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Research Article

Assessment of Toxicity and Therapeutic Effects of Goose Bone in a Rat Model

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Goose bone is traditionally used in the treatment of many ailments including in bone fracture. The aim of the present study was to evaluate the subacute toxicity of goose bone in a rat model by investigating some hematological and biochemical parameters in rats. Subsequently, a histopathological study was performed to confirm the presence of pathological lesions in the rat's vital organs including the liver, kidney, heart, brain, pancreas, lung, spleen, and stomach. Adult Wistar rats were divided into four groups ($n = 8$) and were orally administrated with three doses (30, 60, and 120 mg/kg) of goose bone once daily for 21 days as compared to control animals (received only drinking water). Goose bone did not cause any significant changes on body weight, relative organ weight, and percentage water content at any of the administered doses. There were also no significant alterations in hematological parameters seen. All three doses administered significantly reduced the triglyceride levels as well as the atherogenic index of plasma (AIP). Animals treated with 120 mg/kg doses had significantly reduced alkaline phosphatase (ALP) activity as compared to the control group. There was no significant alteration on other serum biochemical parameters seen. Additionally, histopathological findings confirmed that there was no inflammatory, necrotic, or other toxicological feature seen for all three doses. It is concluded that goose bone is nontoxic and is safe for consumption besides having the potential to be investigated for the treatment of high triglycerides or liver-related disorder.

1. Introduction

From ancient times, various traditional drugs have been utilized in the treatment of several chronic diseases not only for curing but also for prevention of diseases. Goose bone is a traditional remedy utilized in Malay traditional medicine for several years. It has been widely applied for both external and internal complications, especially in the treatment of fractured bones. Nutritionally, goose bone is rich in protein (35%), carbohydrate (6%), fat (11%), and dietary fiber (5%). Additionally, there is also high calcium (14.6%) content with some other essential minerals including sodium (0.41%), potassium (0.14%), and iron (0.004%) identified. The folklore claims on its use may be substantiated by the fact that calcium is an important mineral for the bones, joints, nerve, and muscle [1–3].

Besides bone fractures, it is also believed that regular consumption of goose bone can boost the immune system. Goose bone is traditionally applied to ameliorate fever, particularly in children. Nowadays, goose bone is processed into powder and is inserted into capsules which are used for treatment. Nevertheless, although goose bone treatment is very popular in folk medicine, there are no scientific data to support its use or to evaluate its toxic effects. Therefore, the aim of this study is to assess the possible toxic effect of goose bone in a rat model by investigating the biochemical, hematological, and histopathological parameters.

2. Materials and Methods

2.1. Chemicals and Reagents. Ketamine hydrochloride injection was purchased from Popular Pharmaceuticals Ltd., Dhaka, Bangladesh. All chemicals and reagents used were of analytical grade.

2.2. Preparation of Goose Bone. The geese (*Anserinae anserine*) were reared under a tropical environment, in a farmhouse located in Pasir Mas, Kelantan, Malaysia. The geese were fed with mixtures of bran, inner husk of grain, *sago* (starchy food in hard white grains), vegetables, and rice. The geese were allowed water *ad libitum*. The geese were ready to be slaughtered based on an Islamic law or "halal" manner when they were one year old. Animals of both genders were used, as long as they have reached the optimum age and appeared to be of an average size of an adult goose.

Then, the geese were slaughtered and the bones were separated from the meat. For powder preparation, only the bones were used. The bones (except for the head and the feet) were burned, crushed, and grinded until it became a fine powder and the color turned blackish. Goose bone powder was prepared in a dry environment to avoid fungal growth and oxidization. Subsequently, the powder was inserted into hard-shelled capsules purchased from pharmacies and was packed in a plastic bottle together with a silica gel substance to preserve it. The capsules have been patented (PI2017701257).

2.3. Experimental Animals. Adult Wistar rats of both sexes (150 to 250 g) at 16–18 weeks were utilized. Animals were bred and reared in the animal house at the Department of Biochemistry and Molecular Biology, Jahangirnagar University, at a constant temperature ($23 \pm 2^{\circ}$C) and humidity (44% to 56%). The rats were kept in sterile plastic cages containing soft wood-chip bedding and were exposed to a natural 12 h day-night cycle. The rats were allowed free supply of water and a standard laboratory pellet diet. The experimental protocol was approved by the Biosafety, Biosecurity and Ethical Committee of Jahangirnagar University, Savar, Dhaka, Bangladesh (ethical number: BBEC,JU/M2019 (7) 4) which was in agreement with the internationally established principles of the US guidelines.

2.4. Experimental Design. The rats ($n = 32$) were familiarized to the lab environment 7 days before experimentation. The animals were allotted into four groups ($n = 8$ rats each) with a balance mix of gender [4].

Group A (normal control): rats were given a normal diet and water *ad libitum*.

Group B (treatment 1): rats administered with goose bone (30 mg/kg) dissolved in normal saline for 21 days (again, all rats were given a normal diet and water *ad libitum*).

Group C (treatment 2): rats administered with goose bone (60 mg/kg) dissolved in normal saline for 21 days (again, all rats were given a normal diet and water *ad libitum*).

