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The Effect of Bovine Femur and Rib Bone Extract on α-Amylase Enzyme and Blood Sugar Levels in Streptozotocin (STZ)-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a non-communicable disease that remains a global health problem with increasing prevalence, and the search for natural sources that may help regulate blood glucose levels is ongoing. Therefore, this study aimed to develop a protein-rich extract from bovine femur and rib bones prepared by the boiling technique for 24 hours, and evaluate its effects on fasting blood glucose (FBG) in diabetic rats and its inhibitory activity against α-amylase. Twenty-eight Wistar rats were divided into seven groups: Normal group (NG): no streptozotocin (STZ) induction; Negative control group (NC): induced with STZ (50 mg/kg) without treatment; Positive control group (PC): STZ induction + acarbose (0.9 mg); Treatment groups: STZ induction + bone extracts at 500 mg/kg (R1 and F1) and 1000 mg/kg (F2 and R2) orally, once daily for 30 days. FBG were assessed before induction (day 0), after induction (day 6), and 30 days post-treatment. The results showed that FTIR analysis revealed a characteristic amide absorption consistent with proteins and peptides, and the extracts demonstrated α-amylase inhibitory activity with IC_{50} of 37.41 and 66.87 mg/L for rib and femur extract, respectively. Administration of bone extracts decreased FBG in both groups (F and R) compared with the control groups (NC and PC), although the differences were not statistically significant ($p>0.05$). These results indicate that bone extracts have the potential to be used as an adjunctive therapy for diabetes management.

Keywords: Bovine bone extract, Femur and rib bones, α-amylase inhibition, Blood glucose levels, Streptozotocin.

Introduction

In Indonesia, diabetes remains a major non-communicable disease, posing a significant public health challenge. As mentioned by the International Diabetes Federation (IDF), the global incidence of diabetes mellitus (DM) among adults aged 20 – 79 years is estimated at 537 million. This figure is predicted to rise by 19.73% in 2030 and by 21.77% in 2045. Over the past decade, the prevalence of diabetes mellitus (DM) in Indonesia has continued to rise, increasing from an estimated 10.7 million cases in 2011 to approximately 19.5 million in 2021.[1] With this sharp escalation, Indonesia currently ranks fifth among countries with the highest number of diabetes cases. According to the Basic Health Research report, diabetes mellitus in Indonesia had a prevalence of 10.9% in 2018, and the most recent Indonesian Health Survey in 2023 documented a further increase to 11.7%.[1-3] Diabetes mellitus (DM) is an abnormality of glucose metabolism, which usually begins with a disturbance in glucose homeostasis that causes problems with insulin, such as insulin resistance. Out of the various forms of diabetes, type 2 diabetes mellitus (T2DM) is the most common, accounting for over 90% of all cases worldwide. The increasing prevalence of T2DM has emerged as a critical public health problem due to its strong association with serious disease complications, such as diabetic retinopathy, neuropathy, gastropathy, and nephropathy.[4]

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Glucose absorption from the digestive system is a complex mechanism that regulates blood glucose levels. The catalytic activity of the enzyme α-amylase is a key factor contributing to postprandial hyperglycemia.[5] This enzyme facilitates the digestion of complex carbohydrates by hydrolyzing α-1,4-glycosidic bonds in starch, producing oligosaccharides that are subsequently converted into glucose. Excessive α-amylase activity facilitates rapid glucose absorption, aggravating postprandial hyperglycemia. Consequently, α-amylase inhibition has been recognized as a promising therapeutic intervention for managing hyperglycemia, particularly in patients with type 2 DM. Enzyme inhibition is a therapeutic strategy that delays carbohydrate digestion, thereby attenuating postprandial blood glucose elevation, particularly in individuals with DM type 2.[6]

Alpha-amylase Enzyme inhibition serves as a therapeutic strategy to delay carbohydrate digestion, thereby attenuating postprandial elevations in blood glucose. Nowadays, the approach to therapy based on natural ingredients is increasingly popular because it is considered safe and has fewer side effects compared to synthetic agents, such as miglitol, voglibose, and acarbose.[7] They work by inhibiting carbohydrate-digesting enzymes, such as amylase, but often cause negative reactions, such as digestive problems (stomach discomfort and diarrhea).[8] One of the natural ingredients that is gaining attention are those derived from animal by-products. Animal by-products, such as bovine (*Bos taurus*) bones that are high in protein[9] can reduce blood glucose concentration by delaying glucose absorption, thereby controlling postprandial hyperglycemia.[10]

