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Hepatotoxicity evaluation of geophagic clay soil from Uzola, Edo State, Nigeria in albino rats

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Abstract
The effect of oral administration of geophagic soil from Uzola on the liver of albino rats was evaluated in this study. Major oxides composition analysis showed the presence of SiO_2 (55.70%), MnO (0.01%), Al_2O_3 (23.81%), Fe_2O_3 (3.03%) etc. Forty-five elements were detected and quantified. Zirconium had highest concentration (344.4ppm) followed by Barium (287ppm) while Molybdenum, Silver, Cadmium and Antimony had the lowest concentration of <0.1ppm. Rats were divided into 4 groups (i.e. A, B, C and D). Group, A which served as the control, was orally administered with distilled water while groups B, C and D were orally administered with 400, 800 and 1200 mg/kg body weight dose of the soil solution respectively. The rats were sacrificed 24 hours after soil solution administration for 1, 7 and 21 days. Liver alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and concentrations of serum total bilirubin, conjugated bilirubin, total protein and albumin were determined. There was a significant increase ($p < 0.05$) in liver alanine aminotransferase and aspartate aminotransferase activities and serum alanine aminotransferase activities in the experimental animals. Serum total protein concentration also increased significantly ($p < 0.05$) in the experimental animals when compared to the control group but was reversed to control levels after 21 days administration. Available results show that geophagic clay soil from Uzola, Edo State, Nigeria may have a deleterious effect on liver synthetic functions and may also cause an overproduction of aspartate aminotransferase and alanine aminotransferase in the liver at the doses considered.

Keywords: Geophagy; Major oxides; Liver function; Aminotransferases; Hepatotoxic; Uzola

INTRODUCTION

Pica is the deliberate eating of non-food or non-nutritive substances [1]. Many different types of pica have been described in literature such as ingestion of baby powder, charcoal, calcium hydroxide (lime), ash, uncooked starch and ice [2].

Geophagy, which is the most common type of pica, involves deliberate eating of earth such as clay. It is an ancient practice that is still widespread in many parts of the world such as in Asia, Africa, South America, North America and parts of Europe [3]. Geophagy is a conspicuous though poorly understood behaviour. In humans, the practice is variously regarded as a global health issue or an anthropological idiosyncrasy; indeed, it is widely viewed as an aberrant behaviour or a symptom of metabolic dysfunction [4].

Paradoxically, geophagy is usually considered an adaptive behaviour in nonhuman primates and a wide range of

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mammals [5,6]. It is thought by many to be an adaptive behavior to rectify nutritional deficiencies particularly of micronutrients in pregnant women [7-9]. For humans, adaptive benefits must eclipse the costs of ingesting geohelminths [10] and soil-based toxins, such as lead, copper and potassium (to a hyperkalemic extent) [4]. Despite such costs, an adaptive function is probable given its persistence in human history. Also the taste of clay is claimed to diminish nausea, discomfort and vomiting in "morning sickness".

Although the practice of geophagy is recognised to be more common among some groups of people, it is not limited to certain geographical areas or human societies [11]. Areas of high prevalence include societies of low economic status living within the tropics [12]. Immigration of people from such societies into the UK allows for the possibility of a cultural transfer of the practice [7]. Children consume considerable quantities of soil regardless of geography and socio-economic status. Ingestion varies from the incidental to the incredible, ranging from 75mg/day in Amherst, USA [13] to 650g reported *in vivo* in a single Gambian boy [14].

A single Nigerian village produces 500 tons of soil yearly for consumption across West Africa [15]. In parts of Africa such as Cameroon, Nigeria, Ghana, Sierra Leone, Malawi, Zanzibar and Zambia, soil eating is common amongst females, especially children and pregnant women [16-19]. The prevalence of soil eating amongst pregnant women in Kenya, Ghana, Namibia and Tanzania has been reported in the literature [16-19]. In Zambia and Zimbabwe, the main sources of geophagic soils, which 90% of the rural women ingest, are giant termite mounds. Also in Zambia, brown and white earths are sold on the streets. Unlike the practice of pica, geophagous people are very specific and particular about the type of soil they eat.

8% - 65% of African-American women practice pica. This was confirmed where 46 of the 150 women in the USA and 33 of the 75 in Mexico interviewed, practiced pica. Blocks of magnesium carbonate are available at pharmacies in both Mexico and California and women purchase those for consumption instead of eating clay. Bangladeshi women residing in Bangladesh and also in the UK consume baked clay known as sikor [20,21]. Although the overall prevalence of this habit amongst the Bangladeshi community has not been determined, it is known to be mostly practiced by pregnant women [21]. A study done on Swedish children playing in specific playgrounds found that they either deliberately or by accident consume soil contaminated with different metals including arsenic [22]. In Germany, healing soil is commercially available and used as medicine against diarrhoea and gastric hyper-acidity. Clayey soil may be contaminated with bacteria and other harmful substances.

