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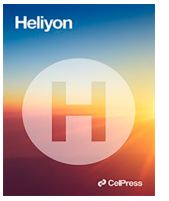

Research article

Biochemical, toxicological, and microbiological assessment of calcined poultry manure for potential use as bone scaffold material

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ABSTRACT

The biosafety of thermally calcined poultry manure as a hydroxyapatite source for potential use as bone-making material was investigated in this study. In vitro assays were used to determine the sensitivity of the antioxidant properties to the thermal calcination temperature used to process the poultry manure (750, 800, and 850 °*C*). The effect of the extract of both calcined poultry manure (local) and analytical grade hydroxyapatite (foreign) at various concentrations of 100%–25 % inclusion at (100 mg/kg) body weight intubation for 21 days on kidney, liver, and serum of animal model used was assessed. The results show that the thermally calcined poultry manure-derived hydroxyapatite generally possessed good antioxidant properties with the poultry manure treated at 750 °*C* having the most promising antioxidant properties compared to those treated at 800 and 850 °*C*, and hence a more likely improved anti-toxicity potential. The various blends of the analytical high-grade hydroxyapatite and thermally calcined poultry manure hydroxyapatite samples are safe compared to the normal control rats with regards hepatic function and renal function parameters with the equal blend of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite (1:1) possessing the lowest activity concentrations. In addition, the enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations of the experimental animals administered the varied compositions of the analytical high grade and thermally calcined poultry manure-derived hydroxyapatite, were lower when compared to normal control rats. The microbiological evaluation suggests that the calcined poultry manure inclusion at various concentrations could not pose a negative effect on various pathology in the liver, kidney, and blood.

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1. Introduction

There is a global emergency for biologically safe and suitable materials to serve as bone substitutes in orthopedic applications [1]. This is on account of the high prevalence of musculoskeletal-related diseases, which is put at approximately 1.71 billion people globally [2]. The high prevalence rate makes it unrealistic to rely on grafting from humans to meet their bone replacement needs, hence the need for biomaterials that can perform the same functions [3]. In recent times, there has been extensive studies in the development of biomaterials to meet the bone implant demands [4,5]. Hydroxyapatite, HAp (calcium orthophosphate - Ca5(OH)(PO4)3) is one of the highly sought-after bioceramics for bone implant application because of its similarities in chemical constitution with human bone and its capacity for bone regeneration due to its osteoconductive and osteointegration properties [6]. It also possesses comparable strength to human bone, is biocompatible, non-toxic, and has a good Ca^{2+} and PO^{4-} ions release rate [7,8]. On these accounts, it has been applied in grafting of bones, filling of bones and teeth, and coating of orthopedics and dental implants, either as sole additions or as composites in polymer or ceramic-based systems [9–12]. However, it has been observed that synthetic hydroxyapatite can be toxic, in addition to requiring high energy-intensive production methods and cost and requires high energy-intensive production costs. Thus, interest in developing non-toxic, economically sustainable, and production-efficient hydroxyapatite with the use of natural sources has been the subject of interest to researchers [13,14].

Development of hydroxyapatite from natural sources such as plants, mineral sources, animal shells, bones, and wastes with the adoption of techniques such as thermal calcination, hydrothermal, sol-gel, chemical precipitation, mechanochemical treatment, among others, have been reported in works of literature [8,13–16]. The rationale for the selection of these natural source materials is largely due to their high source of calcium and phosphorus, which are the core constituents of hydroxyapatite. In most of these studies, obtaining the exact stoichiometric ratio of Ca:P of 1.67:1 or obtaining pure hydroxyapatite have served as research limitations. In addition, assessment of the microbiological and biochemical viability in terms of biosafety and toxicity has not been widely reported.

In this study, biochemical and toxicological evaluation via *in vitro* and *in vivo* assessment of thermally calcined poultry manure which contains hydroxyapatite with associated minerals such as SiO_2, FeO, and K_2O is investigated to establish its biosafety and applicability for bone-making applications. In addition, microbiological assessment by considering the viability of bacteria and fungi on the thermally calcined poultry manure for use as prosthetics was investigated.

The biocompatibility and structural resemblance of thermally calcined poultry manure (TCPM) to human bone makes it a novel avenue to investigate its viability as a sustainable source of hydroxyapatite (HA), an essential component in bone regeneration. Remarkably, TCPM is high in calcium and phosphorus, two crucial elements of hydroxyapatite, making it a perfect option for use in biological applications. Nonetheless, biosafety assessment is essential to guarantee the appropriateness of TCPM. The mineral composition of hydroxyapatite enhances its osteogenic potential as well as cellular activities essential for bone healing, which have also been observed to be modelled by the TCPM [16]. Despite the significant chemical composition similarity of TCPM to pure hydroxyapatite, comprehensive biochemical assays are needed to establish the safety and efficacy of TCPM as reliable source of HA, for consideration as bone scaffold material [8,13].

In addition, prostheses are often in direct contact with the user's skin. The skin provides a natural physical barrier against the invasion of microorganisms, although many microorganisms such as bacteria and fungi are normal flora majority of which are *Staphylococcus aureus, S, epidermis* [17,18], although some of these strains can multiply, produce biofilm for adhesion on skin surface and cause infection [19].

2. Materials and method

2.1. Poultry manure processing

The poultry manure used as natural source biomaterial for the investigation was sourced from the Animal Farm, Federal University of Technology, Akure, Nigeria. The poultry manure was air-dried and then combusted using a steel drum for 3h. After sieving, the ash was subjected to thermal treatment (calcination) at 750, 800, and 850 $^{\circ}C$ for 5h using a heat-treatment furnace.

2.2. Preparation of Working concentration

For the products (750, 800, and 850 $^{\circ}C$) w/v was prepared according to Nwanna et al. [20] method to get an extract concentration of 100ug/ml which was used for the *in-vitro* assays.

2.3. Methods (IN-VITRO assays)

In-vitro antioxidant activities were carried out on the following products (750, 800, and 850 $^{\circ}C$) to determine their antioxidative potential relative to toxicity with body fluid.

2.3.1. Determination of total phenol

The total phenol content of the thermally calcined poultry manure products was determined by mixing 0.2 ml of the products with 2.5 ml of 10 % Folin ciocalteau's reagent and 2 ml of 7.5 ml sodium carbonate. The mixture was held at 45 °C for 40 min, and the absorbance measured at 700 nm in the spectrophotometer with gallic acid used as standard phenol. This procedure conformed with the method of Singleton et al. [21].

2.4. Determination of total flavonoid

A colourimeter assay designed by Bao [22] was used to determine the total flavonoid content of the thermally calcined poultry manure products. It entailed adding 0.2 ml of the products to 0.3 ml of 5 % $NaNO_3$ at zero time. Then, 0.6 ml of 10 % $AlCl_3$ was added after 5min, and on attaining an additional 6min, 2 ml of 1M NaOH was added to the mixture, alongside 2.1 ml of distilled water. At 510 nm, the absorbance was measured against the reagent blank, and the flavonoid content was reported as mg rutin equivalent.

2.4.1. Determination of ferric-reducing property

The procedure reported by Pulido et al. [23], was used to determine the ferric-reducing property of the thermally calcined poultry manure products. It required mixing 0.25 ml of the products with 0.25 ml of 200 mM sodium phosphate buffer pH 6.6, and 0.25 ml of 1 % KFC. The mixture was held for 20min at 50 °C, and subsequently centrifuged for 10min at 2000 rpm after 0.25 ml of 10 % TCA was added to the mixture. The absorbance was measured at 700 nm after mixing 1 ml of the supernatant with 1 ml of distilled water and 0.1 % of $FeCl_3$.

2.4.2. DPPH free radical scavenging ability

The procedure of Gyamfi et al. [24] was adopted to determine the free radical scavenging ability of the calcined poultry manure products against DPPH (1, 1- diphenyl-2-picryhydrazyl). The method required measuring the absorbance at 516 nm of the mixture of the products with 1 ml of 0.4 mM methanolic solution of the DPPH which was left in the dark for 30min.

2.4.3. Nitric oxide (NO) radical scavenging ability

The procedure of Jagetia and Baliga [25] was adopted to determine the NO radical scavenging ability. The method required mixing 5 mM sodium nitroprusside in phosphate saline with the calcined poultry manure products, afterwards incubating the mixture at 25 °C for 150min. The absorbance was measured at 546 nm after the reaction mixture was added to the Greiss reagent.

