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THE POTENCY OF BOVINE BONE GELATIN AS ANTIHYPERTENSIVE AGENT: A REVIEW

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ABSTRACT

This review was purposed to understand the effectiveness of bovine bone gelatin as an antihypertensive agent. This review concerning the effectiveness of bovine bone gelatin as an antihypertensive agent. Hypertension, also called as a silent disease, has become the main cause of coronary heart disease and stroke that contributes to the malfunction of human organs. Changes of lifestyle alongside with science enhancement, provides new inventions regarding methods of hypertension therapy by minimizing the use of synthetic drugs. Collagen tissue of bovine bone gelatin is known to contain angiotensin converting enzyme (ACE) inhibitor, an active peptide that plays a role in lowering blood pressure supporting with the large amount of Gly (27%), Pro (17.6%), and Hyp (14.4%) and repeating pattern of Gly-X-Y. A study was carried out *in vivo* using injected spontaneously hypertensive rats (SHR) with 30 mg/kg and was able to reduce blood pressure by 15 mmHg. Antihypertensive test with SHR tail-cuff at 30 mg/kg bovine gelatin hydrolysate RGL-(Hyp)-GL and RGM-(Hyp)-GF were 31.3 mmHg and 38.6 mmHg respectively. A study conducted using bovine and porcine gelatin with 30–50 kDa (permeate P1) and 1–2 kDa (permeate P3) was able to reduce blood pressure by 22 mmHg and 21.33 mmHg. In addition, it is still possible conducting research to find out other peptides of bovine bone gelatin that can be used as a future alternative antihypertensive agent.

Key words: Antihypertensive activity; bioactive peptide; bovine bone gelatin; hypertension

INTRODUCTION

Hypertension referred to as a silent disease is the main factor causing heart disease and stroke. In an uncontrolled condition, this silent disease will cause damage to other organs such as the brain, kidneys, eyes, and movement organs (Budiarto *et al*., 2018; Caraka *et al*., 2021). Hypertension is often occurred in both developed and developing countries, the symptoms are not felt and according to CDC, hypertension is characterized by an increase in systolic blood pressure exceeding 140 mmHg and diastolic pressure exceeding 90 mmHg. Instantaneous lifestyle of people worldwide causes hypertension to remain at a high rate (Centers for Disease Control and Prevention (CDC), 2012).

The renin-angiotensin-aldosterone system is a major determinant in maintaining arterial blood pressure, one of which targets a component of angiotensin-converting enzyme (ACE), known as dipeptidyl-carboxypeptidase glycolyzed zinc. ACE acts as regulator of arterial blood pressure and electrolyte balance through the renin-angiotensin-aldosterone system (Mendoza Torres *et al*., 2015). Renin-angiotensin-aldosterone system plays an important role in controlling arterial pressure including sodium balance (Schweda, 2015). Hypertension occurs due to the expression of angiotensin converting enzyme (ACE) affected by the environment. This expression correlates with blood pressure (Munro *et al*., 2017) and an occurrence of imbalance disruption between enzymes might cause hypertension.

A partial hydrolysis product of collagen called gelatin has an antihypertensive agent. It is known that gelatin originated from animal protein and contains bioactive peptides which function as an antihypertensive agent, in the form of ACE inhibitors (Aluko, 2015; Mills *et al*., 2016; Rosendorff *et al*., 2015). ACE inhibitors work by inhibiting the performance of an enzyme to produce angiotensin II that affects the narrowing of blood vessels (Annweiler *et al*., 2020). Bovine bone is determined to have collagen and its amino acid structure functioned as a bioactive peptide and ACE inhibitor working as an antihypertensive agent (Banerjee & Shanthi, 2012).

Hypertension therapy using synthetic drugs has a series of negative effects, such as coughing, headache, nausea, vomiting, diarrhea or constipation, nervousness, skin rash, fatigue, and sudden weight loss (Kingman *et al*., 2017). Administration of antihypertensive drugs using bovine bone gelatin might be a potential option in the future. This implementation is carried out to provide knowledge of other usage regarding bovine bone gelatin as an antihypertensive agent. Furthermore, this implementation is also conducted to find out how much potential antihypertensive can occur as well as a basis for further research on a laboratory scale.