In 1980, [23] published a case study on a group of 11 preschool children from Atlanta, United States of America. Eight of these children had a history of geophagia and seven of them tested positive for *Toxoplasma*. One child was simultaneously infected with *Toxoplasmosis* and *Toxocaragondii*. It is thought that the cause of the infection could probably have been the result of oocysts from cat faeces in the soil [23].

Heavy metal poisoning due to geophagia is possible. Lead toxicity/poisoning not only affects the consumer but could also affect an unborn baby in pregnant females [21]. In a survey done by [18] on geophagous pregnant women to determine the silica content in their stools, it was concluded that stools from geophagous women had higher mean silica content than the control group who did not eat soil. There was also a difference in the stool silica content, which was higher in those who ate soft stone in comparison to those who consumed soil from termite mounds.

Even though a lot of work has been done on geophagic soils around the world, there is dearth of information on the safety of consumption of geophagic soils from Nigeria. This work will therefore not only contribute to the little knowledge of these Nigerian clay soils in general but will also ascertain the safety of consumption of the Uzola geophagic soil in particular.

EXPERIMENTAL

Experimental animals. Sixty male albino rats (*Rattus norvegicus*) with weights ranging from 190-230g were obtained from the animal laboratory of the Department of Biochemistry, Faculty of Medical Sciences, University of Jos, Nigeria. The rats were kept in standard cages and allowed to acclimatize to animal house conditions (i.e. temperature: 28 ºC– 31 ºC; photoperiod: 12 h natural light and 12 h dark; humidity: 50%–55 %) for 7 days. They were allowed free access to normal rat pellet and tap water. Rats were divided into 4 groups A, B, C and D. Group, A which served as the control, was orally administered with distilled water while groups B, C and D were orally administered with 400, 800 and 1200 mg/kg body weight dose of the soil solution respectively. The animals were sacrificed 24 hours after extract administration for 1, 7 and 21 days.

Soil material. Geophagic clay soil was obtained from Uzola, Uhunwode Local Government Area in Edo State, South- South, Nigeria.

Assay kits. Assay kits for alanine aminotransferase and aspartate aminotransferase were products of Randox Laboratories, United Kingdom. All other reagents used were of analytical grade and were all prepared in all glass distilled water.

Preparation of aqueous solution of geophagic soil. Wet geophagic clay soil was air-dried to constant weight. A lump of the clay soil was then pulverized using mortar and pestle. The resultant fine soil was thereafter dissolved in distilled water to make the required doses of 400mg/kg, 800mg/kg and 1200mg/kg.

Soil oxides and elemental composition analysis. Soil major oxides, trace/minor elements and rare earth metals were analysed using X-ray diffraction and Inductively Coupled Plasma Mass Spectrometry [24]. This was carried out at Acme Analytical Laboratories (Vancouver) Ltd, Canada

Serum preparation. Rats were anaesthetized by placing them in a jar containing diethyl ether-soaked cotton wool. They were then quickly brought out of the jar and their jugular veins cut. Blood samples were collected into sample bottles and allowed to clot at room temperature for 20 minutes and thereafter centrifuged at 1398 x g for 15 minutes. Clear colourless serum was collected with the aid of Pasteur pipette.

Tissue homogenate preparation. Anaesthetized rats were quickly dissected and their liver taken out and washed clean of blood. The removed livers were then quickly placed in ice-cold 0.25M sucrose solution to retain their integrity. Each liver was dried using a tissue paper and weighed. A weighed portion of each liver was cut into pieces using a scissors and then homogenized in ice-cold 0.25M sucrose solution (1:5 w/v). The homogenates were turned into specimen bottles, kept frozen and used between 24 hours of preparation.

Measurement of enzyme activity and liver function indices. The method of [25] was used for the assay of aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2). [26] method was used for the determination of serum total protein. Serum total and conjugated bilirubin was determined using the method described by [27] while serum albumin was determined using the method of [28]. All measurements were done using Spectronic 21 digital spectrophotometer (Bausch and Lomb, Rochester NY).

Statistical analysis. Data are expressed as mean of 5 replicates± Standard Deviation (SD). The obtained data were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) software. All significant differences were determined by one-way analysis of variance (ANOVA) and Post Hoc multiple comparison was done using Duncan's multiple range test. Significance level was set at $p< 0.05$.

