Tropical Journal of Drug Research March 2026; 3 (2): 366 - 381
ISSN: 2476-8065 (Electronic)
Available online at http://www.tjdr.org
https://doi.org/10.26538/tjdr/v3i2.1

Original Research Article

EFFECTS OF CHRONIC CONSUMPTION OF CALABASH CHALK ON OXIDATIVE STRESS MARKERS AND LIPID PROFILE IN WISTAR RATS

Atim I. Okpo-Ene[1], *Adie U. Polycarp[1], Umoh N. Okon[2], Obi M. Paulica, Agonna O. Odeh[1], Eme O. Efiom[1]

[1]*Department of Human Physiology, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria.*
[2]*Department of Medical Bacteriology, Virology and Mycology, Faculty of Medical Laboratory Science, University of Calabar, Calabar, Nigeria.*
*For correspondence: Email: Polycarp.physiol@unical.edu.ng, +2348134205676

Sent for review: 10 June 2025 Revised accepted: 25 February 2026

Abstract

Purpose*:* Calabash chalk consumption has been reported to cause various systemic alterations, which may affect some homeostatic mechanisms in the body. This study aimed to demonstrate the effect of chronic consumption of calabash chalk on oxidative stress markers and lipid profile.
Methods*:* Ten Wistar rats weighing 160-180 g were divided randomly into 2 groups of 5 rats each. Group one was the control group and received 1 ml of distilled water daily, while group two served as the test group and received 1 ml of calabash chalk suspension orally daily. In addition, all animals were given food and water ad libitum. The experiment lasted for 28 days, followed by animal sacrifice; oxidative stress markers- {superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione peroxidase (GPx)}, lipid profile- {triglycerides (TG), total cholesterol (TC), very low-density lipoproteins (VLDL), high-density lipoproteins (HDL), and low-density lipoproteins (LDL)}, cardiac risk ratio, and atherogenic index of plasma (AIP) were studied.
Results*:* SOD, CAT, and GPx concentrations were significantly lower ($p<0.01$) in the test group compared to the control. Conversely, MDA concentration was significantly higher ($p<0.01$) in the test group compared with the control, while serum TG, HDL, VLDL, and TC concentrations were not significantly different between the 2 groups. However, LDL, AIP, and cardiac risk ratio were significantly increased compared with the control.
Conclusion: Chronic consumption of calabash chalk reduces the efficiency of antioxidant defenses, leading to increased oxidative stress.

Keywords: Oxidative stress, calabash chalk, lipid profile, cardiac risk ratio, atherogenic index

Tropical Journal of Drug Research is indexed by Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals
(DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Calabash chalk is a naturally occurring substance found in West African countries. It is also known as kaolin, edible clay, and marble chalk. Due to migration, this West African substance has been carried worldwide. It is known as “La-Craie” in French, “White dirt” or “White clay” in the United States of America, “Mabele” in Congo, and “Nzu” in Nigeria, amongst others.[1]

Chalk is not a food substance but is readily consumed by numerous people in different parts of the world.[2] This is a form of geophagy and Pica.[3] The practice of geophagy is usually associated with pregnant women. Calabash chalk is largely consumed by females, especially during pregnancy.[4] It helps to prevent morning sickness and excessive salivation in pregnant and breastfeeding women.[1]

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of oxygen reactive species (ROS) in the cells and tissues and the ability of a biological system to detoxify these reactive products (excess free radicals and insufficient antioxidants in the body, resulting in cell damage, i.e., cell membrane, cytoplasmic proteins, lipids, and nucleic acids).[5] Research has shown that oxidative stress is one of the factors responsible for the onset and/ or the progression of several chronic and degenerative diseases, including Cardiovascular disease, Parkinson's disease, Alzheimer’s, Rheumatoid arthritis, Barrett's esophagus, and kidney disease.[6] Some known causative factors of oxidative stress include environmental pollution, smoking and other uses of tobacco, heavy metals, ionizing radiation, over-exposure to the sun, excessive consumption of alcohol, and stress.[7]

Reactive oxygen species (ROS), superoxide anions (O2-), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), and reactive nitrogen species (RNS), like nitric oxide (NO) and peroxynitrite ($ONOO^-$), are produced by the mitochondria during both physiological and pathological conditions and by endothelial and inflammatory cells.[8] ROS produced under normal conditions has a protective effect. They are involved in processes such as protein phosphorylation, apoptosis, activation of specific transcriptional factors, and differentiation. They can also modulate immune-mediated responses against extrinsic pathogens and activate protective signaling pathways to counter inflammation.[9] The overproduction of ROS, leading to their accumulation under abnormal conditions, can contribute to the immediate activation of the inflammatory process. This can explain why ROS plays key roles in the pathogenesis of numerous chronic inflammatory disorders, including reflux esophagitis, Barrett's esophagus, Helicobacter pylori (H. pylori)-induced gastritis, and inflammatory bowel diseases such as ulcerative colitis and Crohn's disease.[10]