Bovine bones, such as *Bos taurus* bones when boiled for a long time produces a broth high in protein and other beneficial nutrients, such as collagen, minerals, and bioactive peptides.[11] Proteins in bone extracts play an important role in glucose metabolisms in the body by regulating insulin sensitivity and glucose homeostasis through the activation of AMP-activated kinase (AMPK), a pathway relevant in the control of glucose absorption by cells.[12,13] Previous studies have indicated that

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bones commonly used for broth preparation are the leg bones (femur) and ribs, derived from both bovine (*Angus*) 24-month and pigs (*Landrace*) 8-month. These bones are considered suitable because they differ in density and porosity, which influence the extraction of minerals and protein. Rib bones, being more porous, allow higher initial release of calcium, magnesium, and trace metals, while femur bones, which are denser and richer in mineral matrix, provide a more sustained release during prolonged cooking. Therefore, femur and rib bones are regarded as good raw materials for bone extract.[14]

Presently, there have been studies reporting the positive effects of femur bone extract. The administration of bovine femur bone extract to streptozotocin (STZ)-induced rats can decrease some biomarkers, such as bilirubin, creatinine, urea, triglycerides (TG), total lipid (TL), total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 sICAM-1, and Malondialdehyde (MDA).[15] Bone extract has also been shown to have beneficial effects on the skin, particularly in preventing wrinkles and maintaining elasticity.[16] Additionally, in an *in vivo* study, the administration of femur bone extract to rat model of ulcerative colitis was found to reduce histological injury, decrease the expression of IL-6, TNF-α, and IL-1β, and increase the expression of INF-γ, IL-10, and IL-4.[17] Therefore, it can be said that bone extract has the potential to improve blood glucose levels, especially for type 2 DM.[15]

Besides the femur bone, rib bones are also often used by the community as a basic ingredient for making broth. Bovine rib bones are often chosen for making broth because they have an ideal combination of bone, connective tissue, and meat. The collagen content in the connective tissue is quite high, so when boiled, it produces gelatin. This gelatin plays an important role in providing a thick texture as well as supporting digestive and joint functions.[18]

Based on the foregoing, there is the need to investigate the potential of femur and rib bone extracts as complementary antidiabetic therapy. The present study investigated femur and rib bone extracts derived from bovine (*Bos taurus*) for their potential inhibitory activity against α-amylase enzyme and blood glucose levels in streptozotocin (STZ)-induced diabetic rat model.

Materials and Methods

Bone extract preparation

Bone extracts were made from two different types of *Bos taurus* bones (4 years old), the femur and rib bones. The bones used in this study were sourced from the Pammolongan Slaughterhouse, Makassar City, South Sulawesi, Indonesia (GPS coordinate: -5.189315,119.496648) on December 6, 2024.

The bones were cut crosswise to obtain pieces weighing 100 – 130 g. The bone fragments were immersed in distilled water and heated at 50°C for 15 minutes to eliminate residual meat, fat, and blood and reduce the risk of microbial contamination. This technique was executed three times. A slow cooker was used to prepare the bone extracts, using a ratio of 1 part bone (by weight) to 4 parts distilled water (by volume) (1 kg of bone to 4 L of distilled water). Acidic water was prepared by combining 1000 mL of distilled water with 20 mL of vinegar, which was subsequently used to boil the broth. Bones were added after the water temperature has reached 100°C. The cooking process was maintained for 24 hours at a temperature of 100 ± 2°C. Acidic water was used to preserve the first volume of the formulation. After boiling, the broth extract was left to cool at room temperature for three hours. Thereafter, the bone extracts were filtered in order to eliminate the bones and residual matter that formed during the cooking process. The bone extracts were subsequently stored in the refrigerator. A portion of the bone (femur and rib) extracts were freeze-dried.[15,17]