RESULTS

Major oxides and elemental composition of Uzola geophagic soil. Tables 1 and 2 show the major oxides and elements detected in the geophagic soil. A total of eleven oxides were detected in the soil sample. SiO_2 was the highest oxide present (55.70%) while MnO was the lowest at 0.01%. 14.33% of the soil sample was lost on ignition. A total of forty-five elements were detected. Zirconium (Zr) had the highest concentration (344.4ppm) followed by Barium (Ba) (287ppm) while Molybdenum (Mo), Silver (Ag), Cadmium (Cd) and Antimony (Sb) had the lowest concentration of <0.1ppm.

Liver and serum alanine aminotransferase. Table 3 shows the toxicity effect of oral administration of the geophagic soil on serum and liver ALT in albino rats. All the experimental animals administered with the various concentrations of the soil solution showed a significant increase ($p< 0.05$) in liver ALT activity. Similarly, all the rats treated with the soil solution also showed a significant increase ($p< 0.05$) in serum ALT activity when compared with the control group.

Liver and serum aspartate aminotransferase. The toxic effect of the soil solution on liver and serum AST in albino rats is as shown in Table 4. All the experimental animals except group B animals treated for 1 day and groups B and C treated for 21 days with the soil solution did not show any significant increase ($p< 0.05$) in liver AST activity when compared with the control group. There was however no significant difference ($p< 0.05$) in the serum AST activity of all the experimental animals when compared to the control group.

Serum total and conjugated bilirubin. The effect of the soil solution on serum Total and Conjugated Bilirubin is presented in Table 5. All the animals treated with the various doses of the soil solution did not show any significant difference ($p< 0.05$) in the serum concentration of Total and Conjugated Bilirubin when compared to the control animals.

Serum albumin and total protein. Table 6 presents the effect of the soil solution on Serum Albumin and Total Protein Concentrations. There was also no significance difference ($p< 0.05$) in serum albumin concentration in all the experimental animals but serum total protein was increased significantly($p< 0.05$) in rats treated with the 1200mg/Kg dose for 1 day and all the animals treated with the various doses of the soil solution for 7 days.

DISCUSSION

Major oxides and elemental composition of Uzola geophagic soil. The mineral composition of edible clays has not been completely documented. However, because of their natural origin, clays may contain numerous contaminants including heavy metals. Result of this study showed that the trace elements in the Uzola geophagic soil was relatively high (Table 1 and 2) when compared to the adequate daily intake of trace

Table 1: Major oxides composition of Uzola geophagic soil

Oxides	Quantity (%)
LOI	14.33
SiO_2	55.70
Al_2O_3	23.81
Fe_2O_3	3.03
CaO	0.06
MgO	0.19
Na_2O	0.02
K_2O	0.39
MnO	0.01
TiO_2	1.39
P_2O_5	0.08
Cr_2O_5	0.015

Table 2: Elemental composition of Uzola geophagic soil

Elements	Quantity (ppm)	Elements	Quantity (ppm)	Elements	Quantity (ppm)
Barium	287.00	Yttrium	34.70	Zinc	48.00
Beryllium	6.00	Lanthanum	71.70	Silver	<0.10
Cobalt	10.90	Cerium	147.80	Nickel	13.50
Cesium	3.50	Praseodymium	17.90	Arsenic	<0.50
Gallium	31.20	Neodymium	67.70	Gold	<0.50
Hafnium	8.60	Samarium	12.36	Cadmium	<0.10
Niobium	37.00	Europium	2.60	Antimony	<0.10
Rubidium	25.40	Gadolinium	10.19	Bismuth	0.10
Tin	5.00	Erbium	4.14	Mercury	0.04
Strontium	82.40	Thulium	0.60	Thallium	<0.10
Tantalum	2.60	Ytterbium	3.71	Selenium	<0.50
Thorium	17.50	Lutetium	0.59	Zirconium	344.40
Uranium	4.30	Molybdenum	<0.10	Holmium	1.49
Vanadium	110.00	Copper	16.00	Dysprosium	8.12
Tungsten	1.80	Lead	12.30	Terbium	1.52

Table 3: Effect of oral administration of Uzola geophagic soil on liver and serum alanine aminotransferase activity in albino rats