2.4.4. ABTS scavenging ability

The 2, 2′-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability of the calcined poultry manure products was determined following the procedure reported by Re et al. [26]. It required adding 2.0 ml of the ABTS solution to 0.2 of the appropriate dilution of the products and the absorbance was measured at 732 nm on holding for 15mins. The Trolox equivalent antioxidant capacity was afterwards determined.

2.4.5. Hydroxyl (OH) radical scavenging ability

The procedure reported by Halliwell and Gutteridge [27] was adopted in determining the ability of the calcined poultry manure products to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose. A reaction mixture containing 120 μl, 20 mM deoxyribose, 400 μl, 0.1M phosphate buffer pH 7.4, 40 μl, 20 mM hydrogen peroxide, and 40 μl, 500 μM FeSO4 was prepared for freshly prepared extract (0–100 μl) of the calcined poultry manure products to be added. The volume was then made to 800 μl with distilled water and incubated at 37 °C for 30min. The reaction was halted by the addition of 0.5 ml of 2.8 % TCA, followed by the addition of 0.4 ml of 0.6 % TBA solution. The absorbance was measured at 532 nm after the tubes were incubated in boiling water for 20min.

2.4.6. IN-VITRO antioxidant enzyme assays

2.4.6.1. Reduced glutathione (GSH) levels. The level of reduced glutathione (GSH) in the liver issue supernatants was determined using the procedure of Sabir et al. [28]. 0.2 ml of the sample solution was added to 1.8 ml of distilled water, subsequently sulfosalicylic acid was added to 3 ml of the precipitating agent. The reaction mixture was centrifuged at 3000 g for 4 min; afterwards 0.5 ml of the tissue supernatant was added to 4.5 ml of Ellman reagent. 0.5 ml of the diluted precipitating agent, 4 ml of phosphate buffer and 0.5 ml of Ellman's reagent was used to prepare a blank. Within 30 min of colour development, the absorbance of the reaction mixture was measured at 412 nm against a reagent blank. From the GSH standard curve, the concentration of the GSH was extrapolated.

2.4.7. Superoxide dismutase (SOD) activity

The procedure explained in details by Fridovich [29] was utilized to determine the activity of SOD in the homogenates. To prepare a 1 in 10 dilution, 1 ml of the sample was made with 9 ml of distilled water. Then, to equilibrate in the spectrophotometer, an aliquot of the diluted sample was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2). 0.3 ml of freshly prepared 0.3 mM adrenaline was added to the mixture which was quickly mixed by inversion to initiate the reaction. The reference cuvette consisted of 2.5 ml buffer, 0.3 ml of the substrate (adrenaline), and 0.2 ml of water. After every 30 s, the increase in absorbance at 480 nm was monitored for 150 s.

2.4.8. Catalase activity

The procedure by Fridovich [29] was also used to determine the catalase activity. 70 μl of the sample was mixed with 920 μl $NaPO_4$ buffer pH 7 containing 0.1 mM EDTA. The reaction commenced by the addition of 10 μl of H_2O_2. The absorbance read at 240 nm (10-s intervals) for 180 s, was used to monitor the decrease in H_2O_2 concentration. The H_2O_2 extinction coefficient (E) = 39.4 $mM^{-1}cm^{-1}$ at 240 nm and 1 mmol H_2O_2 $ml^{-1}min^{-1}$ was expressed as 1 unit of CAT.

2.4.9. Toxicology experimental design

2.4.9.1. Acute toxicity test. This was carried out on the above-named samples with higher in-vitro antioxidant activities, the products were used to formulate products for the subsequent experiments.

2.4.9.2. Animal experiment. In this study, forty-five albino rats (Wistar strain) which weighed 220–260 g, purchased from the Central Animal House of the University of Ibadan, were used for the animal experiment. They were kept in rubber cages and housed in the animal house of the Department of Biochemistry, Federal University of Technology, Akure, Nigeria. The rats were fed with commercial rat chow (Vital Feeds Nigeria Limited) and water ad libitum. They were maintained in the laboratory at 28 ± 1^{o} C temperature, 11 ± 1 % relative humidity, and 12h light and 12 h dark cycle. The rats were acclimatized and randomly allocated into 5 groups as shown below with six rats per 5 groups according to (Nwanna et al., 2023). The methodology and general experimental protocols adopted in the study were subjected to ethical review and approval was obtained (FUTA/ETH/22/71).

3. Methodology

Group 1. administered sample A (100 % AHGH) at 100mg/ kg body weight.

Group 2. administered sample B (25 % TPPM +75 % AHGH) at 100 mg/kg body weight.

Group 3. administered sample C (50 % TPPM+50 % AHGH) at 100 mg/kg body weight.

Group 4. administered Sample D (100 % TPPM) at 100 mg/kg body weight.

Group 5. Normal control.

The foreign is analytical high-grade hydroxyapatite (AHGH). The local is thermally processed (calcined) poultry manure (TPPM).

3.1. Administration of treatments

Oral administration of treatments through intra gastric route using the stomach tube for the safe ingestion of (100 mg/kg) body weight of the formulated extracts to the 5 tested groups for 21 consecutive days. Afterwards, the albino rats were fasted overnight, euthanized by cervical dislocation, then liver and kidney samples were collected and prepared for biochemical analyses in accordance with Nwanna et al. [30].

3.1.1. Kidney function test

3.1.1.1. Determination of creatinine, urea, and bilirubin. Creatinine urea and bilirubin levels in the kidney were determined by using commercial kits (Randox Laboratory, Antrim, UK).

3.1.2. IN-VIVO antioxidant enzyme activities

3.1.2.1. Determination of in-vivo glutathione peroxidase (GPx) activity. The procedure by Godin et al. [31] was used for the determination of the GPx activity. 0.2 ml of 0.4M phosphate buffer (pH7.0) was reacted with 0.1 ml of 10 mM sodium azide together with 2 ml plasma homogenate, 0.2 ml of 10mMglutathione and 0.1 ml of 0.2 mM of hydrogen peroxide. The mixture was incubated for 10min, afterwards the reaction was terminated by the addition of 0.4 ml of 10 % TCA to the reaction mixture. The supernatant after centrifuging the mixture at $3200\times g$ for 2min, was assayed for glutathione content using ELLMAN'S reagent (19.8 mg of DTNB in 100 ml of 0.1 % sodium nitrate). The GPx activity was expressed as mg of GPx consumed/min/g protein.

3.1.2.2. Determination of reduced glutathione content (GSH). The procedure by Sabir et al. [28] was used to estimate the GSH in plasma: Reduced glutathione (GSH) concentration. Aliquots of 0.5 ml of the tissue homogenates were mixed with 0.2 M phosphate buffer, pH 8.0, and 0.1 ml of 0.01 M Ellman's reagent, [5,5′-dithiobis-(2-nitrobenzoic acid)] (DTNB). Tubes were centrifuged at 3000 g for 15 min at room temperature. Using the clear supernatants, the absorbance was read in a spectrophotometer (Jenway) at 420 nm, and the activity of GSH was expressed as mg of GSH consumed/min/g protein.

3.1.2.3. Determination of AST, ALT, and ALP activities in the liver. The enzyme activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were measured in the liver using standard assay kits from Randox chemicals, United Kingdom following the manufacturer prescribed procedure.

3.1.2.4. Determination of tissue total protein content. The total protein was determined using the Biuret method as described in by the manufacturer manual (Randox Laboratories Ltd.). 20 µl of the supernatant was mixed with 1 ml of Biuret reagent (100 mM NaOH, 16 mM Sodium-Potassiumtartrate, 15 mM Potassium iodide, and 6 mM $CuSO_4$), afterwards the mixture was incubated for 30 min at 25 °C and the absorbance was measured at 546 nm using Spectrophotometer. The total protein content was calculated from the bovine serum

albumin standard curve.

3.1.3. Microbiological evaluation

3.1.3.1. Sterilization of samples. Two different powdery materials were used, namely Local (A, which is thermally calcined poultry manure product) and Foreign (B, which is analytical high-grade hydroxyapatite), the two materials were sterilized by exposing them to ultraviolet light for 12 h and subsequently placed in a hot air oven for 12 h at 60 °C.