Group D (treatment 3): rats administered with goose bone (120 mg/kg) dissolved in normal saline for 21 days (again, all rats were given a normal diet and water *ad libitum*).

The doses were chosen based on the normal doses taken by human which is approximately 500 mg/day. Calculation of dose in animals based on human dose was done according to the formula as suggested by Reagan-Shaw et al. [5]:

$$\begin{aligned}
\text{human equivalent dose (mg/kg)} &= \text{rat's dose (mg/kg)} \times \left(\frac{\text{Rat } K_m}{\text{human } K_m}\right),\\
\text{rat's dose (mg/kg)} &= \frac{\text{human equivalent dose (mg/kg)}}{\left(\text{rat } K_m/\text{human } K_m\right)},\\
\text{rat's dose (mg/kg)} &= \frac{8.33\,(\text{mg/kg})}{(6/37)}\left[\frac{500\,\text{mg}}{60\,\text{kg}} = 8.33\,\text{mg/kg}\right],\\
\text{rat's dose (mg/kg)} &= 51.37\,\text{mg/kg},
\end{aligned} \tag{1}$$

where K_m = correction factor which is estimated by dividing the average body weight (kg) of the species to its body surface area (m^2). Assuming the average human body weight is 60 kg with a body surface area of 1.62 m^2, K_m factor for human is calculated by dividing 60 by 1.62, which is 37 (the K_m value for human is 37 and that for rat is 6).

Based on the calculated dose of 51.37 mg/kg, a rounded value at 60 mg/kg was taken as the middle dose range. From this value, the low dose of 30 mg/kg and a high dose at 120 mg/kg were selected. During the experimental period, the animals were monitored for behavioral changes in their feeding and drinking habits and for some physiological

changes including reduced activity and diarrhea in order to detect any sign of abnormalities.

2.5. Harvesting of Organs. At the completion of the experiment, the animals were not given any food or water for one day before ketamine hydrochloride injection (500 mg/kg) [6] via the intraperitoneal veins. Subsequently, blood (5 mL) was taken from the inferior vena cava and was transferred to two tubes. The first tube contained ethylene diamine tetraacetic acid (EDTA) for hematological analyses while the second tube was a plain tube for serum biochemical analyses. The harvested organs were weighed and were kept in 10% formalin for subsequent histopathological examination.

2.6. Measurement of Body and Relative Organ Weights as well as Percentage Water Content. The animal's body weights were measured weekly throughout the experimental period. For measurement of relative organ weight and percentage of water content, tissue samples (liver, kidney, heart, lung, spleen, caecum, pancreas, brain, testes, thymus, caput, stomach, ovary, cowper's gland, and fallopian tube) were removed and weighed as soon as sacrifice. The relative organ weight was estimated by dividing the weight of each organ with the final body weight of each animal based on the following formula [7]:

$$\text{relative organ weight } (\%) = \frac{\text{weight of wet organ}}{\text{rat's body weight}} \times 100. \tag{2}$$

The percentage of water content was estimated based on every wet organ by subtracting the dry weight of each organ, respectively [8].

2.7. Preparation of Serum. Following sacrifice, the blood samples were transferred to dry test tubes and were left to coagulate naturally for approximately 30 min. Serum was yielded following centrifugation (2000 rpm × 10 min).

2.8. Hematological Parameters. The blood parameters were analyzed using an automated hematology analyzer (8000i, Sysmex, Japan) for all groups. These included determination of eosinophils, neutrophils, white blood cells (WBCs), monocytes, lymphocytes, red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH), platelets (PLT), mean corpuscular hemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), red cell distribution width-standard deviation (RDW-SD), red cell distribution width-coefficient of variation (RDW-CV), mean platelets volume (MPV), platelets distribution width (PDW), platelets larger cell ratio (P-LCR), and procalcitonin (PCT).

2.9. Serum Biochemical Analysis. Liver function tests (aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH), total protein (TP), total bilirubin (TB), albumin (ALB), globulin (GLB), and albumin/globulin (A/G) ratio); kidney function tests (uric acid, urea, and creatinine); lipid profiles (triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C)), serum electrolytes including sodium (Na^+), chloride (Cl^-), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and phosphate (PO^{4+}) ions, pancreatic function tests (the enzymes such as lipase and amylase), and also glycemic condition such as serum glucose (GLU) concentrations were established based on standard tests using an automated chemistry analyzer (Dimension EXL with LM Integrated Chemistry System, Siemens Medical Solutions Inc., USA). Another important factor lipid profile such as serum low-density lipoprotein cholesterol (LDL-C) level was established according to the Friedewald formula [9]:

$$\text{LDL-C} = \frac{\text{TC} - \text{HDL-C} - \text{TG}}{5}. \tag{3}$$

Atherogenic indices including cardiac risk ratio (CRR), atherogenic index of plasma (AIP), atherogenic coefficient (AC), and Castelli's risk index-2 (CRI-2) were estimated according to the following formulae [10--13]:

$$\begin{aligned} \text{CRR} &= \frac{\text{TC}}{\text{HDL-C}}, \\ \text{AC} &= \frac{(\text{TC} - \text{HDL-C})}{\text{HDL-C}}, \\ \text{AIP} &= \log\left(\frac{\text{TG}}{\text{HDL-C}}\right), \\ \text{CRI-2} &= \frac{\text{LDL-C}}{\text{HDL-C}}. \end{aligned} \tag{4}$$