Determination of protein content of bone extract

The protein content was determined using the spectrophotometric method with Bradford reagent and BSA (Bovine Serum Albumin) standard solution.[19] The Bradford reagent was made by mixing 0.05 g of CBB (Coomassie Brilliant Blue) G-250, 25 mL of 95% ethanol, and 50 mL of phosphoric acid (H_3PO_3). The BSA standard solution was prepared in five different concentrations: 1.0, 0.8, 0.6, 0.4, and 0.2 mg/mL. Distilled water was used as blank. The blank and standard solutions were added to 3 mL of Bradford solution, then vortexed until homogeneous, and incubated at room temperature for 15 minutes. For the sample, 0.05 mL of either bone extract was added to 3.95 mL of distilled water, corresponding to a dilution factor of 1:80, then the diluted sample was added to 3 mL of Bradford solution, vortexed until homogeneous, and left to incubate at room temperature for 15 minutes. The absorbance of standard, bone extract, and blank solutions was measured at 575 nm using a UV-Vis spectrophotometer.

Bone extract functional group analysis using FTIR

As much as 2 mg of either dry bone (femur and rib) extract and 200 mg of potassium bromide (KBr) were ground until homogeneous (mixed) and placed in a disk press, then the mixture was vacuumed to remove air from the disk. The disk that has been pressed was then placed into the FTIR instrument, and the absorptions were recorded at 400 cm^{-1} - 4,000 cm^{-1} wavenumber.[20]

Determination of α-amylase enzyme inhibitory activity

The α-amylase inhibitory activity of the bone extracts was determined using iodine and spectrophotometric methods.[21] Acarbose and bone extracts (500 µL) were prepared in five different concentrations (20, 30, 40, 60, and 70 mg/L) and added to the α-amylase enzyme (500 µL, 2 mg/mL), then incubated at 37°C for 10 minutes to allow interaction between the enzyme and the inhibitor. After incubation, a substrate in the form of a starch solution (500 µL, 1% w/v) was added to the mixture, and incubated for 10 minutes to allow the enzymatic reaction to occur. The enzymatic reaction was stopped by adding iodine solution 500 µL, 0.5% w/v), which then gave a response with the unhydrolyzed starch residue to form a blue-coloured complex. The amount of residual starch is reflected in the intensity of the blue colour formed, which was then measured using a spectrophotometer at a wavelength of 575 nm. The higher the measured absorbance, the more the amount of starch not hydrolyzed by amylase, indicating enzyme inhibitory activity by the test compound. As a comparison, a control without an inhibitor was also made to determine the normal enzyme activity. Enzyme inhibitory activity was estimated using the formula (1):

$$\text{Percent inhibition } (\%) = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

IC_{50} value which represents the concentration required to inhibit 50% of α-amylase activity was calculated using linear regression. The linear regression equation was obtained as;

y = bx + a, with the x representing sample concentration, and y percentage inhibition. The IC_{50} value was determined using the formula (2):

$$IC_{50} = \frac{50 - a}{b} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Animals

Twenty-eight (28) male Wistar rats, each weighing between 150 - 180 grams and aged six to eight weeks, were obtained from the Animal Care Facility of Hasanuddin University, Makassar, Indonesia. The rats were acclimatized for 7 days and housed in the Biochemistry Laboratory, Faculty of Medicine, Hasanuddin University under controlled conditions (temperature 22 - 25°C, relative humidity 55 - 60%, and a light/dark cycle-12/12h), and with access to standard diet and water *ad libitum*.

Ethical approval

All experimental procedures involving animals were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee. The study protocol was reviewed and approved by the Ethics Committee of the State University Hospital, Hasanuddin University, Dr. Wahidin Sudirohusodo Hospital, Makassar, with Protocol Number UH24121021 and approval number 74/UN4.6.4.5.31/PP36/2025.

Experimental design
Twenty-eight male Wistar rats were divided into seven groups of 4 rats per group. The number of rats in each group was determined using Federer's formula[22] $(t-1)(n-1) \geq 15$. This is based on the Institutional Animal Care and Use Committee Guideline,[23] and Hasanuddin University's ethical recommendations (protocol number UH24121021), which states that the use of experimental animals in experiments should be minimized.
The groups include Normal Control (NG), Negative Control (NC), Positive Control (PC), Femur Groups 1 and 2 (F1 and F2), and Rib Groups 1 and 2 (R1 and R2). The normal control (NG) was not induced with STZ. It was only given a normal diet and drinking water without treatment, the negative control (NC) received STZ injection (50 mg/kg body weight) without the administration of acarbose or bone extract, the Positive Control (PC) received STZ injection (50 mg/kg body weight) followed by the administration of acarbose at a dose of 0.9 mg.rat, this dose is based on the dose given to humans, which is 50 mg,[24] and converted to dose in rats [50 x conversion factor (0.018) = 0.9] and the result is 0.9 mg for the rats' dose.[25] The Femur Groups 1 and 2 (F1 and F2) received STZ injection (50 mg/kg body weight) followed by the administration of femur bone extract at doses of 500 mg/kg body weight and 1000 mg/kg body weight, respectively. The Rib Groups 1 and 2 (R1 and R2) received STZ injection (50 mg/kg body weight) followed by the administration of rib bone extract at doses of 500 mg/kg body weight and 1000 mg/kg body weight. Acarbose and bone extracts were administered orally once daily for 30 days. The study followed a completely randomized design.