3.1.3.2. Source of isolates. The bacteria isolates used were *Staphylococcus aureus, S, epidermidis,* and *Pseudomonas aeruginosa* while the fungi isolate used was *Candida albicans.* The isolates were of clinical origin and collected from the Post-graduate Research Laboratory, Department of Microbiology, FUTA. All the isolates were subcultured on blood agar and incubated for 24 h at 37^0C except for *Candida albicans* which was incubated at 25^0C for 48 h.

3.1.3.3. Standardization of isolates. A loopful of the bacterial and fungal culture was aseptically picked into sterile distilled water in a testube, this was repeated until the turbidity matched 0.5 McFarland's standard (1.5×10^8 cfu/ml) before use as described by Cheesbrough [32].

3.1.4. Experimental design

1 1.0 g of sample A was dispensed into 9.0 ml of sterile distilled water in a test tube to make (10 % weight/volume)
2 0.5 g of sample A was dispensed into 9.5 ml of sterile distilled water in a test tube to make (5 % weight/volume)
3 0.1 g of sample A was dispensed into 9.9 ml of sterile distilled water in a test tube to make (1 % weight/volume)
4 0.5 g of sample B was dispensed into 9.5 ml of sterile distilled water in a test tube to make (5 % weight/volume) – positive control
5 0.5 g of Peptone was dispensed into 9.5 ml of sterile distilled water in a test tube to make (5 % weight/volume) – Negative control
6 Test tube containing 10 ml of sterile distilled water only
7. Test tube containing 10 ml of sterile distilled water only

These six groups were set up for each microbial isolate, 200 μL of the standardized inoculum was dispensed into each test tube except 7. The tubes containing bacteria were incubated at 37 °C for 48 h while the one of fungi was incubated at 25 °C for 48 h in a water bath shaker incubator. All the setups were in triplicate.

3.2. Assessment of viability/survival of microorganisms in the test samples

The setups in the experimental design were examined six (6) hourly by withdrawing 100 μL from each sample, the 100 μL was pour-plated on nutrient agar and potato dextrose agar for bacteria and fungi and incubated for 24 h at 37 °C and 25 °C respectively. The plates were examined for the presence of visible colonies and counted and recorded as colony-forming units (CFU).

4. Results and discussion

4.1. In vitro assessment

Analysing the antioxidant capacity of TCPM requires the use of total phenol (TP) and total flavonoid content (TFC) tests, as they reduce oxidative stress, which is linked to structural and functional changes in bone structure (Table 1). Given that scavenging free radicals is essential for preserving cellular integrity during bone formation, TCPM's high TP and TFC suggest that it possesses this capacity [33,34]. Results showed that the 750 °C sample had the highest total phenol content followed by the 850 °C sample, while the 800 °C sample had the least total phenol content, while the three samples showed no significant ($p < 0.05$) difference for flavonoid contents. Comparatively, the total phenol and flavonoid contents of the TCPM in the present study are significant ($p < 0.05$) higher than that observed by Poveda-Giraldo and Alzate [35] and Vanessa et al. [36] for biorefinery valorized marigold and fertilizer calcined *Cucurbita moschata* fruits, respectively. The trend of results obtained for the total phenol content of the samples was replicated for the ferric-reducing antioxidant power (FRAP) of the samples (Table 1). The ability of TCPM to donate electrons is measured by the

Table 1
The FRAP (mg/gAAE) (total antioxidant property) total phenol (mg/g GAE) and total flavonoid (mg/gQE) of the aqueous extract of the different samples.

Samples	FRAP (mg/gAAE)	Flavonoid (mg/g gQE)	Phenol (mg/g GAE)
750 °C	1.11 ± 0.01^a	0.03 ± 0.001^a	13.31 ± 0.04^a
800 °C	0.64 ± 0.01^b	0.03 ± 0.001^a	8.27 ± 0.04^c
850 °C	0.75 ± 0.01^b	0.04 ± 0.001^a	12.29 ± 0.04^b

Data represents means of duplicate determinations. Values with the same letter along the same column are not significantly different ($p > 0.05$) while those with different letters are significantly different ($p < 0.05$).

ferric-reducing antioxidant power (FRAP) assay, indicating its potential as an antioxidant. Since oxidative stress impairs osteoblast activity and mineralization, thus, the high FRAP and total phenol values observed with the aqueous samples may indicate that the TCPM can effectively reduce oxidative stress, which is essential for bone health [16].

Furthermore, the assays for DPPH, NO, and ABTS provide additional insight into the antioxidant activity of TCPM by testing its capacity to scavenge particular free radicals (Table 2). The DPPH assay evaluates TCPM's capacity to donate hydrogen to stable DPPH radicals, whilst the NO and ABTS assays evaluate its ability to scavenge nitric oxide and ABTS radicals, respectively. Inferentially, the strong scavenging action of TCPM in these tests suggests that it may be able to treat bone disorders brought on by oxidative stress. Because hydroxyl radicals are very reactive and cause damage to bone tissue, the Hydroxyl Radical (OH*) Scavenging Ability assay is essential. The potential of TCPM to mitigate oxidative damage inside bone microenvironments and enhance bone matrix integrity and regeneration is highlighted by its capacity to scavenge OH* radicals, which was best evident with the 750 °C sample, an indication of its superior potential [37]. Together, these tests' evaluations shed light on the biosafety of TCPM and its potential as a source of HA for bone regeneration. The power of TCPM to improve bone tissue regeneration and resilience against oxidative stress is demonstrated by its high antioxidant and radical scavenging abilities [8]. Therefore, TCPM presents a promising option for producing HA that is both sustainable and efficient, with potential advantages for biological applications such as bone tissue manufacturing and regeneration.

To determine if TCPM may be used as a bone scaffold material, *in vitro* antioxidant enzymes (superoxide dismutase [SOD] and catalase [CAT]) and molecules (reduced glutathione [GSH]) need to be evaluated (Table 3). To support healthy cell growth and tissue regeneration, bone scaffold materials must have the capacity to neutralize reactive oxygen species (ROS). Reduced glutathione functions as a strong antioxidant, directly scavenging free radicals and preserving the redox balance within cells. Superoxide radicals are converted to hydrogen peroxide by SOD, and hydrogen peroxide is further reduced in oxidative damage by CAT, which breaks it down into water and oxygen. Remarkably, the result showed that the 750°C-sample had better antioxidant molecule (reduced glutathione) levels than 800 and 850 °C samples, while the 850°C-sample had better antioxidant enzyme (superoxide dismutase and catalase) activities than 750 and 800 °C samples during *in vitro* study. The *in vitro* evaluation of the TCPM had better GSH, CAT and SOD concentrations compared to the modified chicken manure reported by Soe et al. [38]. The result indicates TCPM at 750 °C had more antioxidant molecules as the thermal degradation of reduced glutathione is minimized at this temperature in comparison to higher temperatures. Considering GSH is heat-sensitive and can be destroyed by extreme heat, the GSH levels are lower in the 800 °C and 850 °C samples. Meanwhile, the higher SOD and CAT activities exhibited by the 850 °C sample may be indicative that elevated temperatures induce modifications in the chemical structure of TCPM, potentially augmenting the activation of enzymes or liberating minerals that function as cofactors for these enzymes. Though it comes at the expense of GSH degradation, the material's capacity to degrade reactive oxygen species (ROS) may be enhanced by the increased activity of SOD and CAT at 850 °C.