2.10. Histopathological Findings. The liver, brain, spleen, lung, stomach, kidney, heart, and pancreas tissue samples were fixed in formalin (10%) followed by paraffin embedding fixing. The specimens were sliced into slices of 5 μm thickness by means of a rotary microtome. The specimens were then tainted using hematoxylin and eosin dye [14]. Photomicrographs were taken by using a normal-spectrum fluorescence microscope (Olympus DP 72) at 40x magnification. The microscope was connected to a digital camera (Olympus, Tokyo, Japan). The pathologist who conducted the histopathological examination was unaware to the treatment groups.

2.11. Statistical Analysis. The results were expressed as mean ± standard deviation (SD). Data were evaluated by using SPSS (version 16.0, IBM Corporation, New York, USA). Data from the treatment groups were evaluated against the control by using a one-way ANOVA followed by Dunnett's multiple comparison tests where $p < 0.05$ was deemed as statistically significant.

3. Results

3.1. The Effects of Goose Bone on Body Weight, Relative Organ Weight, and Percent Water Content of Rat's Organs. During the experimental period, rats' body weight increased gradually although these changes were not significantly different from the first to the third week (Figure 1) as compared to the control. There was also no significant difference in both relative organ weight and percentage of water content of different rat's organs (Tables 1 and 2).

3.2. The Effects of Goose Bone on the Hematological Parameters. There was no significant difference among the control and treatments groups in terms of hematological parameters (Table 3).

No significant changes were established when all treatment groups were compared against the control group by using one-way ANOVA followed by Dunnett's multiple comparison tests.

3.3. The Effects of Goose Bone on Serum Biochemical Parameters. Goose bone did not confer any effects on parameters for liver function including ALT, AST, ALP, GGT, LDH activities, and TB levels. In fact, ALP levels were significantly decreased at the highest dose (120 mg/kg) while ALT, AST, TP, ALB, GLB, and A/G which are important biomarkers of liver function remained unchanged at the three dose levels (Figure 2 and Table 4) indicating that goose bone does not affect the function of this major organ and may help to protect against liver damage especially when used in high doses.

The effects of goose bone on the renal function were analyzed by measuring serum urea, uric acid, and creatinine levels and major electrolytes such as Na^+, K^+, Cl^-, Mg^{2+}, P, and Ca^{2+} levels (Table 5). All three doses did not significantly affect the biomarkers for renal function or alter body electrolytes levels.

Lipid profiles (TC, TG, HDL-C, and LDL-C) and atherogenic indices (CRR, AC, AIP, and CRI-2) are reliable markers for cardiovascular diseases. Goose bone significantly reduced serum TG levels (Figure 3) and AIP at all three doses (Table 6).

Goose bone did not affect pancreatic function and blood glucose levels where there were no significant changes on the serum amylase, lipase, and glucose levels (Table 7).

3.4. Histopathological Examination. Histopathological examination of the liver, kidney, lung, brain, stomach, pancreas, spleen, and heart did not show any morphological and pathological changes following the administration of goose bone in all three doses (30, 60, and 120 mg/kg) when compared with the control (Figure 4) again confirming its safety on these organs.

4. Discussion

To our knowledge, this is the first study to confirm the safety profile of goose bone. Daily oral administration of goose bone in a rat model at 30, 60, and 120 mg/kg for 21 days did not cause mortality, change in food habit and water intake, body and organ weight, and biochemical findings, confirming that goose bone is safe when administered at these doses. Additionally, there were no histopathological changes in the organs including the liver, kidney, heart, pancreas, stomach, brain, lung, and spleen confirming that goose bone is safe.

Toxic agents are responsible for abnormal metabolic reactions in the body that may alter the growth of animals and cause deposition of water in vital organs like the liver, heart, lung, spleen, brain, kidney, and reproductive organs including the testes, caput, cowper gland (in males), and ovary and fallopian tube (in females) [15]. Therefore, body and relative organ weights as well as percentage water content are important parameters for toxicological studies. In the present study, no significant difference was observed in body and relative organ weights as well as percentage water content were compared with those of the control group during the experimental period indicating that goose bone does not affect these parameters and rats' homeostasis.

Analysis of hematological parameter is important when assessing physiological and pathological conditions in the body. Deprivation, stress, abnormal body metabolic activities, and response of the body to injury or lesions are closely-related with abnormal findings for hematological parameter [16]. In fact, hematological parameters like RBCs, HGB, HCT, MCV, MCH, MCHC, RDW-SD, and RDW-CV can help to unravel important information about anemic condition and other erythrocyte cell-related disorders such as polycythemia and thalassemia. WBCs and its differential count including neutrophils, lymphocytes, eosinophils, monocytes, and basophils play important roles in the immune system in combating infections where the overproduction of these parameters are important hallmarks of inflammation and stress-related disorders [17, 18]. Platelets and platelet indices including MPV, PDW, and P-LCR are important indicators for early diagnosis of thromboembolic, atherosclerosis, and ischemic heart disease [19–21]. The levels of both ESR and PCT can yield important information about inflammatory condition in the body. In our present study, there were no significant changes in the hematological findings indicating that administration of goose bone causes no toxic effect on the animal's body.

