay powder added to the portion of rat feeds is summarized in Table 1. The animals were fed for approximately 21 days which is equivalent to clay eating by humans for a period of one year (365 days) assuming that life expectancies for rats and humans are equivalent. The life expectancy is estimated as three (3) years or 1, 095 days for rats [7] while for humans is estimated as 52.2 years (19,053 days) according to The National Population Commission [8]. Therefore, clay consumption by humans in one year would be equivalent to clay consumption of rats in approximately 21 days.

2.4 Collection of Blood and Liver Samples

The rats were fasted overnight and sacrificed under light chloroform anesthesia. Blood samples were collected via cardiac puncture and transferred to the appropriate sample bottles. They were then centrifuged at 1000 rpm for 15 min after which the serum formed was collected for antioxidant assay. Liver samples were also collected transferred to bottles containing 10% formalin and taken to the laboratory for biochemical analysis.

2.5 Determination of Antioxidant Markers

Catalase (CAT) was determined by the method of Shina [9], Reduced Glutathione (GSH) was determined using Ellman's reagent [10] and Superoxide Dismutase (SOD) was determined by the auto-oxidation method of pyrogallol described by Marklund and Marklund [11].

2.6 Histopathological Examination

The liver samples excised and preserved in 10% formalin solution. They were processed and embedded in paraffin wax. Sections 4-6 microns were made and stained with hematoxylin / eosin and photomicrographs were made.

2.7 Statistical Analysis of Data

Data obtained from this study were expressed as mean ± S.E.M. One-way analysis of the variance

Table 1. Summary of proportions of nzu added to rats' feeds

Groups	Clay Intake	Amount of rat Feed (g)	Amount of Clay (g)	% of Clay
A	Control	15.00	0.00	0.00
B	Minimum	14.85	0.15	1.00
C	Medium	14.70	0.30	2.00
D	Maximum	13.80	1.20	8.00

(ANOVA) and Turkey post hoc test was used for the establishment of significance differences set at ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Results

The results obtained for the antioxidant markers in the fed rats is presented in Fig. 1. The results showed that there was significant ($p < 0.05$) increase in catalase activity in rats fed with 1.0% and 8.0% clay compared to control (0 clay) and group fed with 2.0%. However, no significant ($p > 0.05$) difference was obtained for the group fed with 2.0% when compared to control. The results obtained for GSH and SOD also showed

no significant ($p > 0.05$) difference in the fed groups when compared to control. Histopathological changes indicated mild periportal and intraparenchymal inflammation in group fed with 8.0% as shown in Fig. 2.

3.2 Discussion

Edible clay had been reported to contain pathogenic microorganisms and dangerous substances that have been implicated in liver damage and generation of reactive oxygen species [4]. Antioxidant enzymes like SOD, CAT and GSH usually provide chief defense against oxidative stress and reactive oxygen species (ROS) [12]. The activities of these enzymes may