4.2. *In vivo assessment*

Figs. 1–6 show the effect of the extract of both local (thermally calcined poultry manure (TCPM), designated local) and foreign (analytical grade hydroxyapatite (AHGH), designated FRN) at various concentrations of 100%–25 % inclusion at (100 mg/kg) body weight intubation for 7, 14, and 21 days on kidney, liver, and serum of animal model used. The effects of 100 % foreign (100 % FRN), 25 % local+75 % FRN, 50 % local+50 % FRN, and 100 % local on liver function markers enzymes AST, ALP, and ALT in the serum and liver of experimental animals were evaluated (Figs. 1–6). Results showed that administration of the varied compositions of the analytical grade hydroxyapatite (FRN) and TCPM (Local) samples significantly ($p < 0.05$) reduced the activities of AST, ALP, and ALT in the serum and liver of experimental animals in a duration-dependent manner when compared to the normal control group, an indication that the administration of the samples will not aggravate the expression of the enzymes. ALT and AST are enzymes that serve as markers for liver injury. ALT is more specific to liver damage, while AST is found in various tissues, including the liver, heart, and muscles. ALP is associated with bile duct function and bone metabolism, and elevated levels can indicate liver or bone disorders [39]. Comparatively, the 50 % local+50 % FRN sample had the lowest ALP, ALT and AST activities, which may be indicative of its ability to maintain the index of hepatocellular injury better than the other blends of foreign and local hydroxyapatite samples. Meanwhile, the various blends of the foreign (analytical high-grade hydroxyapatite) and local (thermally calcined poultry manure) hydroxyapatite samples are safe compared to the normal control rats, and safer compared to that reported for birds fed thermally calcined Zinc oxide nanoparticles at 500 °C [40].

The effects of 100 % FRN, 25 % local+75 % FRN, 50 % local+50 % FRN, and 100 % local on kidney protein and function markers, creatinine, urea and bilirubin of experimental rats were evaluated (Figs. 7–10). Elevated blood levels of creatinine, a waste product of muscle metabolism, usually signify compromised renal function. Urea is yet another important indicator of kidney function, just like

Table 2
ABTS (mmmol/g TEAC) (%DPPH (1, 1- diphenyl-2-picryhydrazyl), % Hydroxyl radical (OH) and % Nitric oxide (NO) of the aqueous extract of the different samples.

Samples	ABTS (mmmol/g TEAC)	DPPH %	OH %	NO %
750 °*C*	9.44 ± 0.01^{a}	21.10 ± 0.11^{a}	90.78 ± 2.86^{a}	13.31 ± 0.04^{a}
800 °*C*	6.72 ± 0.01^{b}	5.58 ± 0.11^{c}	73.68 ± 2.72^{c}	8.27 ± 0.04^{c}
850 °*C*	9.38 ± 0.01^{a}	8.45 ± 0.11^{b}	81.57 ± 2.72^{b}	12.29 ± 0.04^{b}

Data represents means of duplicate determinations. Values with the same letter along the same column are not significantly different ($p > 0.05$) while those with different letters are significantly different ($p < 0.05$).

Table 3
The effect of the extracts on *In-vitro* antioxidant enzymes reduced glutathione (GSH) catalase (CAT) and Superoxide dismutase (SOD) in unit/mg protein.

Samples	GSH (unit/mgprotein)	CAT (unit/mgprotein)	SOD (unit/mgprotein)
750 $^{\circ}C$	2.97 ± 0.01^{a}	4.41 ± 0.11^{c}	92.15 ± 2.86^{a}
800 $^{\circ}C$	1.70 ± 0.01^{b}	5.09 ± 0.11^{a}	84.31 ± 2.72^{b}
850 $^{\circ}C$	2.55 ± 0.01^{a}	6.02 ± 0.11^{a}	94.36 ± 1.72^{a}

Data represents means of duplicate determinations. Values with the same letter along the same column are not significantly different ($p > 0.05$) while those with different letters are significantly different ($p < 0.05$).

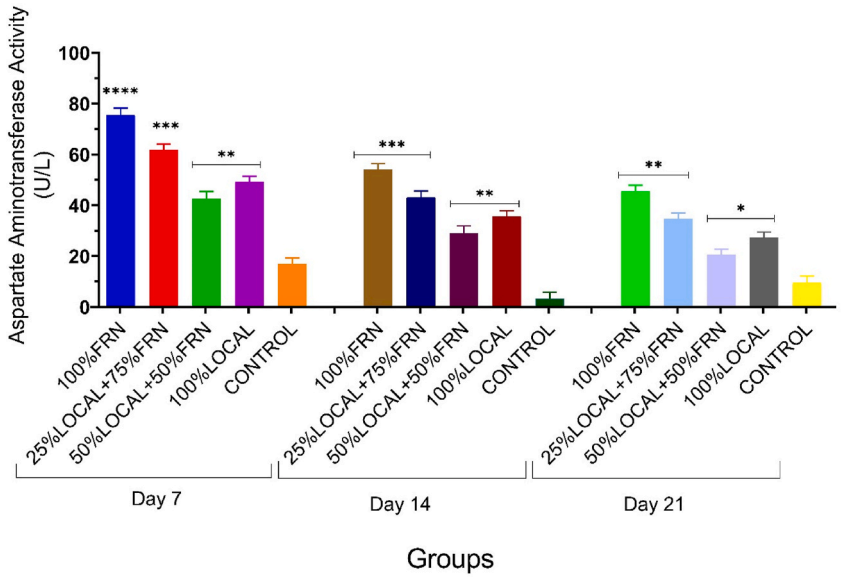

Fig. 1. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Serum Aspartate Aminotransferase Activities on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control
KEY: Analytical High-grade hydroxyapatite (AHGH) is Foreign (FRN)
Thermally calcined poultry manure (TCPM) is LOCAL.

creatinine. Increased blood urea nitrogen (BUN) levels may be a sign of impaired renal clearance and an indication of the body's protein metabolism. Haemoglobin breaks down into bilirubin, which is normally handled by the kidneys and liver. Increased bilirubin levels may be a sign of hepatic failure or problems excreting bile, which can impact the health of the kidneys [39]. Interestingly, results showed that the renal function parameters expressed a trend that was consistent with that observed for hepatic function parameters, with 50 % local+50 % FRN having the least creatinine, urea and bilirubin concentrations. More so, the various blends of the local and foreign TCPM samples are safe compared to the normal control rats and safer compared to that reported for birds fed thermally calcined Zinc oxide nanoparticles at 500 °C [40].

The effects of 100 % FRN, 25 % local+75 % FRN, 50 % local+50 % FRN, and 100 % local on serum and hepatic enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations of experimental rats were evaluated (Figs. 11–13). On day 7 of samples' administration, 100 % foreign (100 % AHGH) had the least serum and hepatic enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations, while 100 % local (100 % TCPM) had the highest serum and hepatic enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations. Additionally, the enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations of the experimental animals administered foreign (100 % AHGH), local (100 % TCPM) or blends of foreign and local (AHGH + TCPM) hydroxyapatite, were significantly ($p < 0.05$) when compared to normal control rats. Although the antioxidant status of the analytical high-grade hydroxyapatite and thermally calcined poultry manure-administered rats was similar to that observed for functional foods studied by Soe et al. [38] and Oguntuase et al. [39], results further showed that on days 14 and 21 the antioxidant status of the experimental animals was further depleted which may be indicative of the toxicity of the various blends of the hydroxyapatite

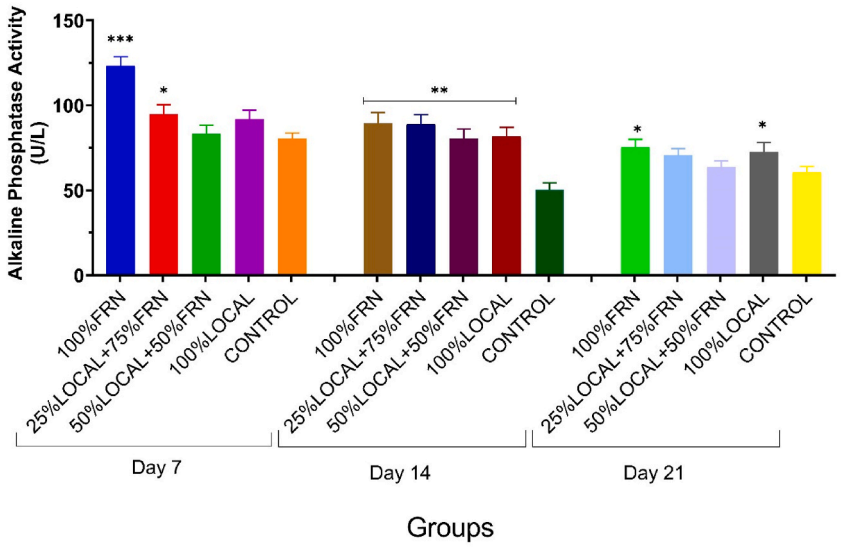

Fig. 2. Effects of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Serum Alkaline Phosphatase Activities on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