General Characteristics of Hypertension Disease

Hypertension is a major risk factor of cardiovascular disease which is known to be the number one cause of death in Indonesia (Tasmini *et al*., 2018) and an estimated 6% of deaths in the world have a correlation with hypertension (Zhang *et al*., 2020). Hypertension is a state of systolic blood pressure >120 mmHg and >80 mmHg. This disease is grouped into 3 stages, such as

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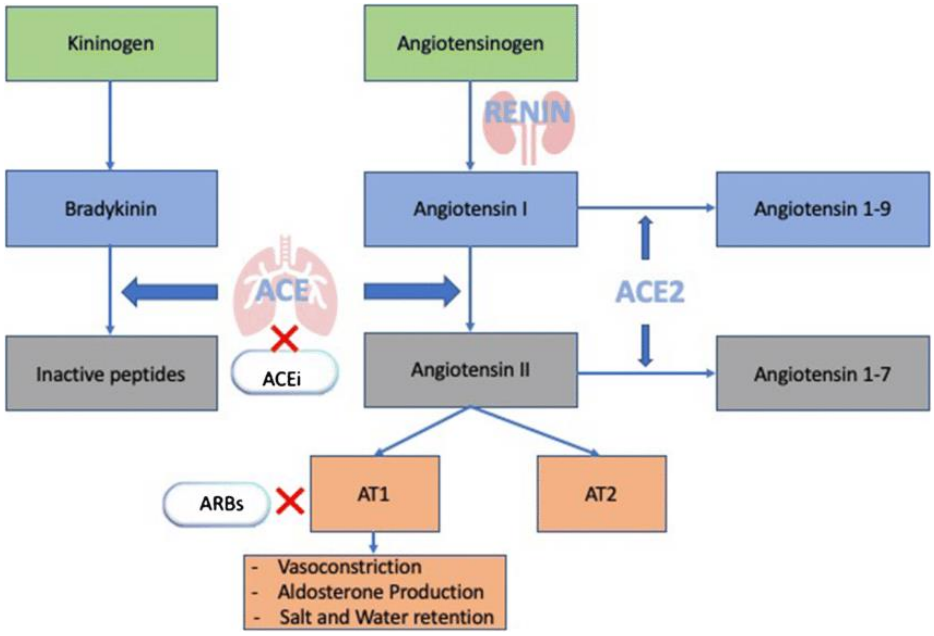
stage 1 130-139 / 80-89 mmHg, stage 2 140-159 / 90-99 mmHg and stage 3 ≥160 / ≥100 mmHg (Chen & Chauhan, 2019). Lifestyle and behavior can affect blood pressure levels in the form of alcohol consumption, smoking and cases of metabolic syndrome by visceral obesity, insulin resistance, oxidative stress, endothelial dysfunction, activated renin-angiotensin system, increased inflammatory mediators, and obstructive sleep apnea (Lackland & Voeks, 2014). Excess consumption of salt and fatty foods can also be a factor in causing hypertension (Imelda *et al*., 2020).

The long-term impact of a hypertensive patient is the emergence of other diseases such as chronic kidney disease and as much as 28.4% of patients with chronic kidney disease in the United States from 1980 to 2010 suffered from hypertension (Xie *et al*., 2019). Untreated metabolic syndrome is at risk of having cardiovascular disease such as coronary heart disease and stroke (Tasmini *et al*., 2018).

The Mechanism of Hypertension Treatment

Hypertension treatment is given to patients based on the level of blood pressure. A successful hypertension treatment according to JNC 8 (The Eight Joint National Committee) is a blood pressure of 150/90 mmHg in patients with the age of over 60 years and 140/80 mmHg in patients under 60 years. Treatment of hypertension can be done by administering antihypertensive, one of which is Angiotensin Converting Enzyme (ACE) inhibitors (James *et al*., 2014). The mechanism of hypertension treatment using ACE inhibitor is shown by Figure 1 (Turner & Kodali, 2020).



Source: Turner & Kodali (2020).

Figure 1. Mechanism of ACE inhibitor (ACEi) and angiotensin receptor blocker (ARB). ACE is angiotensin-converting enzyme; ACE2 is angiotensin-converting enzyme 2; AT1 is angiotensin II receptor type 1; AT2 is angiotensin II receptor type 2.