Superoxide dismutase (SOD), a key antioxidant defense in most living cells exposed to oxygen, is produced during oxygen metabolism.[11] It catalyzes the dismutation of the superoxide anion radical into oxygen and hydrogen peroxide. There are three types of SOD present in humans; SOD1, in the cytoplasm; SOD2 in the mitochondria; and SOD3, which is extracellular.[12] Decrease/inactivation/mutation of SOD leads to high levels of free radicals, which result in cellular damage and disease.[13]

Catalase (CAT) is a common enzyme that catalyzes the breakdown of hydrogen peroxide to water and oxygen in most living organisms exposed to oxygen. It is key in protecting the cells from reactive oxygen species.[14]

Malondialdehyde (MDA), a dialdehyde, is a by-product of lipid peroxidation. It is widely used as a biomarker to assess lipid oxidative damage and oxidative stress in biological samples.[15] It is highly reactive and interacts with proteins, lipoproteins, DNA, and RNA.[15]

Glutathione peroxidase (GPx) is an enzyme family that reduces lipid hydroperoxides to alcohols and free hydrogen peroxide to water and oxygen.[16]

A lipid profile, or panel, is a group of blood tests used to detect abnormalities in lipid levels. They include tests for low-density lipoproteins, high-density lipoproteins, total cholesterol, and total triglycerides. These tests can indicate risks for cardiovascular diseases and other diseases.

MATERIALS AND METHODS

Preparation of kaolin suspension

Blocks of non-salted Calabash chalk were purchased from Watt Market in Calabar South, Calabar, Cross River State, Nigeria. The calabash chalk was ground to a powder using a manual grinder. A 40 g portion of the ground powder was dissolved in 1 liter of distilled water, yielding a suspension with a concentration of 40 mg/ml. The concentration of the suspension was calculated as follows:

$$\text{Concentration (mg/mL)} = \frac{\text{Mass of chalk (mg)}}{\text{Volume of water (mL)}}$$
$$= \frac{40 \times 1000 \text{ mg}}{1000 \text{ mL}} = 40 \text{ mg/mL}$$

This concentrate/suspension was filtered with Whatman filter paper to remove all impurities. The suspension was stored in a plastic jug in a cool, dry place.

Lethal effect of Calabash chalk

Research has shown that no mortality was recorded after oral treatment with calabash chalk, even at a concentration of 5000 mg/kg.[17]

Ethical Approval

Ethical approval (234PHY3823) for the research was obtained from the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Cross River State, Nigeria.

Experimental animals

Ten (10) adult albino male rats of the Wistar strain weighing between 160 and 180 g were used for these experiments. They were housed in the animal facility of the Physiology Department, University of Calabar, at a temperature of 28 ± 2 0C and 12/12 hours of light and dark cycles. The animals were allowed to acclimatize for two weeks before the onset of the experiments. The animals were fed with standard feed pellets and were allowed access to food and water ad libitum. Five animals were kept in each cage (wooden cages with wire net covers), and the cages were always kept clean.

Experimental design

The animals were randomly divided into 2 groups of 5 rats each. Group 1 served as the control group and was administered 1 ml of distilled water every morning for 28 days, while group 2 served as the test group and was administered 1 ml of calabash chalk suspension every morning for 28 days.[18] The calabash chalk and distilled water were administered via the orogastric route. Food and water intake were determined daily, and their body weights were taken before and after the experiment. The rats were humanely sacrificed using chloroform on day 29.

Assay of Catalase (CAT)

Catalase activity was assayed using the UV spectrophotometric method, which depends on monitoring the change of 240 nm absorbance at high levels of hydrogen peroxide solution (>30mM).[19]

A 20% homogenate was prepared in phosphate buffer (0.067 M, pH 7.0), and the homogenate was employed for the assay. The samples were read against a control without homogenate but containing the H_2O_2-phosphate buffer. To the experimental cuvette, 3.0 ml of H_2O_2-phosphate buffer was added, followed by the rapid addition of 40 µl enzyme extract, and mixed thoroughly. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240 nm. The enzyme solution containing H_2O_2-free phosphate buffer served as the control.

One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

Assay of Malondialdehyde (MDA)

Malondialdehyde was assayed using the thiobarbituric acid (TBA) test, which involves reacting MDA with TBA to form a pink-colored adduct, which is measured spectrophotometrically.

Into two test tubes labeled "test" and "blank" were added 10 µl of serum and 10 µl of distilled water, respectively. Then, 0.5 ml of 25% TCA (trichloroacetic acid) and 0.5 ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were added. The mixture was boiled for 40 minutes in a water bath (HH-S4 Water bath, Jiangsu Jinyi Instrument Tech. Co., Ltd, China) and cooled in cold water. Then, 0.1 ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelengths of 532 nm and 600 nm against the blank.

Assay of superoxide dismutase (SOD)

Superoxide dismutase uses the photochemical reduction of riboflavin as an oxygen-generating system and catalyzes the inhibition of NBT (Nitro blue Tetrazolium) reduction, the extent of which can be assayed spectrophotometrically.[20] The incubation medium contained a final volume of 3.0 ml, 50 mM potassium phosphate buffer (pH 7.8), 45 M methionine, 5.3 mM riboflavin, 84 M NBT, and 20 M potassium cyanide. The amount of homogenate added to this medium was kept below one unit of enzyme to ensure sufficient accuracy. The tubes were placed in an aluminum foil-lined box maintained at 25°C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600 nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the enzyme. One unit of enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT (Sigma Aldrich, USA). The values were calculated as units/mg protein.