The liver is the major site for detoxification and elimination of toxic substance from the body. Any foreign substances that may affect the liver function can alter ALT, AST, ALP, GGT, TB, TP, ALB, GLB, and albumin-globulin (A/G) ratio activities [22, 23]. Generally, damage to the parenchymal liver cell is associated with elevation of these enzymes in the blood [24]. ALT and AST are the major intracellular cytoplasmic enzymes where their elevation in the serum indicates some injuries in the liver cells [25]. On the other hand, ALP and GGT are indicators for hepatobiliary damage [26] while AST and LDH, mostly found in the heart, liver, kidney, and skeletal muscle, are indicators of both myocardium and hepatocellular injuries [23]. TB indicates liver injury or necrosis and measures the binding, conjugation, and excretory capacities of the hepatocytes

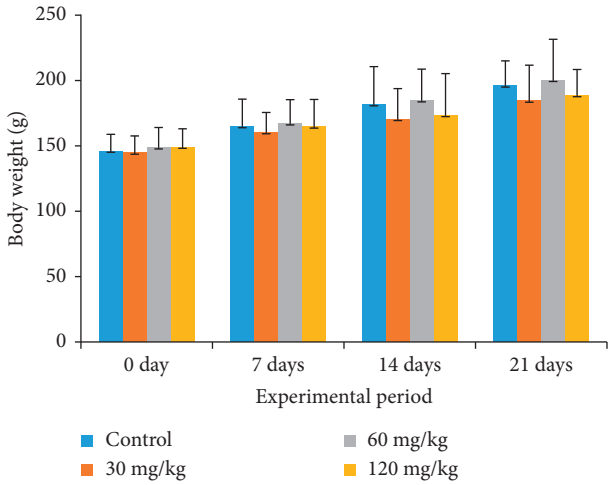

Figure 1: The effects of goose bone on body weight (g) gain. Results are expressed as mean ± SD. ($n = 8$). Nonsignificant changes were found when all treatment groups were compared against the control group ($p < 0.05$) as determined by one-way ANOVA followed by Dunnett's multiple comparison tests.

Table 1: The effects of goose bone on relative organ weight.

Organs	Control	30 mg/kg	60 mg/kg	120 mg/kg
Heart	0.384 ± 0.01	0.359 ± 0.01	0.367 ± 0.01	0.320 ± 0.02
Kidney	0.781 ± 0.07	0.807 ± 0.03	0.801 ± 0.05	0.795 ± 0.07
Lung	0.671 ± 0.87	0.713 ± 0.14	0.657 ± 0.13	0.634 ± 0.02
Liver	3.732 ± 0.40	3.890 ± 0.26	3.849 ± 0.11	3.742 ± 0.45
Spleen	0.226 ± 0.01	0.233 ± 0.02	0.232 ± 0.01	0.211 ± 0.01
Caecum	0.276 ± 0.02	0.265 ± 0.04	0.257 ± 0.04	0.255 ± 0.05
Pancreas	0.129 ± 0.06	0.156 ± 0.04	0.172 ± 0.11	0.124 ± 0.06
Brain	0.864 ± 0.01	0.874 ± 0.05	0.871 ± 0.07	0.829 ± 0.15
Testes	0.113 ± 0.09	0.121 ± 0.08	0.118 ± 0.09	0.097 ± 0.06
Thymus	0.133 ± 0.01	0.149 ± 0.06	0.139 ± 0.07	0.147 ± 0.05
Caput	0.287 ± 0.07	0.268 ± 0.09	0.254 ± 0.05	0.276 ± 0.05
Cowper gland	0.151 ± 0.01	0.159 ± 0.01	0.156 ± 0.00	0.152 ± 0.01
Stomach	0.659 ± 0.03	0.630 ± 0.01	0.590 ± 0.03	0.608 ± 0.02
Ovary	0.075 ± 0.03	0.077 ± 0.01	0.070 ± 0.02	0.072 ± 0.03
Fallopian tube	0.306 ± 0.01	0.280 ± 0.01	0.282 ± 0.01	0.270 ± 0.01

Results are expressed as mean ± SD ($n = 8$). Nonsignificant changes were found when all treatment groups were compared against the control group ($p < 0.05$) as determined using one-way ANOVA followed by Dunnett's multiple comparison tests.

[27]. TP and albumin activities are important indicators of liver function since they provide important information on the severity of liver necrosis and the capacity for protein synthesis [28]. In this study, daily oral administration of goose bone to rats at the three doses extracts no adverse effect on liver function markers. Furthermore, microscopic examinations of the rats' liver did not confer any cellular necrosis or centrilobular degenerative changes, infiltration of any inflammatory cells, or changes in cell sizes and architecture, thus further confirming its safety as also supported by the histopathological findings.