The angiotensin converting enzyme I (ACE) is a key of enzyme for regulating blood pressure *in vivo* (Miralles *et al.*, 2018). ACE activates the renin-angiotensin-aldosterone pathway, promoting the conversion of angiotensin I to angiotensin II. ACE inhibitors are angiotensin-converting enzyme inhibitors that block the conversion of angiotensin I to angiotensin II. Angiotensin II is a powerful vasoconstrictor

that, when inhibited, causes blood vessels to dilate and aldosterone output to decrease, lowering blood pressure (Chen *et al*., 2018; Knežević *et al*., 2017). ACE inhibitors work by inhibiting the peptidyl dipeptides converting enzyme that hydrolyzes angiotensin I to angiotensin II and activates bradykinin. ACE inhibitory was the 1st medications applied to lower blood pressure in hypertension and can be found as synthetic ACE inhibitors drugs such as benazepril, captopril, enalapril, fosinopril, lisinopril, zestril, moexipril, perindopril, quinapril, accupril, ramipril, and trandolapril (Iwaniak *et al*., 2014). However, the ACE inhibitor drugs often cause effects such as angioedema, coughing, and hypotension (Miralles *et al.,* 2018). Thus, alternative source for hypertension treatment and aimed for reducing blood pressure with less adverse effects have found on natural polypeptides from food proteins. Those findings said that the polypeptides having a similar structure with synthetic drugs and showing potential ACE inhibitory activities both *in vivo* and *in vitro* (Cao *et al.,* 2019; FitzGerald *et al.,* 2004).

Collagen as Natural Inhibitor Peptides

Collagen is a renewable natural resource that can be used as medical and chemical materials, food resources, and etc. (Pal & Suresh, 2016). Bone, skin, and another part of animal husbandry contain a large number of collagens, exist in the connective tissue, and categorized as by-products (Blanco *et al.,* 2017). However, the usage of collagen is limited. This is caused by collagen's amino acid composition does not match with human needs and hard to be digested. Research and development for improving the use of collagen is done. Collagen is hydrolyzed to obtain the bioactive peptides in smaller sizes which have been found as peptides and amino acids compound (Chen *et al.,* 2020). Some of collagen peptides, as bioactive peptides, were reported having a special function, such as ACE inhibitory activity, called cryptic peptides (Banerjee & Shanthi, 2016).

Bioactive peptides are a specific part of food protein with low molecular weight, consisting of 2-30 amino acids released during the metabolic process (Ryder *et al*., 2016). Bioactive substances also beneficial to one's health and help to prevent diseases from developing. The major benefits of bioactive peptides are ACE inhibitor, antioxidant, antidiabetic, antiobesity, and antibacterial (Jakubczyk *et al*., 2020). The location of particular amino acid residues is crucial for robust antihypertensive action (Daliri *et al*., 2017). The *in vitro* treatment revealed that it can create ACE inhibitors when collagen from animal sources binds to a target on the active site (Aluko, 2015; Mills *et al*., 2016; Rosendorff *et al*., 2015). The ACE inhibitors sourced from protein are promising for safety. These confirmed ACE inhibitory peptides are containing 10-12 amino acids and having C-terminal hydrophobic side (Baehaki *et al.,* 2016).

The C-terminal residue was found in hydrophobic side. Historically, the first source of ACE inhibitor found in snake venom, a mixture of peptides and enzymes that most of the inhibitory peptides contained the residues a P as the C-terminal and N, I, and P near the C-terminal. Then, it was theorized that, a bioactive peptide can act as ACE inhibitor having the region of hydrophobic C-terminal with G-X′-Y′ amino acid arrangement (X′ is often P, L, I or A and Y′ often P) (Banerjee & Shanthi, 2012). The structural activity of the ACE inhibitor by polypeptides is related to the presence of C- in tripeptide residues and hydrophobic amino acid residues (Leu, Ala, Val, Ile, Tyr, and Phe) (Escudero *et al*., 2012).

Inhibition occurs because the peptides on the ACE bind to the N- and/or C- ends of the ACE inhibitor, such as hydrophobic amino acids with aliphatic chains (Gly, Ile, Leu, and Val) at the N-end and cyclic amino acids or aromatic chains (Pro, Tyr, and Trp) at the C-end of the ACE inhibitor (Fitzgerald & Meisel, 2000; Vermeirssen *et al*., 2002). Various types of oligopeptides containing ACE inhibitors have been identified from

collagen hydrolysate including Met-Gly-Pro, Gly-Pro-Leu, and Gly-Pro-Val (Campbell *et al*., 2014; Garfinkle, 2017), Ala-Hyp also has potential ACE inhibitors (Kim *et al*., 2001). The potential of ACE inhibitors is found in the Gly-X-Y repeating pattern (Herregods *et al*., 2011). Collagen hydrolyzes the X-Gly peptide from Pro-X-Gly-Pro chain and produces Gly-Pro at the N- end, Pro-X at the C- end (Ackerman *et al*., 1999). The high content of Gly and Pro can be potential precursors for bioactive peptides. Several enzymes are able to produce peptides with hydrophobic amino acid type at their C end thus considered as strong and potential candidates for ACE inhibitor activity (Aluko, 2015; Gomez-Guillen *et al*., 2011; Minkiewicz *et al*., 2011).