Assay of glutathione peroxidase (GPx)

Glutathione peroxidase catalyzes the following reaction:

$$2\,GSH + H2O2 \xrightarrow{\text{Se-GPx}} GSSG + 2H2O$$

Glutathione was assayed by its reaction with DTNB (5, 5'-dithiobis-(2-nitrobenzoic acid)) to give a compound that absorbs at 412 nm. To 0.4 ml of buffer (Sigma Aldrich, USA), 0.2 ml of EDTA, 0.1 ml of sodium azide (Sigma Aldrich, USA), 0.2 ml of reduced glutathione (Sigma Aldrich, USA), and 0.1ml of H_2O_2 were added to two test tubes labeled as "test" and "control". To the test 0.2 ml of sample was added, and to the control 0.2 ml of water was added. The contents were mixed well and incubated at

37°C for 10 mins; the reaction was arrested with the addition of 0.5 ml of 10% TCA. To determine the glutathione content, 1.0 ml of supernatant was removed by centrifugation, and that to, 3.0 ml of buffer and 0.5 ml of Ellman's reagent (Sigma Aldrich, USA) were added. The color developed was read at 412 nm. Standards in the range of 40-200 µg were taken and treated similarly. The activity was expressed in terms of µg of glutathione consumed/min/mg protein.[20]

Lipid profile

Determination of the serum cholesterol (TC)

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.[21]

$$Cholesterolester + H2O \rightarrow Cholesterol + Fattyacids$$

Cholesterol + O2 → Cholesterol-3-one + H2O2

$2H_2O_2$ + phenol + aminoantipyrine→Quinoneimine + $4H_2O$

Two test tubes were labeled "test" and "blank" then, 10 µl of the standard reagent and 10 µl of distilled water were added to them, respectively. 10 µl of the sample was added to another test tube. Then, 1000 µl of reagent R1 was added to each test tube and mixed, then, incubated for 10 minutes at 37 0C. The absorbance of all the cuvettes was read and recorded at 546 nm.

CHOL conc (mg/dl) = change in Abs sample x conc of standard change in Abs standard.

Determination of serum triglycerides (TAG)

Serum triglyceride concentration was determined using the enzymatic colorimetric method based on the hydrolysis of triglycerides to glycerol and free fatty acids by lipoprotein lipase. The liberated glycerol is subsequently phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase to form glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol-3-phosphate is then oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2). In the presence of peroxidase, the hydrogen peroxide reacts with 4-aminophenazone and 4-chlorophenol to form a quinoneimine dye, the intensity of which is directly proportional to the triglyceride concentration in the sample and is measured spectrophotometrically[22].

About 5 µl of distilled water was added into a test tube labeled "blank", 5 µl of the standard reagent was added into another test tube and 5 µl of the sample was added into another test tube. Then, 500 µl of reagent R1 was added into each test tube and mixed. This was incubated for 5 minutes at 37 0C. The absorbance of all the cuvettes was read and recorded at 546 nm.

TAG (mmol/l) = change in Abs sample x standard conc. change in Abs standard

Determination of serum lipoproteins (HDL)

High-density lipoprotein cholesterol (HDL-C) was determined using a Biosystems® commercial diagnostic kit based on the precipitation method. The principle of the assay is that very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) in serum are precipitated by phosphotungstate in the presence of magnesium ions, leaving HDL in the supernatant. After centrifugation, cholesterol in the HDL-containing supernatant was quantified enzymatically by the cholesterol oxidase–peroxidase (CHOD-PAP) method and measured spectrophotometrically.[23]

Briefly, 100 µL of serum sample (and standard) was pipetted into centrifuge tubes, followed by addition of 500 µL of diluted precipitation reagent (R1). The mixture was gently mixed and allowed to stand for 10 min at 25°C, then centrifuged at 3500 rpm for 15 min. Thereafter, 50 µL of the supernatant (HDL fraction) was transferred into test tubes labelled "test", "standard" and "blank". Subsequently, 500 µL of cholesterol reagent (CHOL) was added to each tube and incubated for 10 min at 25°C. Absorbance was read at 500 nm against reagent blank. HDL-C concentration (mg/dL) was calculated using the kit formula:

$$\text{HDL-C} = \frac{\Delta\text{Abs}_{\text{sample}}}{\Delta\text{Abs}_{\text{standard}}} \times \text{Conc}_{\text{standard}}$$

Very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were not directly measured but were calculated from triglycerides (TG), total cholesterol (TC) and HDL-C using the Friedewald equations. [24] VLDL-C was estimated as TG/5 (mg/dL), while LDL-C was derived as TC − HDL-C − VLDL-C, as shown below:

$$\text{VLDL-C} = \frac{\text{TG}}{5}$$

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{VLDL-C}$$

The Friedewald approach is widely applied for lipid profile estimation in both experimental and clinical studies, particularly where triglyceride levels fall within acceptable limits for the validity of the equation. [24]