Injection of streptozotocin (STZ)
STZ was prepared in 0.01 M citrate buffer, and was administered as a single intraperitoneal injection at a dose of 50 mg/kg of body weight. To prevent hypoglycemic shock, rats were given 5% glucose 6 hours after STZ injection for 24 h.[26]

Measurement of fasting blood glucose (FBG) levels
FBG in rats was measured on day 1 (before Injection), day 6 (post-STZ Injection), and day 36 (post-30 days of bone extract administration) using the GOD-PAP method (Glucose Oxidase-Peroxidase 4-Amino-antipyrine).[27] The rats were fasted for 16 h before the blood glucose assessment. A 1 mL blood sample was obtained from the lateral tail vein of each rat, placed in a tube, and centrifuged at 6000 rpm for 15 minutes. The serum obtained (10 µL) was pipetted and added to 1000 µL of reagent (consisting of glucose oxidase, peroxidase, 4-amino-antipyrine, and phenol),[27] homogenized, and incubated at room temperature for 10 minutes. The FBG was assessed by measuring the absorbance of the serum samples at wavelength of 505 nm using a UV-Vis spectrophotometer.[27] The rats were considered diabetic if the FBG was > 150 mg/dL.[26]

Determination of treatment doses
The doses of the bone extracts were determined by conducting a toxicity test on rats, which showed that doses ranging from 500 to 2000 mg/kg of body weight were considered safe.[15] The bone extract doses of 500 mg/kg body weight and 1000 mg/kg body weight were chosen, which corresponded to 3 mL and 6 mL of the extract preparation, respectively.

Statistical analysis
GraphPad Prism statistical software version 9.5.1 was used as a statistical analysis tool. Data analysis was done to determine the homogeneity and normality of the data. All experimental results were presented as mean ± standard deviation (SD). Statistical tests were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences between the treatment groups. P-value < 0.05 was regarded as significant. Changes in blood sugar levels [Δ (delta) values], which represent the difference between pre-treatment (baseline) and post-treatment blood glucose, were also evaluated.

Results and Discussion

Protein content in bone (femur and rib) extracts
The protein content in this study was measured using spectrophotometry with the Bradford method. The Bradford method determines the total protein content of the sample. Hydrolyzed proteins are indicated by a colour change to blue when the sample is reacted with the Bradford solution. The blue colour change occurs due to complex formation between the protein and Coomassie Brilliant Blue (CBB). The complex formed is very stable and forms very quickly.[19,28]
The measurement of protein content using the Bradford method revealed that the concentrations of protein in the femur and rib bone broth extracts were 14.56 mg/mL and 23.04 mg/mL, respectively. The protein content in the rib extract was significantly higher than that of the femur extract ($p < 0.05$). This difference can be attributed to the variation in the composition of the constituent tissues between the two types of bones. Rib bones contain protein-rich marrow, and more connective tissue, especially collagen, which is an important contributor to the soluble protein content in bone broth.[29]
The process of boiling bones in hot water causes the denaturation and dissolution of structural proteins, such as collagen, glycoproteins, and proteins from bone marrow into the liquid medium. The thinner rib bones with a more open trabecular structure allow for more effective diffusion of proteins into the boiling water compared to the denser and more compact femur bones.[30]
The protein content in bone extract is an important parameter because it is closely related to its functional potential, including as a source of amino acids, collagen, and bioactive peptides. Previous studies have shown that bone extracts from parts of the bone with abundant soft tissue and marrow tend to have higher nutritional value and biological activity. Therefore, these findings support the fact that the type of bone used in making broth can affect the nutritional quality of the broth.[31]