Urea, uric acid, and creatinine are waste products from protein metabolism that are eventually expelled via the

Table 2: The effects of goose bone on the percentage of water content of different organs.

Organs	Control	30 mg/kg	60 mg/kg	120 mg/kg
Heart	52.90 ± 1.13	55.23 ± 0.83	53.95 ± 2.42	52.84 ± 1.61
Kidney	52.01 ± 5.55	52.32 ± 8.15	51.50 ± 5.16	53.93 ± 3.76
Lung	61.03 ± 1.21	61.84 ± 3.45	61.87 ± 2.73	59.75 ± 1.62
Liver	60.63 ± 4.47	54.40 ± 5.11	54.87 ± 1.24	53.71 ± 1.38
Spleen	47.75 ± 0.67	50.23 ± 2.38	51.38 ± 3.38	46.59 ± 1.85
Caecum	65.55 ± 1.12	63.52 ± 1.92	64.58 ± 1.43	63.82 ± 1.73
Pancreas	41.68 ± 8.65	40.26 ± 2.81	38.27 ± 1.32	37.81 ± 0.37
Brain	57.78 ± 7.46	53.74 ± 5.82	45.51 ± 1.80	32.37 ± 10.58
Testes	41.53 ± 6.30	55.32 ± 3.87	50.13 ± 1.67	31.47 ± 3.49
Thymus	59.58 ± 1.12	54.81 ± 1.01	58.22 ± 1.13	55.71 ± 1.45
Caput	61.30 ± 1.89	65.24 ± 2.00	64.22 ± 1.65	64.85 ± 1.91
Cowper gland	67.68 ± 1.19	62.13 ± 2.68	66.21 ± 1.90	65.98 ± 2.07
Stomach	60.91 ± 2.45	58.75 ± 1.95	58.15 ± 4.21	56.26 ± 1.23
Ovary	56.65 ± 2.76	56.34 ± 1.59	54.20 ± 1.39	57.14 ± 1.87
Fallopian tube	39.01 ± 0.01	29.88 ± 0.01	28.13 ± 0.01	35.20 ± 0.01

Results are expressed as mean ± SD ($n = 8$). Nonsignificant changes were found when all treatment groups were compared against the control group ($p < 0.05$) as determined using a one-way ANOVA followed by Dunnett's multiple comparison tests.

kidneys. Therefore, increase in their serum levels indicates functional damage to kidneys [29, 30]. In our study, there were no significant changes in these renal biomarkers following administration of low to highest dose ranges of goose bone, indicating that goose bone is not toxic to the kidneys as further confirmed by histopathological observations of the kidney tissues.

Serum lipid profile is measured to predict hyperlipoproteinemia, triglyceridemia, liver obstruction, fatty liver disease, and pancreatitis [31, 32]. Elevated levels of plasma triglyceride level are an important risk factor for cardiovascular disease [33] and are related to hypertension [34], diabetes mellitus, and obesity [35]. In fact, high plasma total cholesterol levels are linked to the development of atherosclerosis and other cardiovascular-related disease [36]. In addition, low HDL-C and high LDL-C are imperative predictors for cardiovascular disease [37] where high HDL-C shows a protective role by enhancing cholesterol transport via collection of excess cholesterol from peripheral tissue. On the other hand, atherogenic indices (CRR, ACC, RIP, and CRI-2) are derived from lipid panels where the increase in the atherogenic indices is associated with the development of cardiovascular diseases. In our study, goose bone significantly reduced serum TG levels and AIP at all three administered doses while other parameters of lipid profile and atherogenic indices remain unchanged. In some previous reports, natural products that are high in omega-3 fatty acids have been reported to reduce TG levels. For example, Shearer et al. suggested that fish oil containing omega-3 fatty acid can reduce TG level [38] as also with goose bone which is high in lipid content that is healthy to the body. Similarly, since AIP is a logarithmic ratio of TG and HDL-C, when TG levels are reduced, AIP is also decreased as seen in this case.

Table 3: The effects of goose bone on the hematological parameters in whole blood.