The Profile of Bovine's Bone Gelatin

Gelatin is a natural product obtained through partial hydrolysis of collagen from animal skin and bones with physicochemical properties that are water soluble, transparent, odorless, tasteless (Gomez-Guillen *et al*., 2011) and has reversible properties (sol to gel), expands in cold water, forms a film, affects the viscosity of the material and protects the colloid system (Wonganu, 2020). Gelatin is considered as a miracle food that can be applied in the pharmaceutical, medical, photography and food industries because it forms gel, viscosity and melts in the mouth (Gomez-Guillen *et al*., 2011). It can also be used as a stabilizer, gelling agent, and microencapsulation agent for food products such as jelly, milk, yogurt, ice cream, cheese and canned food (Koli *et al*., 2012), another important characteristic is high digestibility (Deviarny *et al*., 2015). Gelatin has bioactive properties in the form of antimicrobial (antioxidant) and antihypertensive properties by inhibiting angiotensin converting enzyme (ACE) (Gomez-Guillen *et al*., 2011).

Gelatin consists of amino acids such as glycine (Gly), proline (Pro) and 4-hydroxyproline (4Hyd). The general structure of gelatin is –Ala-Gly-Pro-Arg-Gly-Glu-4Hyd-Gly-Pro. The proline and hydroxyproline amino acids play an important role in the effect of the gel on gelatin (Syafiqoh, 2013). The complete content of essential and non-essential amino acids along with their percentage is shown in Table 1.

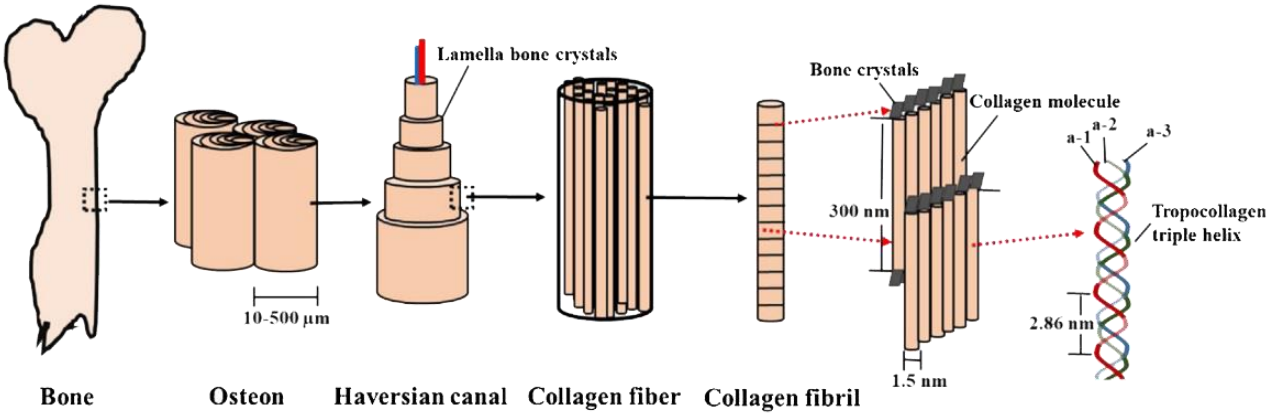
Table 1. Amino acid content in gelatin

Non-Essential Amino Acid	Percentage (%)	Essential Amino Acid	Percentage (%)
Glycine	26.00-27.00	Arginine	8.60-9.30
Proline	14.80-17.60	Lysine	4.10-5.90
Hydroxyproline	12.60-14.40	Leucine	3.20-3.60
Glutamic acid	10.20-11.70	Valine	2.50-2.70
Alanine	8.70-9.60	Phenylalanine	2.20-2.26
Aspartic acid	5.50-6.80	Threonine	1.90-2.20
Serine	3.20-3.60	Isoleucine	1.40-1.70
Hydroxylysine	0.76-1.50	Methionine	0.60-1.00
Tyrosine	0.49-1.10	Histidine	0.60-1.00
Cysteine	0.10-0.20	Tryptophan	0.00-0.30

Source: Nur (2011).