FTIR spectrum of bone extract
Functional groups of femur and rib bone extracts were determined using Fourier Transform Infrared (FTIR) spectroscopy. Based on the FTIR results presented in Figures 1 and 2 and Table 1, both bone extracts had characteristic amide absorption regions, which are usually found in proteins and peptides. In this, study, the proteins and peptides exhibited five characteristic amide absorption signals: amide A, amide B, amide I, II, and III. The first absorption peak exhibited by both bone extracts is the Amide A absorption. The rib bone extract displayed a wavenumber at 3658.72 cm^{-1} and the femur bone extract at 3462.22 cm^{-1}, indicating NH stretching vibration. The normal wave number for Amide A is 4000 - 2500 cm^{-1}. The position of the N-H group in the peptide will shift to a lower frequency when influenced by hydrogen bonding.[32]

Table 1: FTIR Spectral data/Functional group characteristics

No	Absorption Area	Wavenumber (cm^{-1})		Function Group	Absorption Region (cm^{-1})
		Rib	Femur		
1	Amide A	3658.31	3462.22	N-H stretching	2500 - 4000
2	Amide B	2926.01	2922.16	CH_2 stretching asymmetric	2915 - 2935
3	Amide I	1654.92	1691.37	C=O stretching	1600 - 1700
4	Amide II	1550.77	1570.06	C-N stretching and N-H bending	1480 - 1575
5	Amide III	1238.3	1238.3	N-H bending and C-H stretching	1229 - 1301

of Rib and Femur Bone Extract

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The Amide B absorption in the rib bone extract was detected at a wavenumber of 2926.01 cm^{-1} and in the femur at a wavenumber of 2922.16 cm^{-1}. This indicates the presence of a characteristic collagen group, namely; Amide B. The Amide B group has an absorption band at a wavenumber of 2915 cm^{-1} - 2935 cm^{-1}. This wave absorption indicates that Amide B absorption is formed from the asymmetrical stretching of CH_2.[33]

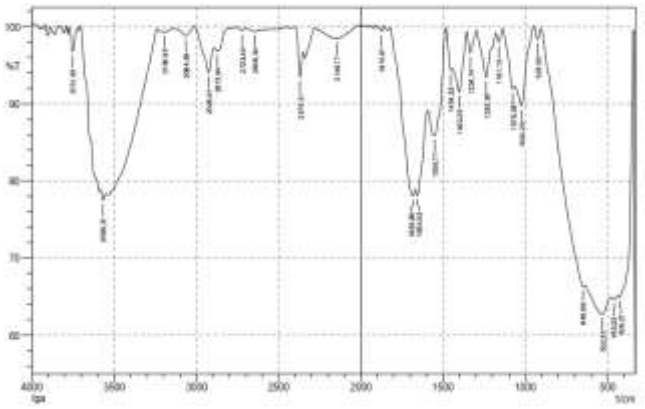

Figure 1: FTIR Spectrum of Rib Bone Extract

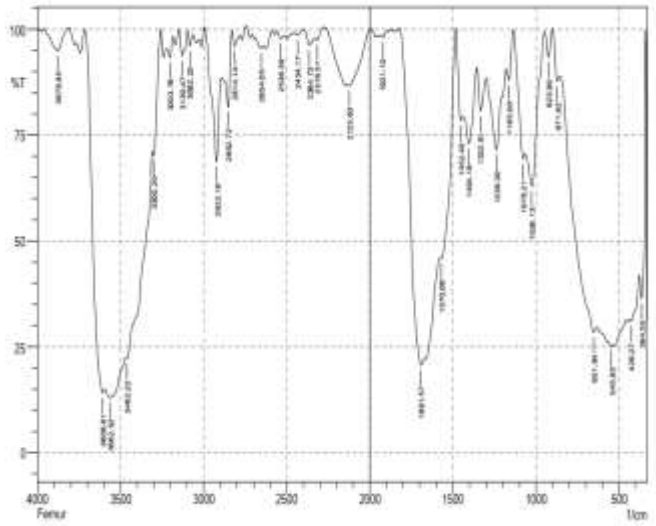

Figure 2: FTIR Spectrum of Femur Bone Extract

The Amide I was detected in the wavenumber range of 1600 cm^{-1} - 1690 cm^{-1}. This wavenumber indicates a characteristic functional group of collagens.[34] The Amide I absorption of the rib bone extract was detected at a wavenumber of 1654.92 cm^{-1} and the femur at 1691.37 cm^{-1}, indicating the presence of C=O group stretching vibrations. The femur absorption band at 1691.2 cm^{-1} is at the upper end of the typical Amide I absorption band (1690 - 1600 cm^{-1}), which originates from the stretching of the carbonyl (C=O) bond in the peptide group (–CONH–) in proteins.[35]