Determination of Atherogenic Index (AI)

The atherogenic index (AI) was calculated from serum lipid parameters to assess the tendency for atheroma formation. It was determined using the formula:

$$\text{AI} = \frac{\text{LDL-C}}{\text{HDL-C}}$$

where LDL-C is low-density lipoprotein cholesterol and HDL-C is high-density lipoprotein cholesterol, expressed in mg/dL.[25]

Determination of Cardiac Risk Ratio (CRR)

Cardiac risk ratio (CRR), also referred to as Castelli's Risk Index-I, was calculated as an index of cardiovascular risk using total cholesterol and HDL cholesterol values. It was computed using the formula:

$$\text{CRR} = \frac{\text{TC}}{\text{HDL-C}}$$

where TC represents total cholesterol and HDL-C represents high-density lipoprotein cholesterol (mg/dL).[26]

Determination of Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase (GPx) activity was determined spectrophotometrically using a commercial assay kit (or standard laboratory protocol) based on the principle that GPx catalyzes the reduction of hydrogen peroxide (or organic hydroperoxides) by reduced glutathione (GSH), producing oxidized glutathione (GSSG). In the presence of glutathione reductase (GR) and NADPH, GSSG is converted back to GSH with concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance due to NADPH consumption was monitored at 340 nm and GPx activity was expressed as U/L (or U/mg protein), according to the manufacturer's instructions.[27]

Statistical analysis

The results are presented as mean ± SEM. Independent t-test analysis was used to compare the two means of the groups. $P<0.05$ was taken to be statistically significant. Computer Software, Graph Pad Prism, SPSS, and Excel Analyzer were used for the analysis.

RESULTS AND DISCUSSIONS

Antioxidant enzymatic families, including Catalases (CAT), Superoxide Dismutases (SOD), and Glutathione Peroxidases (GPx), work cooperatively to protect cells from excessive reactive oxygen species (ROS) derived from endogenous metabolism.[28]

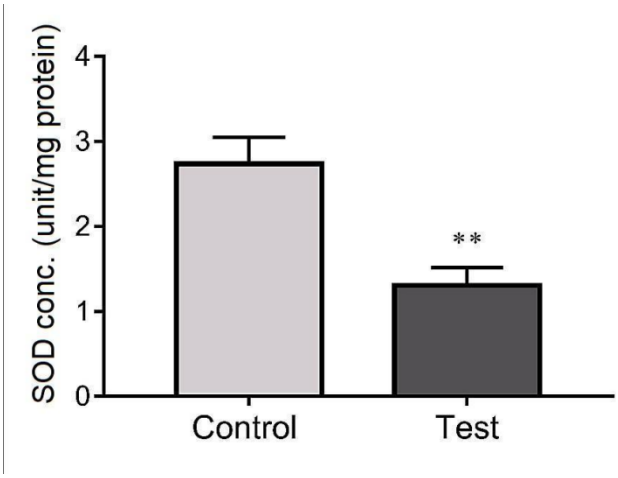

FIG 1: Comparison of Superoxide dismutase (SOD) concentration between the control and test group.
Values are mean ± SEM, n = 5. **p< 0.01 vs control

Superoxide dismutase is a key antioxidant. Its physiological importance is highlighted by the severe pathologies seen in mice genetically engineered to lack these enzymes, e.g., hepatocellular carcinoma. Mice lacking SOD2 die several days after birth amid massive oxidative stress.[28] In this study, its concentration was significantly reduced in the test group and could have led to oxidative stress. Among the defense systems operating against reactive oxygen species, catalase is the most potent antioxidant enzyme; it is known to catalyze the decomposition of hydrogen peroxide into water and oxygen in an energy-efficient manner in cells exposed to oxidative stress.[29] In most living organisms, hydrogen peroxide is a potentially dangerous by-product of oxygen metabolism. According to Allain,[11] in this study, chronic consumption of calabash chalk significantly reduced catalase concentration in the test group. This indicates that chronic consumption of calabash chalk significantly reduced/slowed down the decomposition of hydrogen peroxide by catalase, resulting in oxidative stress.

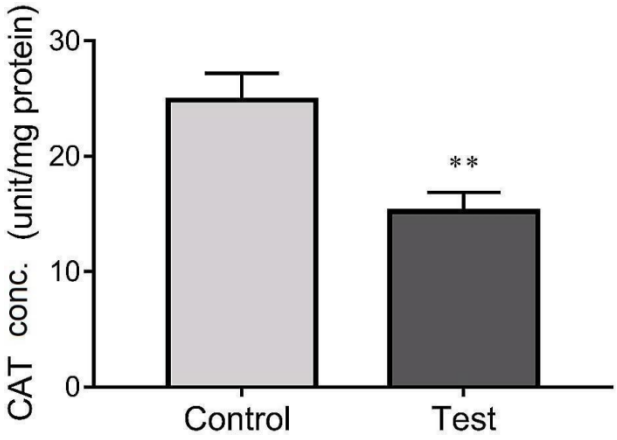

FIG 2: Comparison of Catalase (CAT) concentration between the control and the test group.
Values are mean ± SEM, n = 5. **p< 0.01 vs control

In the test group, malondialdehyde concentration was significantly increased. A high concentration of malondialdehyde indicates increased oxidative imbalance and lipid peroxidation, which is a significant biological consequence of oxidative cellular damage in humans.[30]
Glutathione peroxidase (GPx) was significantly reduced in the test group. Thus, its function to reduce lipid hydroperoxides and hydrogen peroxide was significantly impaired, leading to oxidative stress (GPx3).