Hematological parameters	Control	30 mg/kg	60 mg/kg	120 mg/kg
WBC ($\times 10^9$/L)	6.15 ± 1.31	6.61 ± 0.71	6.40 ± 1.30	4.87 ± 1.03
NEUT (%)	15.75 ± 1.89	14.25 ± 2.50	15.75 ± 1.89	19.75 ± 3.59
LYMPH (%)	81.25 ± 2.87	84.25 ± 2.63	81.25 ± 2.87	76.75 ± 3.86
Mono (%)	1.65 ± 0.39	1.35 ± 0.17	1.65 ± 0.39	1.28 ± 0.22
Eo (%)	1.02 ± 0.36	1.30 ± 0.29	1.18 ± 0.35	1.05 ± 0.13
RBC ($\times 10^{12}$/L)	6.12 ± 2.36	6.11 ± 2.36	6.12 ± 2.36	7.70 ± 0.49
HGB (g/dl)	13.80 ± 1.11	13.80 ± 1.17	13.80 ± 1.12	14.40 ± 0.74
ESR (mm)	3.40 ± 0.11	4.10 ± 0.31	3.90 ± 0.42	4.00 ± 0.34
HCT (%)	35.85 ± 13.85	35.85 ± 13.85	35.85 ± 13.85	44.35 ± 2.79
MCV (fL)	58.70 ± 1.24	58.70 ± 1.24	58.70 ± 1.24	57.63 ± 1.31
MCH (pg)	18.70 ± 0.76	18.70 ± 0.76	18.70 ± 0.76	18.70 ± 0.26
MCHC (g/dl)	31.88 ± 1.23	31.88 ± 1.23	31.88 ± 1.23	32.50 ± 0.85
PLT ($\times 10^9$/L)	692.00 ± 267.70	692.00 ± 267.71	692.00 ± 267.71	803.75 ± 86.32
RDW-SD (fL)	27.70 ± 2.32	27.70 ± 2.32	27.70 ± 2.32	27.53 ± 3.12
RDW-CV (%)	14.25 ± 2.18	14.25 ± 2.18	14.25 ± 2.18	15.28 ± 2.46
PDW (fL)	8.80 ± 0.27	8.80 ± 0.27	8.80 ± 0.27	8.93 ± 0.33
MPV (fL)	8.40 ± 0.54	8.40 ± 0.51	8.40 ± 0.51	8.18 ± 0.26
P-LCR (%)	12.80 ± 2.80	12.80 ± 2.79	12.80 ± 2.79	11.30 ± 1.90
PCT (%)	0.58 ± 0.21	0.58 ± 0.21	0.58 ± 0.21	0.66 ± 0.09

Results are expressed as mean ± SD ($n = 8$). HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelets, RDW-SD: red cell distribution width-standard deviation, RDW-CV: red cell distribution width-coefficient of variation, PDW-CV: platelets distribution width, ESR: erythrocyte sedimentation rate, MPV: mean platelets volume, P-LCR: platelets larger cell ratio, and PCT: procalcitonin.

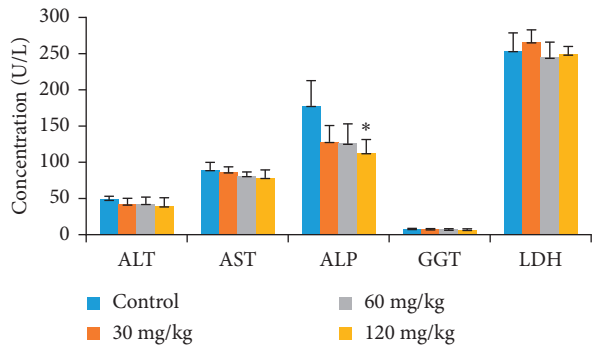

Figure 2: The effects of goose bone on serum hepatic marker enzymes. Results are expressed as mean ± SD ($n = 8$). *denotes level of significant difference when compared to the control group as determined using a one-way ANOVA followed by Dunnett's multiple comparison tests.

Serum amylase and lipase are two major digestive enzymes in animals. Amylase acts on starch in food, breaking it down into smaller carbohydrate molecules while lipase breaks down dietary fats into smaller molecules called fatty acids and glycerol. Both are important biomarkers for diagnosis of acute pancreatitis as they are synthesized and secreted into the intestinal lumen to be released into the circulation in catalytically active forms [39]. However, acute pancreatitis lipase is a more reliable diagnostic marker than amylase for its lipolytic activity and can be assayed very rapidly [40, 41]. The blood glucose level can indicate body utilization of glucose. Estimation of blood glucose level is the first step in diagnoses of diabetes mellitus, since it is a reliable marker, the levels of which are increased in diabetics following meal intake [42].

Table 4: The effects of goose bone on serum total bilirubin (TB), total protein (TP), albumin (ALB), globulin (GLB), and albumin/globulin (A/G) ratio.

Biochemical parameters	Control	30 mg/kg	60 mg/kg	120 mg/kg
TB (mg/dl)	0.20 ± 0.07	0.17 ± 0.04	0.20 ± 0.07	0.22 ± 0.04
TP (g/L)	5.45 ± 0.33	5.48 ± 0.27	5.35 ± 0.44	5.34 ± 0.38
ALB (g/L)	2.70 ± 0.14	2.66 ± 0.14	2.56 ± 0.30	2.71 ± 0.21
GLB (g/L)	2.75 ± 0.18	2.82 ± 0.15	2.79 ± 0.23	2.63 ± 0.17
A/G	0.98 ± 0.01	0.94 ± 0.04	0.92 ± 0.10	1.02 ± 0.02

Results are expressed as mean ± SD ($n = 6$). No significant changes were established when all treatment groups were compared against the control group by using one-way ANOVA followed by Dunnett's multiple comparison tests.