The Amide II absorption region appeared in the range of 1480 cm^{-1} – 1575 cm^{-1}. The Amide II absorption of the rib bone extract was detected at a wavenumber of 1550.77 cm^{-1} and the femur at a wavenumber of 1570.06 cm^{-1}. The existence of the Amide II group indicates the presence of CN stretching and NH bending.[36] The Amide III absorption in both the rib and femur bone extracts was detected at a wave number of 1238.3 cm^{-1}. This range indicates the existence of the Amide III functional group, which shows CH stretching and NH bending. Amide III usually displays at a wavenumber range of 1229 cm^{-1} - 1301 cm^{-1}.[32,37]

α-amylase inhibition activity of bone extracts

Inhibition of the α-amylase enzyme is one of the important approaches in the management of hyperglycemia.[5,8,38] The α-amylase enzyme plays a role in catalyzing the cleavage of α-1,4 glycosidic bonds in starch, producing simple sugars that can be absorbed in the small intestine. Therefore, the inhibition of α-amylase activity may slow down the digestion of carbohydrates in the intestines, thereby reducing post-meal blood glucose spikes.[39]

In this study, an *in vitro* α-amylase inhibitory activity test was conducted on two types of bone extracts, namely; femur and rib bone extracts, and compared with acarbose. The determination of α-amylase inhibitory potential was conducted using the iodine method, which measures the residual starch that remains unhydrolyzed by the enzyme. The greater the amount of residual starch, the higher the enzyme inhibitory activity by the test sample.[40]

The IC_{50} value, which is the sample concentration needed to inhibit 50% of enzyme activity is used as an indicator of the effectiveness of the test sample.[41] The results obtained indicate that both the femur and ribs can suppress the α-amylase enzyme, with varying potential depending on the type of bone. These results indicate that both bone extracts contain bioactive compounds that function as natural α-amylase inhibitors.

The α-amylase inhibitory activity test showed that the bone extract from the rib bones had a superior inhibitory potential compared to the extract from the femur bones, and still lower than the positive control, which is acarbose; however, this difference was not statistically significant ($p > 0.05$). The IC_{50} values of each sample are shown in Table 2 and Figure 3.

Table 2: α-Amylase Inhibitory Activity of Bone Extracts and Acarbose

No	Sample	Concentration (mg/L)	Percentage Inhibition (%)	IC_{50} Value (mg/L)	*p*-value
1	Femur Bone Extract	20	44.42	66.87	$p > 0.05$
		30	45.86		
		40	46.92		
		60	49.19		
		70	50.32		
2	Rib Bone Extract	20	46.27	37.41	
		30	47.87		
		40	50.54		
		60	55.33		
		70	57.72		
3	Acarbose	20	45.69	27.71	
		30	52.28		
		40	55.26		
		60	63.89		
		70	68.20		

The difference in inhibitory activity between the two types of bones is likely due to variations in the content of bioactive peptides, hydrolyzed collagen, and minerals, which affect the interactions with the enzyme's active site.[42] The femur is a long bone rich in bone marrow, and it has high levels of calcium, phosphorus, and type I collagen. These components make the extracts derived from the femur highly potent in providing bioactive compounds that support regenerative, anti-inflammatory, and immunomodulatory functions.[16,43] On the other hand, the rib bones fall into the category of flat bones that contain more cartilage tissue. This cartilage is known to be a major source of type II collagen and glycosaminoglycans, such as chondroitin sulphate and hyaluronic acid, which maintain joint and connective tissue health. The extract obtained from rib bones tends to be richer in gelatin and bioactive compounds that support tissue elasticity.[44,45] The rib bone extract inhibited higher α-amylase inhibitory activity than the femur extract, as indicated by lower IC_{50} value. Hence, the lower the IC_{50} value, the more potent the enzyme inhibitory activity.[41] However, the difference was not statistically significant ($p > 0.05$).