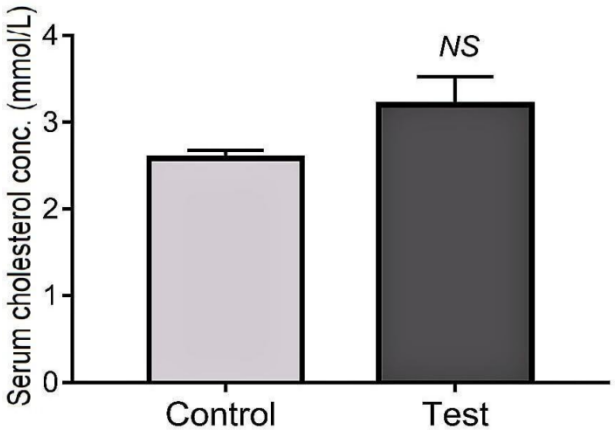

FIG 3: Comparison of serum cholesterol concentration between the control and the test group. Comparison of serum triglyceride concentration between the control and the test group.
Values are mean ± SEM, n = 5, p> 0.05 vs control. NS: not significant.

From the results obtained, serum triglyceride and cholesterol concentrations of the test group's animals were higher than the control group. However, the concentrations were not significantly different. This is indicative of hyperlipidemic or cardiotoxic activity from the chronic consumption of calabash chalk because an increase in serum or plasma levels of triglycerides or cholesterol has been considered to increase the risk of developing atherosclerosis and other cardiovascular diseases. [31-33]

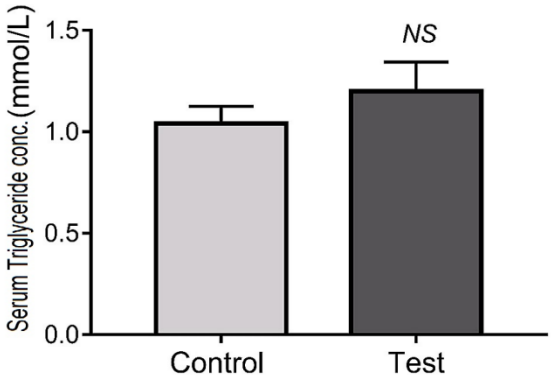

FIG 4: Comparison of serum triglyceride concentration between the control and the test group.
Values are mean ± SEM, n = 5, P> 0.05 vs control. NS: not significant.

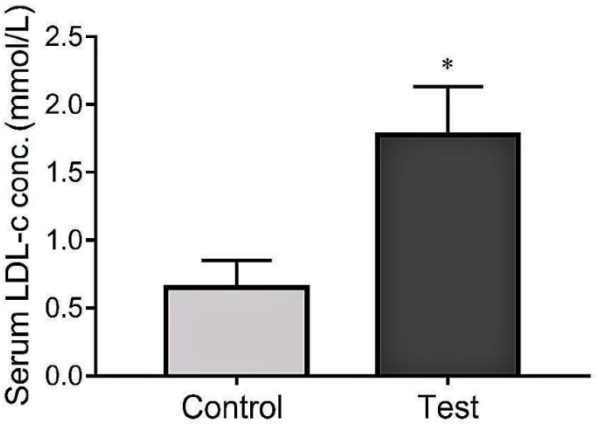

FIG 5: Comparison of the serum LDL-c concentration between the control and the test group.
Values are mean ± SEM, n = 5. * p< 0.05 vs control.

The low-density lipoprotein (LDL) of the test group was significantly higher compared to the control group. Similarly, the very low-density lipoprotein (VLDL) concentration of the test group was higher than that of the control group, though not significantly. Both LDL and VLDL are regarded as "bad cholesterol" and are associated with an increased risk of cardiovascular disease. They are commonly associated with diabetes, hypertension, hypertriglyceridemia, and atherosclerosis.[34] Also, they can increase the risk of blood clots in the arteries and the formation of a thrombus, which can predispose a subject to stroke or heart attack.

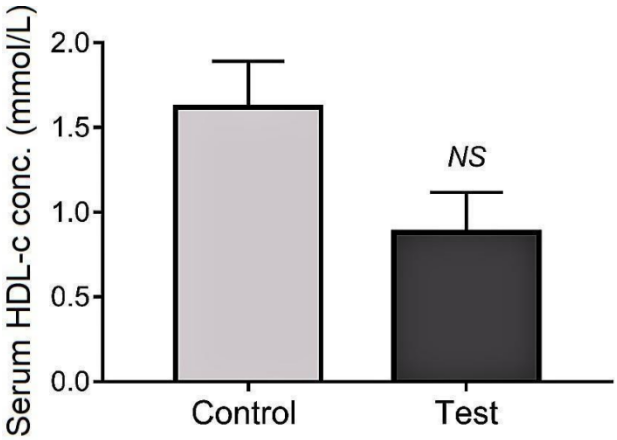

FIG 6: Comparison of serum HDL-c concentration between the control and the test group.
Values are mean ± SEM, n = 5. NS: not significant.