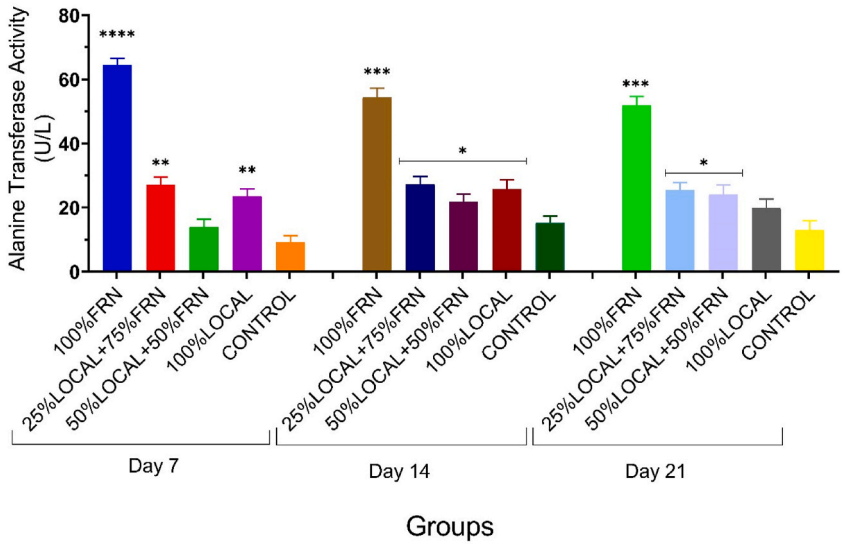

Fig. 3. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Serum Alanine Transferase Activities on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

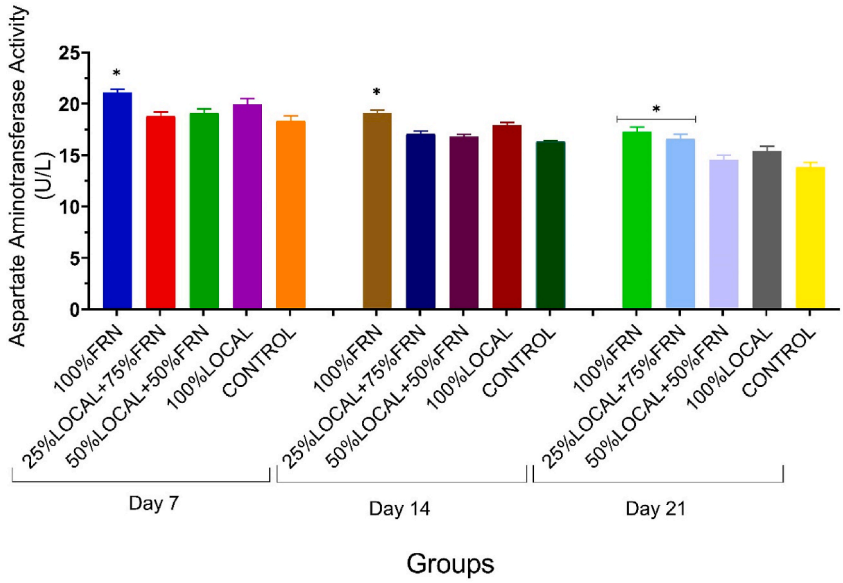

Fig. 4. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Liver Aspartate Aminotransferase Activities on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

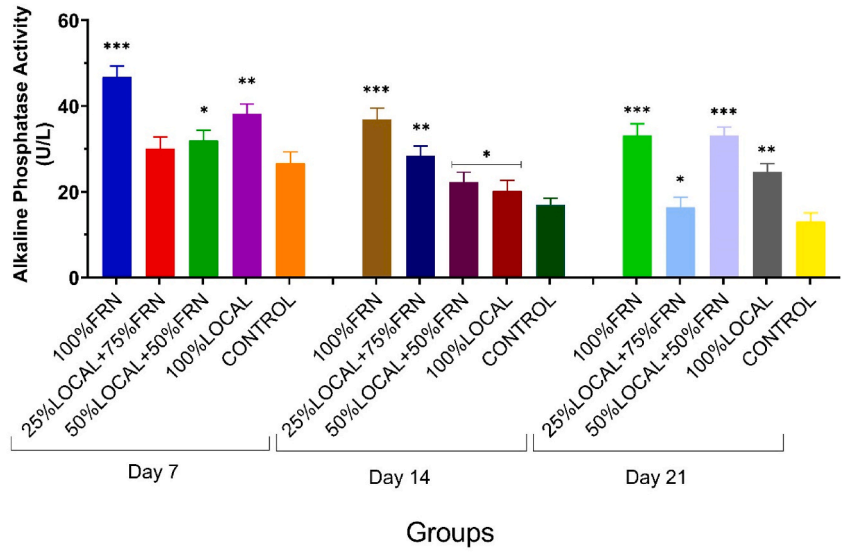

Fig. 5. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Liver Alkaline Phosphatase Activities on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

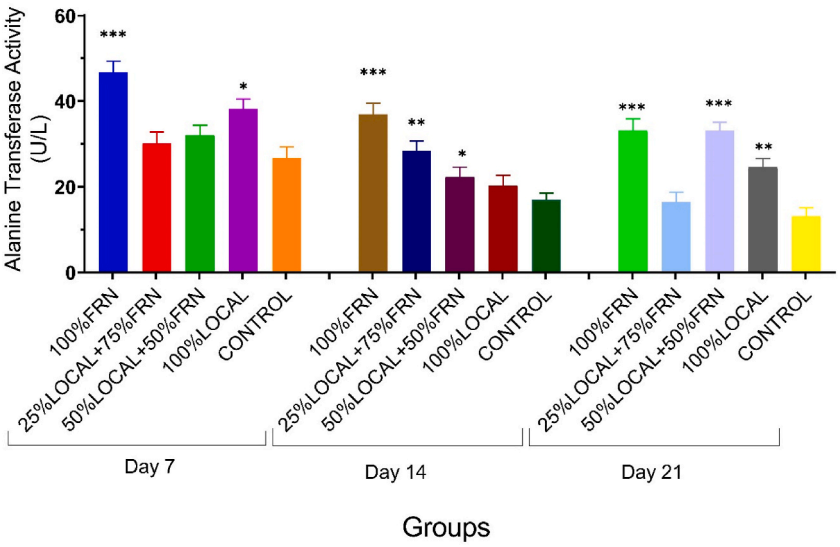

Fig. 6. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Liver Alanine Transferase Activities on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

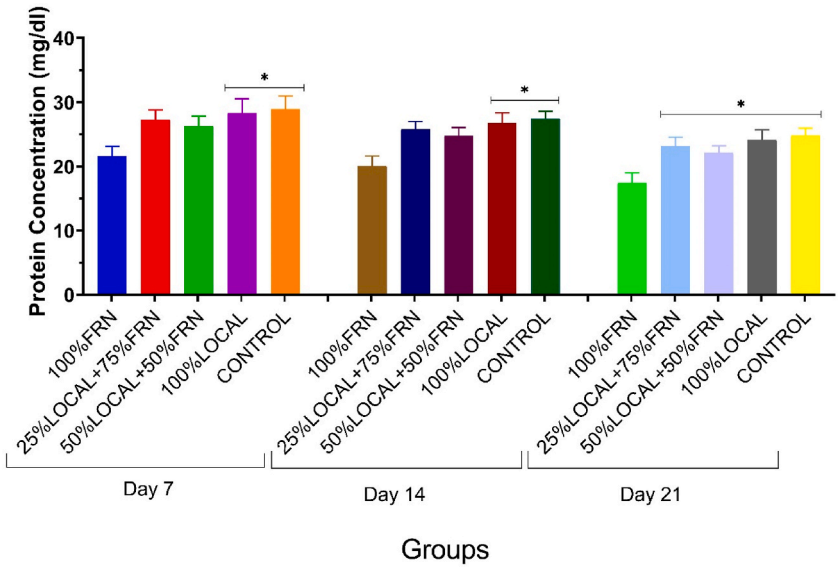

Fig. 7. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Kidney Protein Concentration on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

compositions during prolonged administration [41–43].