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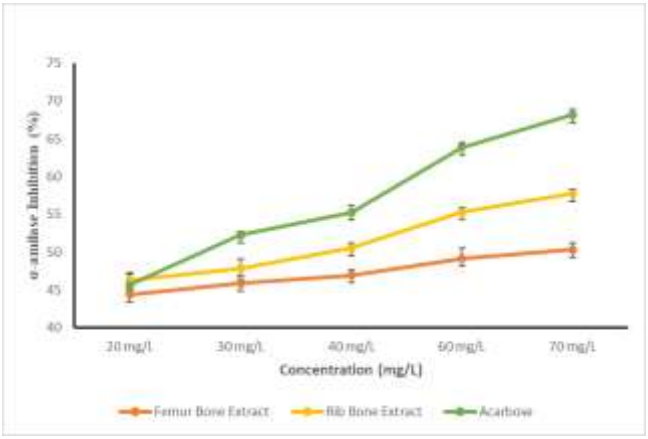

Figure 3: Inhibitory activity of α-amylase enzyme by femur bone extract, rib bone extract, and acarbose

Effect of bone extracts on fasting blood glucose

The initial FBG measurement results showed that the average FBG of the rats before STZ injection was 58.5 ± 7.30 mg/dL for NG, 65.9 ± 22.6 mg/dL for NC, 58.1 ± 9.07 mg/dL for PC, 64.1 ± 21.1 mg/dL for F1, 57.6 ± 2.93 mg/dL for F2, 71.3 ± 17.4 mg/dL for R1, and 60.4 ± 8.52 mg/dL for R2. This shows that all groups of rats at the beginning of the experiment were in a normal condition (normoglycemic). Figure 4 presents the data on FBG in rats.

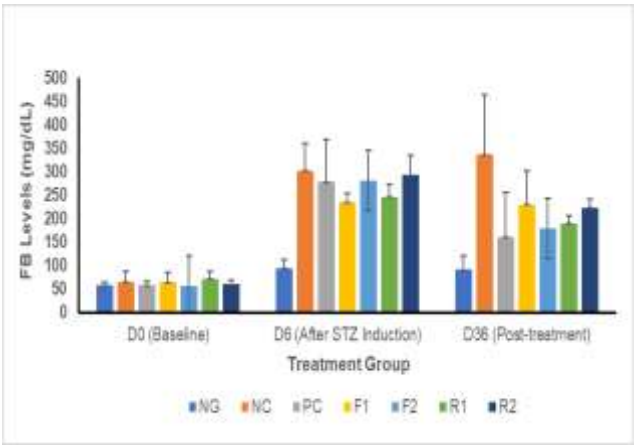

Figure 4: Fasting Blood Glucose (FBG) Level for each treatment group.

D0 (baseline-before STZ Injection), **D6** (after STZ Injection), and **D36** (post-treatment).

Data shown are the mean ± standard deviation (SD), n = 4. **NG:** normal group (water and feed); **NC:** negative control (STZ without treatment); **PC:** positive control (STZ, acarbose); **F1:** STZ, femur bone extract 500 mg/kg bw; **F2:** STZ, femur bone extract 1000 mg/kg bw; **R1:** STZ, rib bone extract 500 mg/kg bw; **R2:** STZ, rib bone extract 1000 mg/kg bw.

After STZ injection, the average FBG levels of the rats increased to 303.0 ± 57.5 mg/dL for NC, 279.0 ± 90.7 mg/dL for PC, 235.0 ± 19.9 mg/dL for F1, 281.0 ± 26.8 mg/dL for F2, 246.0 ± 26.7 mg/dL for R1, and 293.9 ± 42.7 mg/dL for R2. The administration of STZ at a moderate dose, between 40 to 50 mg per kg body weight, can cause partial disruption of insulin secretion, resembling the characteristics of Type 2 Diabetes Mellitus.[46]