Days	Enzyme	A (Control)	B (400mg/kg)	C (800mg/kg)	D (1200mg/kg)
1	Liver Alanine Aminotransferase	55.69 ± 3.50^a	74.33 ± 8.96^b	83.67 ± 12.44^b	67.67 ± 3.06^b
7		55.66 ± 5.50^a	77.33 ± 5.03^b	78.00 ± 6.93^b	72.67 ± 4.51^b
21		55.67 ± 3.90^a	81.67 ± 8.39^b	80.33 ± 13.32^b	82.00 ± 15.00^b
1	Serum Alanine Aminotransferase	18.10 ± 3.64^a	35.33 ± 14.06^{ac}	63.67 ± 12.62^b	56.33 ± 11.03^{bc}
7		18.05 ± 3.61^a	35.33 ± 8.96^b	27.67 ± 5.82^b	25.67 ± 4.30^{ab}
21		18.00 ± 3.63^a	37.67 ± 1.26^b	36.33 ± 9.07^b	38.00 ± 6.25^b

Activities in UI ±SD (n=5); Values with superscripts different from control are significantly different ($p < 0.05$).

Table 4: Effect of oral administration of Uzola geophagic soil on liver and serum aspartate aminotransferase activity in albino rats

Days	Enzyme	A (Control)	B (400mg/kg)	C (800mg/kg)	D (1200mg/kg)
1	Liver Aspartate Aminotransferase	26.67 ± 3.69^a	37.67 ± 12.22^a	63.00 ± 1.73^b	58.67 ± 7.10^b
7		26.67 ± 3.78^a	61.00 ± 5.29^b	50.00 ± 5.57^c	43.00 ± 1.73^c
21		26.67 ± 3.79^a	30.33 ± 6.34^a	37.00 ± 9.52^a	46.67 ± 14.57^b
1	Serum Aspartate Aminotransferase	23.00 ± 5.28^a	23.67 ± 5.69^a	32.00 ± 8.72^a	25.67 ± 5.69^a
7		23.00 ± 5.29^a	32.00 ± 8.52^a	26.33 ± 4.01^a	22.67 ± 4.16^a
21		23.00 ± 5.29^a	30.33 ± 4.04^a	29.00 ± 5.55^a	21.67 ± 2.89^a

Activities in UI ±SD (n=5); Values with superscripts different from control are significantly different ($p < 0.05$).

Table 5: Effect of oral administration of Uzola geophagic soil on serum total and conjugated bilirubin concentrations in albino rats

Days	Enzyme	A (Control)	B (400mg/kg)	C (800mg/kg)	D (1200mg/kg)
1	Serum total bilirubin	11.90±2.94[a]	15.30±5.10[a]	17.00±5.89[a]	13.60±3.89[a]
7		11.90±2.94[a]	18.13±5.74[a]	10.20±0.01[a]	10.20±0.02[a]
21		11.90±2.94[a]	10.20±0.01[a]	11.90±2.94[a]	12.83±2.53[a]
1	Serum conjugated bilirubin	5.10±0.50[a]	8.50±2.94[a]	8.52±2.97[a]	6.80±2.94[a]
7		5.10±0.50[a]	6.80±1.94[a]	5.10±0.01[a]	5.10±0.04[a]
21		5.10±0.50[a]	5.10±0.02[a]	5.10±0.03[a]	6.80±2.94[a]

Concentration in g/L ±SD (n=5). Values with superscripts different from control are significantly different ($p < 0.05$)

Table 6: Effect of oral administration of Uzola geophagic soil on serum albumin and total protein concentrations in albino rats

Days	Parameter	A (Control)	B (400mg/kg)	C (800mg/kg)	D (1200mg/kg)
1	Serum Albumin	45.33±12.00[a]	43.00±9.54[a]	44.33±11.34[a]	40.33±11.15[a]
7		45.33±11.50[a]	49.33±10.12[a]	33.33±6.81[a]	31.00±5.57[a]
21		45.33±11.59[a]	31.00±4.58[a]	32.67±7.57[a]	34.67±3.06[a]
1	Serum Total Protein	52.33±7.10[a]	54.67±1.53[a]	49.00±1.00[a]	75.00±7.50[b]
7		52.33±7.10[a]	73.33±5.03[b]	68.67±4.93[b]	70.00±7.18[b]
21		52.33±7.10[a]	45.00±5.20[a]	57.67±7.64[a]	62.33±5.55[a]