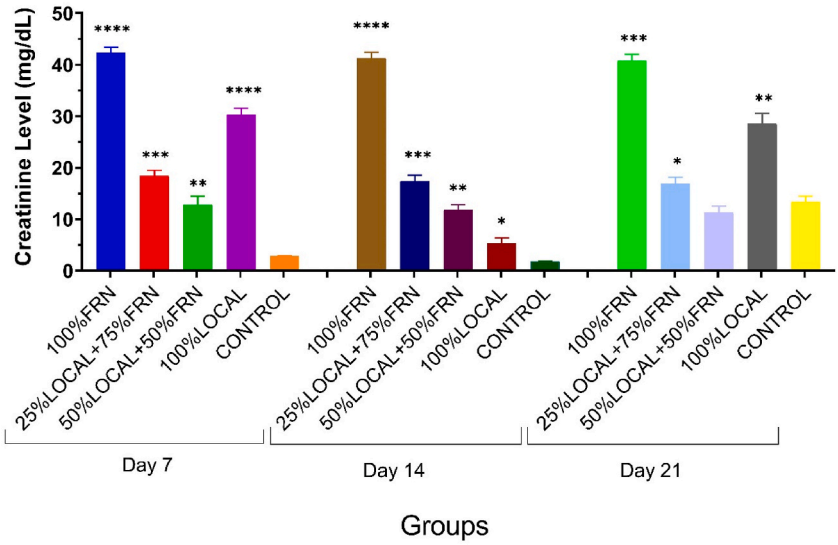

Fig. 8. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Kidney Creatinine Concentration on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

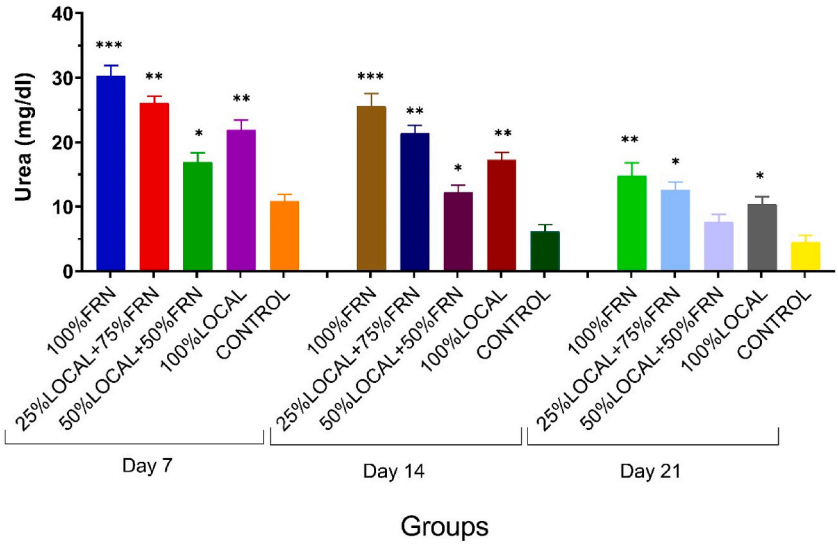

Fig. 9. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Kidney Urea Concentration on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

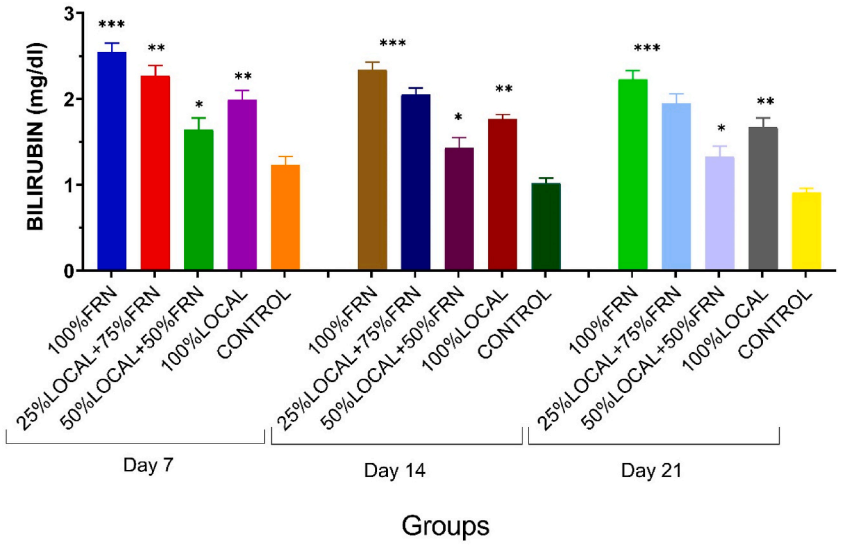

Fig. 10. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Kidney Bilirubin Concentration on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

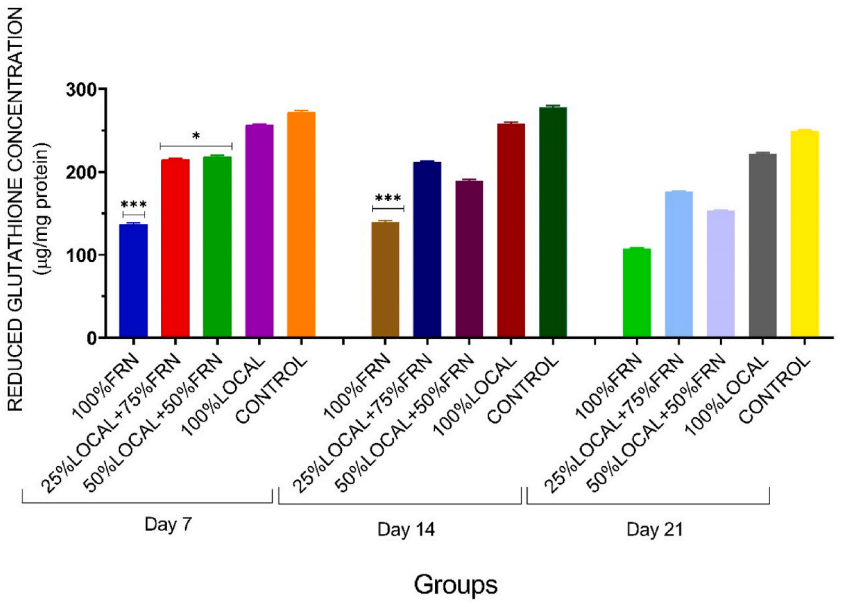

Fig. 11. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Serum GSH Concentration on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

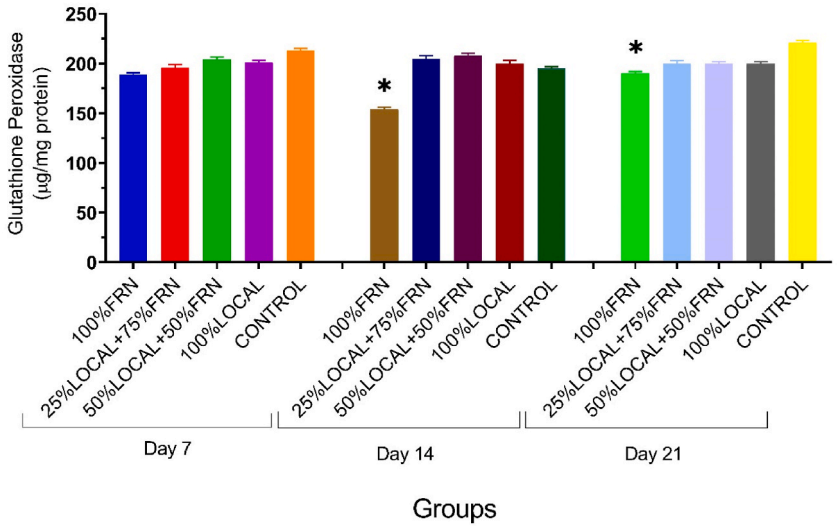

Fig. 12. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Serum Glutathione Peroxidase Activities on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