Another highlight of our research was the histopathological study that further confirmed the nontoxic effect of goose bone. Toxic substances cause degenerative necrosis of hepatocytes, vacuolization of the hepatic lobules [43], degeneration of the kidney glomeruli with inflammatory infiltrates [44], massive separation of cardiac muscle necrotic damage to the myocytes of the heart tissue [45] and islets of Langerhans of pancreas [46], neuronal degeneration of brain [47], damage of alveoli of lung [48], inflammation of the mucous layers of stomach [49], and damage of the hematopoietic and lymphoid elements of spleen [50]. The histopathological findings of the major organs provide a further strong support on its safety. Overall, our study confirms that goose bone is nontoxic, safe, and beneficial to some extent (especially on the liver and hematological profiles) to be consumed at the normal doses taken in humans.

Our study has some limitations. Since the effects of goose bone were investigated only for 21 days, its subchronic and

Table 5: The effects of goose bone on serum renal markers.

Biochemical parameters	Control	30 mg/kg	60 mg/kg	120 mg/kg
Creatinine (mmol/L)	0.35 ± 0.02	0.37 ± 0.04	0.39 ± 0.03	0.36 ± 0.01
Urea (mmol/L)	35.10 ± 1.64	28.20 ± 0.51	39.72 ± 5.07	36.81 ± 6.23
UA (mmol/L)	0.45 ± 0.07	0.43 ± 0.09	0.29 ± 0.14	0.33 ± 0.11
Na^+ (mmol/L)	138.25 ± 6.49	141.25 ± 0.82	141.00 ± 3.67	145.50 ± 4.97
K^+ (mmol/L)	3.72 ± 0.31	4.02 ± 1.23	4.22 ± 0.64	4.62 ± 0.92
Cl^-(mmol/L)	101.00 ± 5.74	104.75 ± 3.89	104.25 ± 2.58	107.00 ± 3.53
PO^{4+} (mmol/L)	8.20 ± 0.75	9.45 ± 1.67	8.61 ± 1.47	8.30 ± 1.06
Ca^{2+} (mmol/L)	10.26 ± 0.78	11.03 ± 1.09	11.05 ± 1.12	11.47 ± 0.52
Mg^{2+} (mmol/L)	0.75 ± 0.06	0.69 ± 0.07	0.80 ± 0.11	0.78 ± 0.08

Results are expressed as mean ± SD ($n = 8$). *The level of significant difference when compared to the control group as determined by using one-way ANOVA followed by Dunnett's multiple comparison tests.

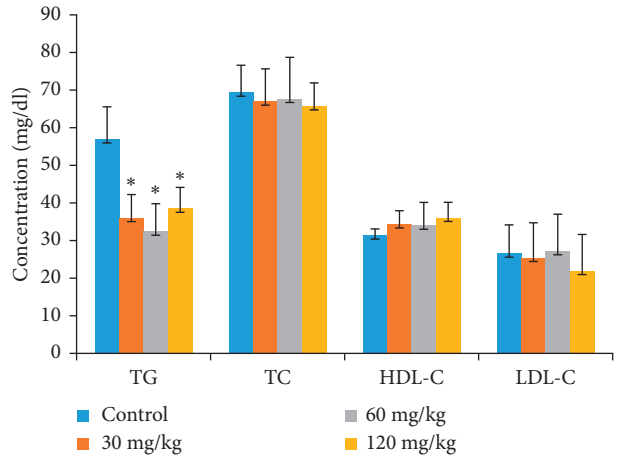

Figure 3: The effects of goose bone on lipid profile. Results are expressed as mean ± SD ($n = 8$). *denotes level of significant difference when compared to the control group as determined using a one-way ANOVA followed by Dunnett's multiple comparison tests.

Table 6: The effects of goose bone on atherogenic indices.

Biochemical parameters	Control	30 mg/kg	60 mg/kg	120 mg/kg
CRR	2.21 ± 0.17	1.96 ± 0.26	2.02 ± 0.30	1.86 ± 0.34
ACC	1.20 ± 0.17	0.96 ± 0.26	1.02 ± 0.30	0.86 ± 0.34
AIP	0.25 ± 0.08	0.21 ± 0.01*	0.20 ± 0.07*	0.18 ± 0.02*
CRI-2	0.84 ± 0.22	0.75 ± 0.28	0.83 ± 0.28	0.64 ± 0.30

Results are expressed as mean ± SD ($n = 8$). *denotes level of significant difference when compared to the control group as determined using a one-way ANOVA followed by Dunnett's multiple comparison tests.

Table 7: The effects of goose bone on pancreatic function.

Biochemical parameters	Control	30 mg/kg	60 mg/kg	120 mg/kg
Amylase (U/L)	2145.25 ± 403.83	2080.00 ± 565.73	2196.00 ± 615.50	2070.50 ± 388.30
Lipase (U/L)	27.13 ± 2.61	29.43 ± 2.09	24.56 ± 2.91	25.86 ± 3.60
Glucose (mmol/L)	10.27 ± 0.88	10.59 ± 0.47	9.47 ± 1.11	9.64 ± 1.54

Results are expressed as mean ± SD ($n = 8$). No significant changes were found when all treatment groups were compared with the control group ($p < 0.05$) as determined by using one-way ANOVA followed by Dunnett's multiple comparison tests.