Streptozotocin (STZ) is a compound similar to glucose that is taken up by pancreatic *β*-cells through the GLUT2 transporter.[47] Inside the cell, STZ releases an alkylating N-methyl-nitrosourea group, causing DNA damage, activation of PARP enzymes, and depletion of NAD^+ and ATP.[48] This process triggers *β*-cell death through apoptosis or necrosis mechanisms. Partial damage to *β*-cells, especially at moderate doses (around 40 - 50 mg/kg bw), reduces insulin secretion without destroying the entire *β*-cells, thus resembling the pathophysiology of Type 2 Diabetes Mellitus.[49,50] Insulin naturally lowers blood glucose levels by increasing glucose uptake by target tissues such as muscles and the liver, through the activation of the IRS–PI3K–Akt pathway and the translocation of GLUT4 to the cell surface. The decrease in insulin production due to *β*-cell damage by STZ disrupts glucose homeostasis.[51,52]

The toxicity of STZ increases further by the increase in reactive oxygen species (ROS), coupled with a reduced capacity of cellular antioxidant systems like glutathione. The accumulation of ROS leads to severe oxidative stress, membrane, protein, and DNA damage, and worsens *β*-cell and other tissue dysfunction.[53]

After 30 days of treatment, FBG levels decreased in all treated groups. The average FBG in the PC group decreased to 161.0 ± 94.9 mg/dL, F1 to 230.0 ± 71.2 mg/dL, F2 to 179.0 ± 21.5 mg/dL, R1 to 190.0 ± 16.4 mg/dL, and R2 to 223.0 ± 19.3 mg/dL, whereas in the NC (STZ) group, FBG increased to 337.0 ± 12.7 mg/dL. The average FBG data on day 6 (post-STZ injection) and day 36 (post-treatment) were statistically compared to the observe effect of the treatment on blood sugar levels.

According to the statistical analysis results, there were no notable differences in the FBG changes observed in the groups before and after the treatment, as indicated by a p-value of 0.07, which is detailed in Table 3. The highest decrease in FBG was observed in the PC group (acarbose) with an average FBG change of -117.51 ± 78.0 mg/dL (Table 3). In the bone extract treatment group, the highest decrease in FBG was found in the F1 group, with an average change of -101.93 ± 21.67 mg/dL (Table 3). In the treatment group with femur and rib bone extracts, both at doses of 500 and 1000 mg/kg bw, a decrease and improvement in FBG were observed; however, FBG levels did not return to the normal range.

Table 3: Changes in Fasting Blood Glucose (FBG) levels in rats following treatment with bone extract

Treatment Group	Change in FBG Level (D36 – D6)	*p* value
NG	-3.25 ± 37.59	
NC	34.54 ± 161.20	
PC	-117.51 ± 78.0	
F1	-5.50 ± 81.22	$p > 0.05$
F2	-101.93 ± 21.67	
R1	-56.00 ± 42.58	
R2	-69.62 ± 60.06	

Values are mean ± SD, n = 4, $*p < 0.05$ indicates a significant difference. FBG (Fasting Blood Glucose). The change in FBG represents the difference between the FBG on day 6 (after STZ induction) and day 36 (post-treatment). **STZ** (Streptozotocin); **NG:** normal group (water and feed-); **NC:** negative control (STZ without treatment); **PC:** positive control (STZ, acarbose); **F1:** STZ, femur bone extract 500 mg/kg bw; **F2:** STZ, femur bone extract 1000 mg/kg bw; **R1:** STZ, rib bone extract 500 mg/kg bw; **R2:** STZ, rib bone extract 1000 mg/kg bw.

This may be caused by several factors, such as the level of *β*-cell damage in the pancreas due to STZ Injection, which is quite severe and irreversible, resulting in low endogenous insulin production even though oxidative stress and inflammation have been suppressed. Furthermore, the thirty-day intervention may not be sufficient to provide maximal regenerative or restorative effects on pancreatic tissue and overall glucose metabolism. The bioavailability of active substances in bone extract may vary among individual rats due to differences in physiological responses, body weight, and the degree of damage to metabolic organs. Bone extract contains complex nutrients that work gradually through the repair of the metabolic environment.[54-56]

Conclusion

The findings from this study have demonstrated that extracts from femur and rib bones inhibit the activity of α-amylase enzyme and decrease blood glucose levels in rats with diabetes. The blood glucose-lowering effect of the bone extracts may be attributed to their protein content. This suggests that bone extract can be a useful supplementary therapy for the management of diabetes mellitus. To optimize the use of bone extract as a functional therapy, further studies are needed on the molecular mechanisms, effective doses, and potential combinations with other antidiabetic agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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