The high-density lipoprotein (HDL) in the test group was low compared to the control group, though not significantly. HDL plays a vital role in reverse cholesterol transport, in which excess cholesterol is removed from the peripheral vessels and transported back to the liver for disposal.[35] It has several other beneficial biological properties, including antioxidative, anti-inflammatory, endothelial/vasodilatory, antithrombotic, and

cytoprotective functions.[36] The reduction of HDL can be considered to be cardiotoxic.

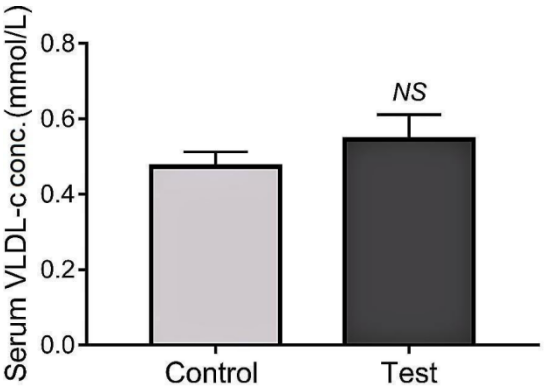

FIG 7: Comparison of the serum VLDL-c concentration between the control and the test group.
Values are mean ± SEM, n = 5. NS: not significant.

The cardiac risk ratio is a screening tool used to assess the risk of developing cardiovascular diseases. The higher the ratio, the higher the risk of developing cardiovascular diseases. The cardiac risk ratio was significantly higher in the test group compared to the control group. A risk ratio greater than 4.5 is considered a high risk for cardiovascular diseases.[37] The cardiac risk ratio in the test group was higher than 4. This result is supported by the low concentration of HDL and high concentrations of LDL, serum cholesterol, and triglycerides in the test group.[38] Chronic consumption of calabash chalk increases the cardiac risk ratio and may predispose to cardiovascular disease.

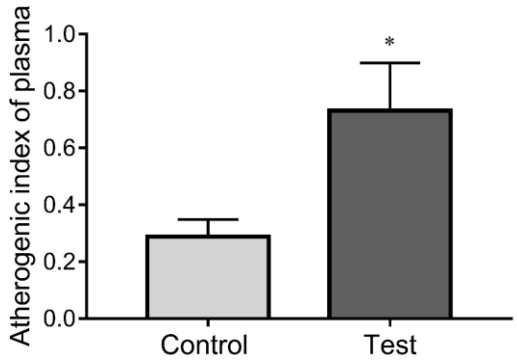

FIG 8: Comparison of the atherogenic index of plasma between the control and the test group.
Values are mean ± SEM, n = 5. * p< 0.05 vs control.

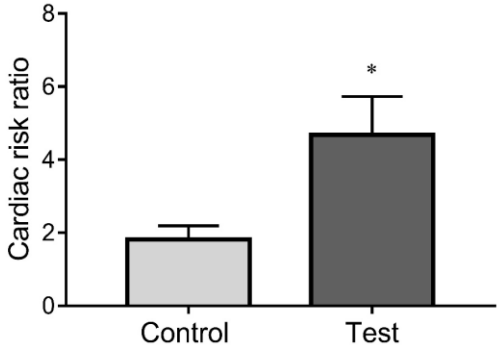

FIG 9: Comparison of the cardiac risk ratio between the control and the test group.
Values are mean ± SEM, n = 5. * p< 0.05 vs control.

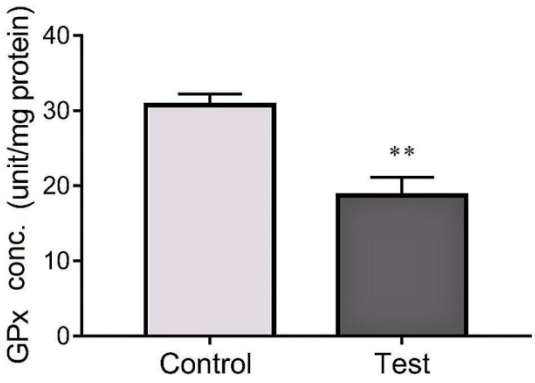

FIG 10: Comparison of Glutathione Peroxidase (GPx) concentration between the control and the test group.
Values are mean ± SEM, n = 5. **p< 0.01 vs control.

The atherogenic index of plasma (AIP) is a logarithmically transformed ratio of molar concentrations of triglycerides to HDL cholesterol. The strong correlation may explain the high predictive value between the atherogenic indexes of plasma with lipoprotein particles.[39] As observed from the result, the atherogenic index of plasma in the test group was significantly increased compared with the control group. From this result, it could be deduced that chronic consumption of calabash chalk has the potential to increase the risk of cardiovascular diseases.