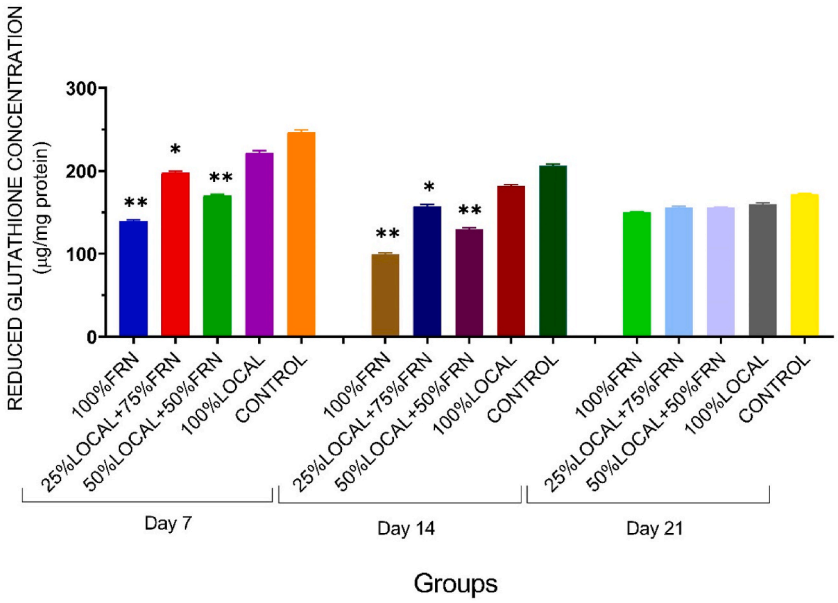

Fig. 13. Effects of varied compositions of analytical high grade and thermally calcined poultry manure-derived hydroxyapatite on Hepatic GSH Concentration on Days 7, 14 and 21. Results are expressed as mean ± Standard Deviation (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group.
Key: 100%FRN: administered 100 % AHGH at 100 mg/kg body weight
25%LOCAL+75%FRN: administered 25 % TCPM +75 % AHGH at 100 mg/kg body weight
50%LOCAL+50%FRN: administered 50 % TCPM +50 % AHGH at 100 mg/kg body weight
100%LOCAL: administered 100 % TCPM at 100 mg/kg body weight
CONTROL: normal control.

4.3. Microbiological evaluation

Prosthetic materials are usually in contact with the skin and, therefore are expected to possess good antimicrobial properties which will enhance the materials to reduce the chance of the infection [19]. In this study, the survival of *Staphylococcus aureus, S, epidermidis, Pseudomonas aeruginosa*, and *Candida albicans* in graded concentrations (10 %, 5 %, and 1 % weight/volume) of local materials used for prostheses over 48 h was carried out (Tables 4–7), forging materials was used as standard. It was noted that there was a gradual reduction in the bacterial and fungal load from 6 h, zero microbial counts were observed at 42 h in both local and foreign materials. The gradual reduction of microbial load with time and total death of microorganisms at 42 h compared with the samples with peptone could mean that the prosthetics materials lack nutrients for the sustenance of microbial growth and could possess antimicrobial properties. Hence, the findings suggest that the product's inclusion at various concentrations could not pose a negative effect on various pathology in the liver kidney, and blood.

5. Conclusion

The biosafety of thermally calcined poultry manure as a hydroxyapatite source for potential use as bone-making material using biochemical, toxicological and microbiological assessments was investigated in this study. From the results, the following conclusions are drawn.

- the thermally calcined poultry manure generally possessed good antioxidant properties with particularly good capacity to scavenge free radicals and reduce oxidative stress, both of which are crucial in preserving cellular integrity during bone formation and contribute to bone health
- The poultry manure treated at 750 $^{\circ}C$ had the most promising antioxidant properties compared to those treated at 800 and 850 $^{\circ}C$, and hence a more likely improved anti-toxicity potential.
- The analytical high-grade hydroxyapatite at (100 %) had a lower level of endogenous enzymes (GPX, GSH) when compared to the positive control while there were increased liver and serum enzymes (ALT, ALP, AST) activities with no variation with the total protein in the kidney.
- Relatively, the 50 % local+50 % FRN sample had the lowest ALP, ALT and AST activities, which may be indicative of its ability to maintain the index of hepatocellular injury better than the other blends of foreign and local hydroxyapatite samples. Meanwhile, the various blends of the analytical high-grade hydroxyapatite and thermally calcined poultry manure hydroxyapatite samples are safe compared to the normal control rats.
- The various blends of the analytical high grade and thermally calcined poultry manure-derived hydroxyapatite showed renal function parameters safe compared to the normal control rats with the blend of 50 % local+50 % FRN having the least creatinine, urea and bilirubin concentrations.
- The 100 % foreign (100 % AHGH) had the least serum and hepatic enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations, while 100 % local (100 % TCPM) had the highest serum and hepatic enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations. Additionally, the enzymatic (glutathione peroxidase) and non-enzymatic (reduced glutathione) antioxidant concentrations of the experimental animals administered foreign (100 % AHGH), local (100 % TCPM) or blends of foreign and local (AHGH + TCPM) hydroxyapatite, were significant when compared to normal control rats.
- Generally, the variation in all the treatment groups relative to the control had positive and better results across all test parameters during the acute toxicity period of 21 days.

Table 4
Survival of *Staphylococcus aureus* in graded concentrations of 750 $^{\circ}C$ calcined poultry manure (local material).

Time (hour)	Experimental groups/Microbial loads (Log cfu/ml)						
	1	2	3	4	5	6	7
Initial	2.31 ± 0.14^{a}	2.31 ± 0.13^{a}	2.32 ± 0.13^{a}	2.31 ± 0.13^{a}	2.30 ± 0.13^{a}	2.30 ± 0.12^{a}	0.00 ± 0.00^{b}
6	2.29 ± 0.13^{a}	2.30 ± 0.13^{a}	2.30 ± 0.13^{a}	2.30 ± 0.13^{a}	2.29 ± 0.13^{a}	2.29 ± 0.13^{a}	0.00 ± 0.00^{b}
12	2.17 ± 0.13^{a}	2.25 ± 0.13^{a}	2.27 ± 0.13^{a}	2.13 ± 0.12^{b}	2.55 ± 0.14^{a}	2.19 ± 0.13^{a}	0.00 ± 0.00^{c}
18	2.03 ± 0.11^{b}	2.08 ± 0.12^{b}	2.18 ± 0.12^{b}	1.99 ± 0.11^{b}	2.85 ± 0.16^{a}	2.01 ± 0.12^{b}	0.00 ± 0.00^{e}
24	$1.72 \pm 0.0.9^{b}$	1.96 ± 0.11^{b}	2.04 ± 0.12^{b}	1.32 ± 0.07^{c}	3.19 ± 0.18^{a}	1.69 ± 0.09^{b}	0.00 ± 0.00^{d}
30	$1.04 \pm 0.0.6^{c}$	1.55 ± 0.09^{b}	1.90 ± 0.11^{b}	0.00 ± 0.00^{d}	3.36 ± 0.19^{a}	0.92 ± 0.05^{c}	0.00 ± 0.00^{d}
36	0.00 ± 0.00^{c}	1.05 ± 0.06^{b}	1.58 ± 0.09^{b}	0.00 ± 0.00^{c}	3.66 ± 0.21^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
42	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.86 ± 0.05^{b}	0.00 ± 0.00^{c}	5.45 ± 0.31^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
48	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	5.45 ± 0.31^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Note: the results are presented as raw data of triplicate. 1: 1.0 g of sample A was dispensed into 9.0 ml of sterile distilled water in a test tube to make 10 % weight/volume; 2: 0.5 g of sample A was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume; 3: 0.1 g of sample A was dispensed into 9.9 ml of sterile distilled water in a test tube to make 1 % weight/volume; 4: 0.5 g of sample B was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – positive control; 5: 0.5 g of Peptone was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – Negative control; 6: Test tube containing 10 ml of sterile distilled water only; 7. Test tube containing 10 ml of sterile distilled water only.

Table 5
Survival of *S. epidermidis* in graded concentrations of 750 °*C* calcined poultry manure (local material).