chronic effects were not investigated. Therefore, future studies should be conducted to investigate the long-term effects of goose bone. Additionally, the dose can be increased based on the Organization for Corporation and Development (OECD) guideline in order to have a more rigorous test. Finally, the use of Hematoxylin-Eosin-Saffron

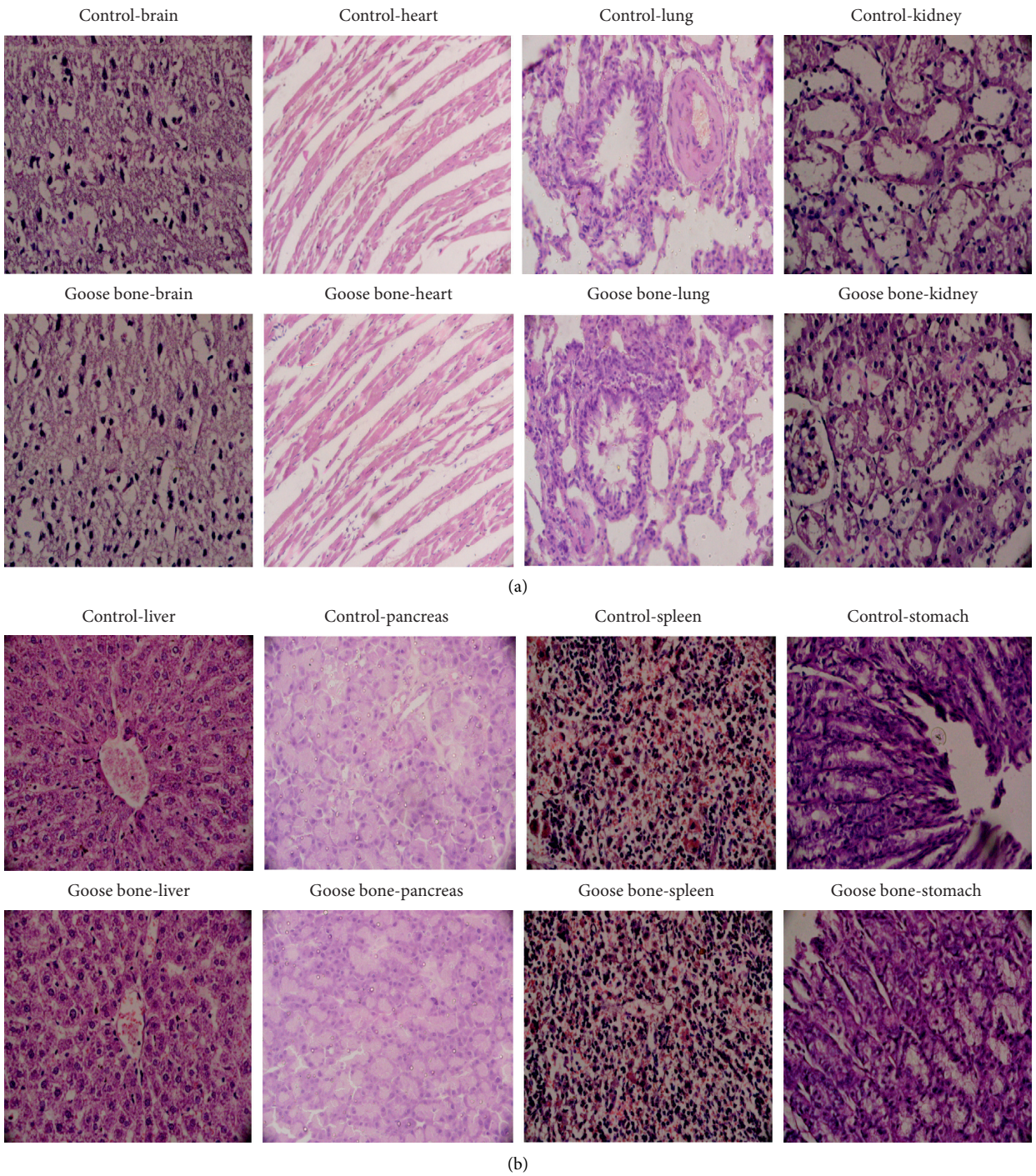

Figure 4: Histopathological photomicrographs of control and treatment groups (120 mg/kg) (40x magnification, scale bar: 20 μm). No morphological and pathological changes were observed between the control and goose bone-administered groups.

may be more ideal in histopathological analysis since saffron stains collagen fibers in the tissues besides contributing to complete removal of eosin.

5. Conclusion

Goose bone is nontoxic and is safe for consumption at the investigated doses. Oral administration of goose bone up to 120 mg/kg did not significantly affect body weight, relative organ weight profile, and percentage water content of vital organs as well as physiological, hematological, and biochemical abnormalities over the three-week treatment period. The histopathological findings on the major organs further confirm its safety. Additionally, goose bone may ameliorate high triglyceride and ALP-related disorders.

Data Availability

The data used to support the findings of this study are enclosed within the article. Additional data are accessible from the corresponding author upon request.

Conflicts of Interest

The goose bone capsules studied in this research were provided by Star Goose Enterprise, who hold a patent (PI2017701257) and provided financial support.

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