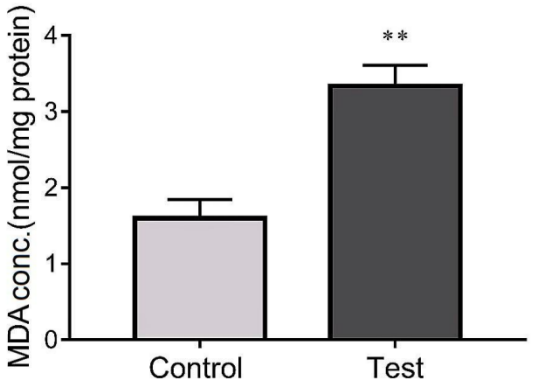

FIG 11: Comparison of Malondialdehyde (MDA) concentration between the control and the test group.
Values are mean ± SEM, n = 5. **p< 0.01 vs control

The mechanism by which this occurs is largely unknown. The exposure to lead, arsenic, barium, and other toxic heavy metals in calabash chalk might be responsible for this outcome. This is corroborated by the reports of the American Health Association (2018).[40]

CONCLUSION

In conclusion, this study found that a prolonged use of calabash chalk causes oxidative stress and lipid metabolism in Wistar rats. The considerable decrease of antioxidant enzymes (SOD, CAT, and GPx) and an increase in malondialdehyde are signs of poor antioxidant defense and high rates of lipid peroxidation. Though it did not influence the total cholesterol and triglycerides, the high LDL, cardiac risk ratio, and atherogenic index indicate that there is a greater risk of cardiovascular dysfunction. Toxic elements like lead and arsenic contained in calabash chalk are likely to cause these effects. It should not then be used on a regular basis, and more research is required to understand the underlying molecular pathway.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

ACHKNOWLEDGEMENT

Authors are very thankful to the Technologists of the Department of Pharmacology and Toxicology, Kaduna State University for their inputs in the course of the laboratory work.

REFERENCES

1. Brevik EC, Slaughter L, Singh BR, Steffan JJ, Collier D, Barnhart P, Pereira P. Soil and human health: current status and future needs. Air Soil Water Res. 2020; 13:1178622120934441. doi:10.1177/1178622120934441.
2. Bonglaisin JN, Kunsoan NB, Bonny P, Matchawe C, Tata BN, Nkeunen G, Mbofung CM. Geophagia: benefits and potential toxicity to humans. Front Public Health. 2022; 10:893831. doi:10.3389/fpubh.2022.893831.
3. Voltura E. Looking at the dietary habits, health, and distributions of parrots through the lens of geophagy to improve management outcomes in conservation arks and parks [dissertation]. College Station (TX): Texas A&M University; 2020.
4. Ben-Alon L, Ayalon SY. Can we eat earth buildings? The mineralogical commonality of earth building and edible earth practices. J Cult Herit Manag Sustain Dev. 2020; 10(2):175-189.
5. Piatoikina AS, Lyakhova AA, Semennov IV, Zhilyaeva TV, Kostina OV, Zhukova ES, Antonova KD, Mikhailov AG. Association of antioxidant deficiency and levels of protein and lipid peroxidation products in first-episode schizophrenia. J Mol Neurosci. 2022; 72(2):217-225.
6. Unim AP, Godwin GC, Irene OE, Clement OO, Gabriel IE. Gastrointestinal and hepatobiliary effects of aqueous extract of Costus afer leaves in diabetic Wistar rats. Pak J Biochem Mol Biol. 2024; 57(2):35-58.
7. Alkadi H. A review on free radicals and antioxidants. Infect Disord Drug Targets. 2020; 20(1):16-26.
8. Goutami L, Jena SR, Swain A, Samanta L. Pathological role of reactive oxygen species in female reproduction. In: [Editor(s) surname initials], editors. Oxidative stress and toxicity in reproductive biology and medicine. Cham: Springer; 2022. p. 201-220.
9. Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN, Lakshminarasaiah U. Antioxidants and human diseases. Clin Chim Acta. 2024; 546:200-206.
10. Lemasters JJ, Jaeschke H. Oxidative stress and inflammation in the liver. In: Arias IM, Alter HJ, Boyer JL, Cohen DE, Shafritz DA, Thorgeirsson SS,