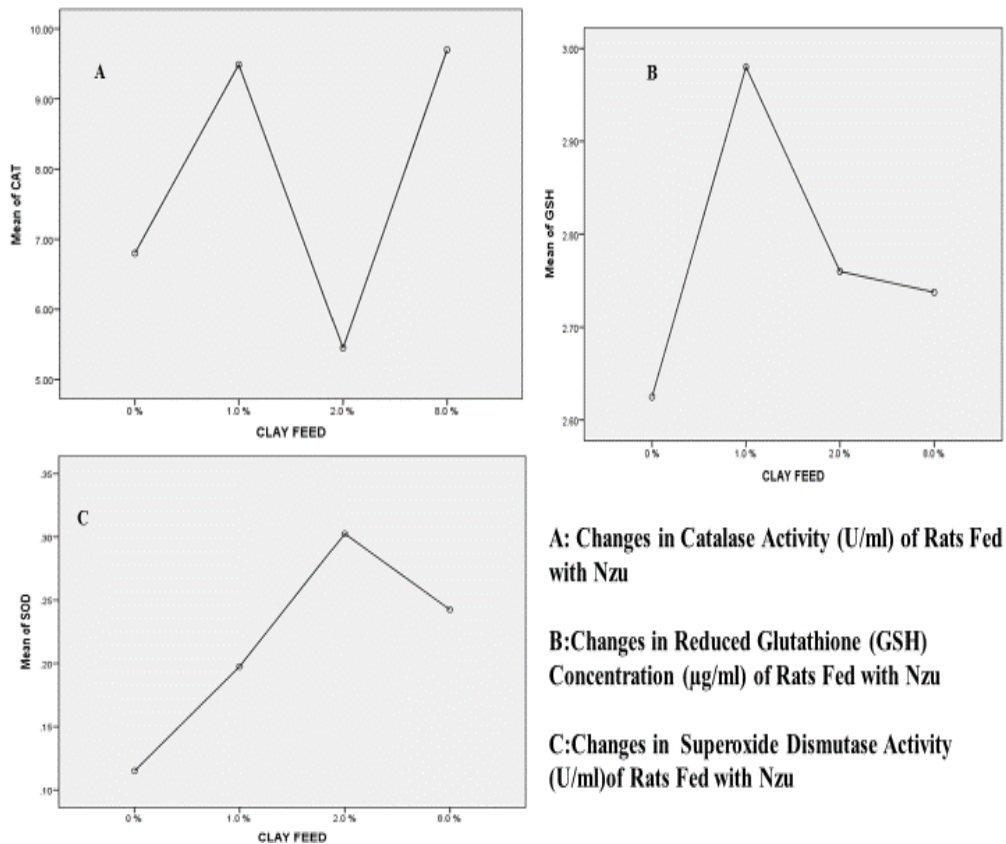


Fig. 1. Changes in antioxidant markers of rat feed fed with varying percentage of nzu
 A: (Control group) 0% clay, B: Fed 1% clay, C: fed 2% clay, D: fed 8% clay

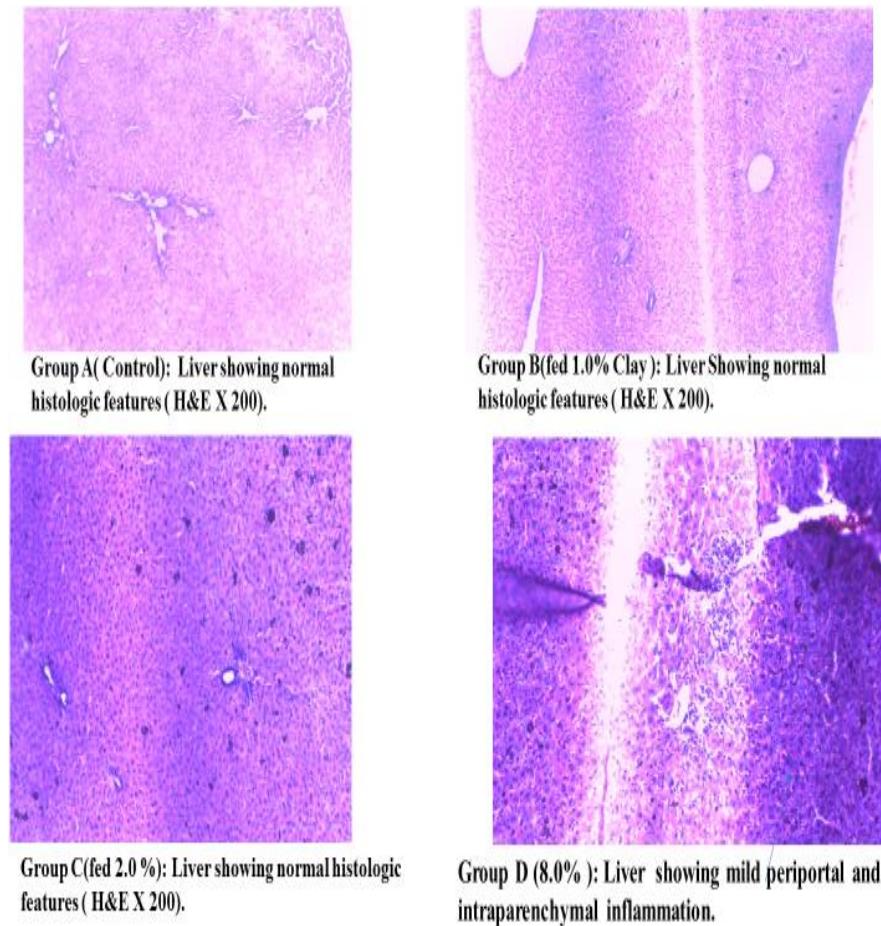


Fig. 2. Histological changes in liver sections of rats fed with varying percentage of nzu