Concentration of albumin in g/L, total protein in µmol/L ±SD (n=5). Values with superscripts different from control are significantly different ($p < 0.05$)

elements in the human body. For example, the Cu content in the geophagic soil was 16 ppm, while the recommended daily intake is 1.0 – 1.5 mg/kg/day [29]. The levels of Co, Mn, Mo, Ni, Pb, Hg and Sr were higher than the tolerable daily intake as well [30-32]. Literature review had shown that prolonged consumption of heavy metal can deplete some essential nutrients in the body causing a decrease in immunological defenses, intrauterine growth retardation, impaired psycho-social behaviors, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer [33]. Heavy metals such as Cu, Zn, Mn, Co and Mo act as micronutrients for the growth of animals and human beings when present in trace quantities, whereas others such as Cd, As, and Cr act as carcinogens [34]. The toxic effect observed may be attributed to elements (like barium, strontium, vanadium, lanthanium, cerium, neodymium and zircomium) present in very high concentrations in the Uzola soil and which may be toxic to the living system. Intentional or accidental human ingestion of soluble barium compounds has been reported to cause gastroenteritis, hypokalemia, acute hypertension, cardiac arrhythmias, skeletal muscle paralysis and death [35-38]. Aluminium chloride has been reported to increase thiobarbituric acid reactive substances and decrease the activities of glutathione S-transferase, superoxide dismutase, catalase and glutathione peroxidase in liver, kidney and brain of rats treated with 34 mg/kg bw for 70 days. Plasma transaminases, lactate dehydrogenase, glucose, urea, creatinine, bilirubin, total lipid, cholesterol, triglyceride and Low Density Lipoprotein-Cholesterol were also increased, while total protein, albumin and High Density Lipoprotein-Cholesterol were decreased [39]. In our study, the concentration of aluminium (as oxide) was 28.81 g/100g soil sample, which is equivalent to 238100mg/kg. This extremely high concentration of aluminium may also be implicated in the toxic effect observed.

Liver and serum transaminases. Alanine transaminase (ALT) is majorly present in the hepatocyte cytoplasm with lesser concentrations found in the kidneys, heart and skeletal muscle [40]. It is therefore a more specific marker of liver damage than aspartate transaminase (AST) since AST may also be elevated in diseases involving other organs such as heart and muscle. Liver is prone to xenobiotic-induced injury because of its central role in the metabolism of foreign compounds and its portal location within the circulation system [41]. In this study, there was an increase in liver ALT activity, which was followed, by a simultaneous increase in serum ALT. This is suggestive of induction or over-secretion of ALT in the liver above normal levels [42, 43]. Liver AST similarly increased following soil solution administration. This confirms overproduction of transaminases in the liver of the experimental animals. However, serum AST remained normal even though serum ALT was increased. This may indicate that there was no compromise of the hepatocytes membrane integrity and the observed increase in serum ALT might have occurred as a result of leakage from other principal sources (e.g. kidney, heart, erythrocytes, skeletal muscle) not examined in this study.

Serum total and conjugated bilirubin. Bilirubin is a yellow pigment found in bile, a fluid made by the liver. A small amount of old red blood cells are degraded and subsequently replaced by new blood cells every day. Bilirubin is the breakdown product of these old blood cells. The liver helps to break down bilirubin so that it can be removed by the body in the stool. Bilirubin (total and conjugated) could be used to assess the excretory function of the liver [44, 45]. Severe hemolysis causes the release of more bilirubin into the blood, which manifests as elevated levels of unconjugated and total bilirubin [46]. Unconjugated and total bilirubin can also increase in the event of low bilirubin conjugation [46]. Results from this study (Table 5) reveal that there was proper conjugation and subsequent excretion of bilirubin by the liver of experimental animals. Moreover**,** the soil solution did not cause any obstruction of the biliary tree since there was no significant increase in serum conjugated bilirubin concentration. This shows proper functioning of liver excretory ability.

Serum albumin and total protein. Assessment of protein in the liver could be used as important indicator of synthetic function of the organ [44, 45]. Albumin is manufactured by the liver and represents a major synthetic protein and is a marker for the ability of the liver to synthesize proteins. The aqueous solution of geophagic soil did not cause any significant change in serum albumin concentrations in this study but caused a significant rise in serum total protein concentrations. Therefore, the elevated levels in the total protein may be due to increase in the synthetic activity of the organ. The soil solution might have stimulated the liver to increase the synthesis of proteins other than albumin (especially globulins) to a level that far exceeded that required by the animals. However, the animals were able to recover after 21 days of soil solution administration.

CONCLUSION. It can therefore be inferred that oral consumption of geophagic soil from Uzola, Edo State at the doses we considered may have a deleterious effect on liver synthetic functions and may also cause an overproduction of AST and ALT in the liver. This soil solution may have deleterious effect on other organs not examined in this study.

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