Time (hour)	Experimental groups/Microbial loads (Log cfu/ml)						
	1	2	3	4	5	6	7
Initial	2.28 ± 0.13^{a}	2.31 ± 0.13^{a}	2.35 ± 0.14^{a}	2.22 ± 0.13^{b}	2.34 ± 0.14^{a}	2.29 ± 0.13^{a}	0.00 ± 0.00^{c}
6	2.07 ± 0.12^{b}	2.08 ± 0.12^{b}	2.11 ± 0.12^{a}	2.04 ± 0.12^{c}	2.15 ± 0.12^{a}	2.08 ± 0.12^{b}	0.00 ± 0.00^{d}
12	1.86 ± 0.11^{c}	2.03 ± 0.12^{b}	2.12 ± 0.12^{b}	1.75 ± 0.10^{c}	2.49 ± 0.14^{a}	2.04 ± 0.11^{b}	0.00 ± 0.00^{d}
18	1.83 ± 0.11^{d}	1.96 ± 0.11^{c}	2.07 ± 0.12^{b}	1.73 ± 0.10^{d}	2.55 ± 0.15^{a}	2.02 ± 0.12^{b}	0.00 ± 0.00^{e}
24	1.29 ± 0.07^{c}	1.46 ± 0.08^{c}	1.93 ± 0.11^{b}	1.07 ± 0.06^{d}	2.88 ± 0.17^{a}	1.32 ± 0.08^{c}	0.00 ± 0.00^{e}
30	1.01 ± 0.06^{c}	1.12 ± 0.06^{c}	1.39 ± 0.08^{b}	1.01 ± 0.06^{c}	3.45 ± 0.19^{a}	1.01 ± 0.06^{c}	0.00 ± 0.00^{d}
36	0.31 ± 0.02^{b}	0.37 ± 0.02^{b}	0.72 ± 0.04^{b}	0.30 ± 0.02^{b}	3.47 ± 0.20^{a}	0.37 ± 0.02^{b}	0.00 ± 0.00^{c}
42	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.31 ± 0.01^{b}	0.00 ± 0.00^{c}	3.58 ± 0.21^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
48	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	3.59 ± 0.21^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Note: the results are presented as raw data of triplicate. 1: 1.0 g of sample A was dispensed into 9.0 ml of sterile distilled water in a test tube to make 10 % weight/volume; 2: 0.5 g of sample A was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume; 3: 0.1 g of sample A was dispensed into 9.9 ml of sterile distilled water in a test tube to make 1 % weight/volume; 4: 0.5 g of sample B was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – positive control; 5: 0.5 g of Peptone was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – Negative control; 6: Test tube containing 10 ml of sterile distilled water only; 7. Test tube containing 10 ml of sterile distilled water only.

Table 6
Survival of *Pseudomonas aeruginosa* in graded concentrations of 750 °*C* calcined poultry manure (local material).

Time (hour)	Experimental groups/Microbial loads (Log cfu/ml)						
	1	2	3	4	5	6	7
Initial	2.39 ± 0.14^{a}	2.39 ± 0.14^{a}	2.39 ± 0.14^{a}	2.40 ± 0.14^{a}	2.40 ± 0.13^{a}	2.39 ± 0.14^{a}	0.00 ± 0.00^{b}
6	2.33 ± 0.13^{a}	2.33 ± 0.13^{a}	2.34 ± 0.14^{a}	2.32 ± 0.13^{a}	2.32 ± 0.13^{a}	2.34 ± 0.13^{a}	0.00 ± 0.00^{b}
12	2.32 ± 0.13^{a}	2.32 ± 0.13^{a}	2.32 ± 0.13^{a}	2.32 ± 0.13^{a}	2.31 ± 0.13^{a}	2.31 ± 0.13^{a}	0.00 ± 0.00^{b}
18	2.19 ± 0.13^{a}	2.22 ± 0.13^{a}	2.20 ± 0.13^{a}	2.19 ± 0.13^{a}	2.20 ± 0.12^{a}	2.24 ± 0.12^{a}	0.00 ± 0.00^{b}
24	1.91 ± 0.11^{b}	1.92 ± 0.11^{b}	1.93 ± 0.11^{b}	1.93 ± 0.11^{b}	2.66 ± 0.15^{a}	1.89 ± 0.11^{b}	0.00 ± 0.00^{c}
30	1.29 ± 0.07^{b}	1.40 ± 0.08^{b}	1.39 ± 0.08^{b}	1.33 ± 0.07^{b}	3.09 ± 0.17^{a}	1.33 ± 0.08^{b}	0.00 ± 0.00^{c}
36	0.75 ± 0.04^{b}	0.86 ± 0.05^{b}	0.90 ± 0.05^{b}	0.75 ± 0.04^{b}	3.40 ± 0.19^{a}	0.70 ± 0.04^{b}	0.00 ± 0.00^{c}
42	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	3.49 ± 0.2^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
48	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	3.60 ± 0.21^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Note: the results are presented as raw data of triplicate. 1: 1.0 g of sample A was dispensed into 9.0 ml of sterile distilled water in a test tube to make 10 % weight/volume; 2: 0.5 g of sample A was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume; 3: 0.1 g of sample A was dispensed into 9.9 ml of sterile distilled water in a test tube to make 1 % weight/volume; 4: 0.5 g of sample B was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – positive control; 5: 0.5 g of Peptone was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – Negative control; 6: Test tube containing 10 ml of sterile distilled water only; 7. Test tube containing 10 ml of sterile distilled water only.

Table 7
Survival of *Candida albicans* in graded concentrations of 750 °*C* calcined poultry manure (local material).

Time (hour)	Experimental groups/Microbial loads (Log cfu/ml)						
	1	2	3	4	5	6	7
Initial	2.42 ± 0.13^{a}	2.42 ± 0.14^{a}	2.42 ± 0.14^{a}	2.40 ± 0.14^{a}	2.42 ± 0.14^{a}	2.43 ± 0.14^{a}	0.00 ± 0.00^{b}
6	2.40 ± 0.14^{a}	2.41 ± 0.14^{a}	2.41 ± 0.14	2.39 ± 0.14^{a}	2.41 ± 0.14^{a}	2.41 ± 0.14^{a}	0.00 ± 0.00^{b}
12	2.39 ± 0.14^{a}	2.39 ± 0.14^{a}	2.40 ± 0.14^{a}	2.40 ± 0.13^{a}	2.40 ± 0.13^{a}	2.39 ± 0.14^{a}	0.00 ± 0.00^{b}
18	2.35 ± 0.12^{a}	2.35 ± 0.13^{a}	2.35 ± 0.13^{a}	2.35 ± 0.14^{a}	2.36 ± 0.15^{a}	2.31 ± 0.13^{a}	0.00 ± 0.00^{b}
24	2.10 ± 0.12^{b}	2.11 ± 0.12^{b}	2.11 ± 0.12^{a}	2.09 ± 0.13^{b}	2.71 ± 0.15^{a}	2.09 ± 0.12^{b}	0.00 ± 0.00^{c}
30	1.34 ± 0.08^{b}	1.36 ± 0.07^{b}	1.37 ± 0.08^{b}	1.33 ± 0.08^{b}	2.86 ± 0.16^{a}	1.33 ± 0.08^{b}	0.00 ± 0.00^{c}
36	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	2.93 ± 0.17^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
42	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	3.07 ± 0.18^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
48	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	3.16 ± 0.18^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Note: the results are presented as raw data of triplicate. 1: 1.0 g of sample A was dispensed into 9.0 ml of sterile distilled water in a test tube to make 10 % weight/volume; 2: 0.5 g of sample A was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume; 3: 0.1 g of sample A was dispensed into 9.9 ml of sterile distilled water in a test tube to make 1 % weight/volume; 4: 0.5 g of sample B was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – positive control; 5: 0.5 g of Peptone was dispensed into 9.5 ml of sterile distilled water in a test tube to make 5 % weight/volume – Negative control; 6: Test tube containing 10 ml of sterile distilled water only; 7. Test tube containing 10 ml of sterile distilled water only.

- The microbiological evaluation suggests that the calcined poultry manure inclusion at various concentrations could not pose a negative effect on various pathology in the liver, kidney, and blood.

Data and code availability statement

Data will be made available on request.

CRediT authorship contribution statement

Kenneth Kanayo Alaneme: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Sandra Boluwatife Fagbayi:** Methodology, Investigation. **Esther Emem Nwanna:** Writing – review & editing, Supervision. **Ochuko Mary Ojo:** Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Kenneth Alaneme reports financial support was provided by Tertiary Education Trust Fund. Kenneth Alaneme reports article publishing charges was provided by National Research Fund. None If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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