increase in respect to the time and level of exposure to substances that cause toxicity in many organisms [13]. From this study, significant increase in catalase activity was observed. This may be due to detoxification of nzu consumed by rats. Increase in catalase activity may result to depletion of the enzyme and provide weak defense against reactive oxygen species generated during metabolism. The non-significant effect on GSH and SOD may be due duration of feeding of nzu. The results also revealed mild periportal and intraparenchymal inflammation in the liver section of rats feed with 8% nzu. Studies conducted with the clay on adult wistar rats have also revealed fragmentation of the parenchymal cells and dilation of sinusoids of the liver sections [4].

4. CONCLUSION

From the study it can be deduced that clay consumption has the potential to elicit the

activities of antioxidant markers providing weak defenses against reactive oxygen species (ROS) and liver damage in the rats.

ETHICAL APPROVAL

The experiment was conducted according to the ethical guidelines for the use of laboratory animals of Rivers State University Port Harcourt, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Agomuo EN, Amadi PU, Adumekwe C. Gestational geophagia affects nephrocardiac Integrity, ATP-driven proton pumps, the Renin–Angiotensin–

- Aldosterone system, and F2-Isoprostane status. Medical Sciences. 2019;7(2):13.
2. Ikewuchi CC, Ikewuchi JC, Ezeka UK, Ifeanacho MO. Effect of “edible clay” (takere) suspension on serum lipid profiles and atherogenic indices of normal Wistar rats. Food science & nutrition. 2019; 7(3):977-86.
 3. Dean JR, Deary ME, Gbafa BK, Scott WC. Characterisation and analysis of persistent organic pollutants and major, minor and trace elements in Calabash chalk. Chemosphere. 2004;57(1):21-5.
 4. Ekong MB, Akpantah AO, Ibok OS, Eluwa MA, Ekanem TB. Differentia effect of calabash chalk on the histology of the liver of adult Wistar rats. Int J Health. 2009; 8:2.
 5. Ogbuagu DH, Nwachukwu IN, Nwazuluahu AC, Kalakiya NP. Haematotoxicological and hepatotoxic inductions of a geophagic substance (Calabash chalk) on the albino rat (*Rattus norvegicus*). Journal of Environmental Toxicology and Public Health. 2017;2:14-26.
 6. Aprioku, JS, Ogwo-Ude EM. Gestational toxicity of Calabash chalk (Nzu) in Wistar rats. International Journal of Applied and Basic Medical Research. 2018;8(4):249-252.
 7. Sofo GA. A Study of the Effects of Clay Eating on Iron Status and Growth Rate Using Rats (Doctoral dissertation, University of Ghana).
 8. The National Population Commission (NPC) 2019.
 9. Sinha AK. Colorimetric assay of catalase. Analytical biochemistry. 1972;47(2):389-94
 10. Ellman GL. Tissue sulphhydryl groups. Archives of Biochemistry & Biophysics. 1959; 82:70-77.
 11. Marklund SL and Marklund G. Involvement of superoxide anion radical in the auto-oxidation of pyrogallol and convenient assay for superoxide dismutase. European Journal of Biochemistry. 1974;47:469.
 12. He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cellular Physiology and Biochemistry. 2017;44(2):532-53.
 13. Yan S, Wang J, Zhu L, Chen A, Wang J. Toxic effects of nitenpyram on antioxidant enzyme system and DNA in zebrafish (*Danio rerio*) livers. Ecotoxicology and environmental safety. 2015;122:54-60.

© 2021 Dokubo et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/52617>