editors. The liver: biology and pathobiology. 6th ed. Hoboken: Wiley; 2020. p. 714-727.
11. Tong Y, Hua X, Zhao W, Liu D, Zhang J, Zhang W. Protective effects of Lactobacillus plantarum CCFM436 against acute manganese toxicity in mice. Food Biosci. 2020; 35:100583.
12. Odu PO, Ujah GA, Uket JM, Odu VK, Inwang UA. Costus afer leaves extract ameliorates stress-induced alterations in hematological and lipid parameters in Wistar rats. Trop J Nat Prod Res. 2025; 9(6):2821-2826. doi:10.26538/tjnpr/v9i6.63.
13. Himoto T, Masaki T. Current trends on the involvement of zinc, copper, and selenium in hepatocarcinogenesis. Nutrients. 2024; 16(4):472.
14. Yuan F, Yin S, Xu Y, Xiang L, Wang H, Li Z, Zhang X, Liu Y. The richness and diversity of catalases in bacteria. Front Microbiol. 2021; 12:645477.
15. Moretti E, Cerretani D, Noto D, Signorini C, Iacoponi F, Collodel G. Relationship between semen IL-6, IL-33 and malondialdehyde in human seminal plasma. Reprod Sci. 2021; 28(8):2136-2143.
16. Muthukumar K, Rajakumar S, Sarkar MN, Nachiappan V. Glutathione peroxidase 3 protects phospholipids during cadmium-induced oxidative stress. Antonie Van Leeuwenhoek. 2021; 114(4):761-771.
17. Tang SP, Mao XL, Chen YH, Yan LL, Ye LP, Li SW. Reactive oxygen species induce fatty liver and ischemia-reperfusion injury by promoting inflammation. Front Immunol. 2022; 13:870239.
18. Watson F, Austin P. Physiology of human fluid balance. Anaesth Intensive Care Med. 2021; 22(10):644-651.
19. Hamza TA, Hadwan MH. New spectrophotometric method for the assessment of catalase activity in biological tissues. Curr Anal Chem. 2020; 16(8):1054-1062.
20. Valotto Neto LJ, de Araujo MR, Moretti RC, Machado NM, Joshi RK, Buglio DS, da Silva FC, dos Santos TC, Pereira LG. Neuroprotective effects of Bacopa monnieri: a systematic review. Antioxidants. 2024; 13(4):393.
21. Kitchawengkul N, Prakobkij A, Anutrasakda W, Yodsin N, Jungsuttiwong S, Amatatongchai M, Pimanprom W, Sananmuang T. Peroxidase-mimicking carbon dots for cholesterol sensing. Anal Chem. 2021; 93(18):6989-6999.
22. Mahboob S, Tahir K, Ali S. Industrial applications of microbial lipases: a systematic review. Int J Biol Biotechnol. 2022; 19:171-182.
23. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20(4):470–475.
24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499–502.
25. Dobiásová M. Atherogenic index of plasma [log(triglycerides/HDL-cholesterol)]: Theoretical and practical implications. Clin Chem. 2004;50(7):1113-1115
26. Castelli WP, Abbott RD, McNamara PM. Summary estimates of cholesterol used to predict coronary heart disease. Circulation. 1983;67(4):730-734.
27. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70(1):158-169
28. Galasso M, Gambino S, Romanelli MG, Donadelli M, Scupoli MT. Catalase regulation and function in cancer. Free Radic Biol Med. 2021; 172:264-272.
29. Sharma S, Bhattarai S, Ara H, Sun G, Bhuiyan MS, Kevil CG, Williams JB, Smith LH. SOD2 deficiency causes lethal cardiomyopathy. Redox Biol. 2020; 37:101740.
30. Bassey IE, Emodi BA, Akpan UO, Iyakndue IF, Anakebe EA, Icha BE, Njoku CI, Udoh US, Umoh UA. Oxidative stress in prostate cancer patients receiving androgen deprivation therapy. JCO Glob Oncol. 2020; 6:1481-1489.
31. Liang S, Wang C, Zhang J, Liu Z, Bai Y, Chen Z, Wong LL, Hernandez F. Triglyceride–glucose index and coronary artery disease: a meta-analysis. Cardiovasc Diabetol. 2023; 22(1):170.
32. Unim AP, Edet OU, Okoi OC, Okpo-ene AI, Chijindu OM, Uquetan US, Essien VA. Evaluation of gastroprotective potentials of papain and fucoidan in Wistar rats. Ibom Med J. 2026;19(1):84-91.
33. Damato A, Vianello F, Novelli E, Balzan S, Gianesella M, Giaretta E, Rossi F, Bianchi A. Clay minerals and animal physiology. Front Vet Sci. 2022; 9:889612.
34. Pirahanchi Y, Sinawe H, Dimri M. Biochemistry of LDL cholesterol [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557758/ (Accessed January 10, 2026).
35. Pownall HJ, Rosales C, Gillard BK, Gotto AM. High-density lipoproteins and atherogenesis. Nat Rev Cardiol. 2021; 18(10):712-723.
36. Xepapadaki E, Zvintzou E, Kalogeropoulou C, Filou S, Kypreos KE. Antioxidant function of HDL. Angiology. 2020; 71(2):112-121.
37. Calcaterra I, Tufano A, Lupoli R, Iannuzzo G, Emmi G, Di Minno MN, Di Minno MND. Cardiovascular disease in antiphospholipid syndrome. Pol Arch Intern Med. 2020; 130:102-110.
38. Packard C, Chapman MJ, Sibartie M, Laufs U, Masana L. Intensive LDL-cholesterol lowering and cardiovascular risk. Heart. 2021; 107(17):1369-1375.
39. Zhu Y, Chen M, Liu K, Gao A, Kong X, Liu Y, Wang Z, Sun J, Qi R, Li H. Atherogenic index of

plasma and restenosis risk. J Atheroscler Thromb. 2022; 29(8):1226-1235.
40. American Heart Association. Lead and mercury exposure and cholesterol levels [Internet]. ScienceDaily. 2018. Available from: https://www.sciencedaily.com (Accessed January 10, 2026).