Gelatin produced from bovine bones is classified as type B by demineralized bone. Type B gelatin using an alkaline solution has an isoelectric point at a pH of 4.7–5.4 (GMIA, 2012). The structure of bone collagen is different, shown in Figure 2 (Cao *et al*., 2020). The bone of the bovine femur has a compact shape, is a high source of collagen and is obtained from a slaughterhouse which has little

contamination, making it suitable as a raw material for making gelatin (as gelling, thickening or stabilizing agent) (Laksana, 2016; Munda, 2013). The collagen molecules in bone bind to the nanoparticles of mineral $Ca_3(PO_4)_2$ or mineral phase closely and form a complex interlocking network. Its hierarchical structure is stabilized by molecular coil, interactions of intermolecular, and mechanical interlocking between hydroxyapatite crystals and spesific amino acid group (sequence of polyaspartic acid, residues of γ-carboxyglutamic acid, and sequences of glutamic acid) (Liu *et al*., 2016; Stock, 2015).



Source: Cao *et al.* (2020)

Figure 2. Hierarchial structure of bone form macro- to nano- scale

The physicochemical composition of beef bones is 33.3% gelatin, 3.85% $CaCO_3$, 57.35% K_3PO_4, 2.05% $Mg_3(PO_4)_2$, 3,45% Na_2CO_3, specific gravity of 1.9 g/cm^3 (Septriansyah, 2000). Collagen is an amino acid protein that is dominated by the polypeptides 28,8 % glycine, 15,5 % proline, and 14,2 % alanine which are the main ingredients for making gelatin (Chi *et al*., 2014; GMIA, 2012). The main raw material for gelatin is a collagen compound with the following breakdown reactions:

$C_{102}H_{149}N_{31}O_{38}$ + H_2O → $C_{102}H_{151}N_{31}O_{39}$
Collagen Water Gelatin

The reaction occurs at a temperature of 60-95°C. An increase of temperature more than 95°C causes the breakdown of gelatin with the following reaction:

$C_{102}H_{151}N_{31}O_{39}$ + H_2O → $C_{55}H_{83}N_{17}O_{18}$ + $C_{47}H_{70}N_{14}O_{18}$
Gelatin Water Semiglutin Hemicollin

The collagen molecule is composed of approximately 20 amino acids in which there is a repeating polypeptide subunit (tropocollagen) containing 1050 amino acid residues in the individual collagen peptide chain (Ichsan, 2012). Collagen consists of three polypeptides twisted together to form a triple helix. The three tendrils of the polypeptide chain are tightly coiled around each other (Chi *et al*., 2014). One tropocollagen unit consists of three polypeptide chains. The pyrrolidine, proline, and hydroxyproline rings take part in forming the polypeptide chain and strengthening the triple helix. Tropocollagen will be denatured by heating or treatment with substances such as acids, bases, urea, and potassium permanganate. Collagen fibers might experience shrinkage if it was heated above the shrinkage temperature (Ts= 45°C) and the split triple helix will have a longer size. The breakdown of these

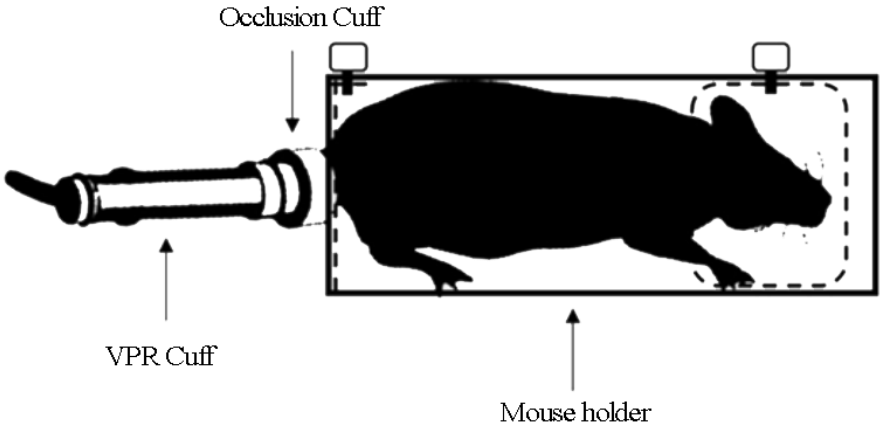
structures into random and water-soluble bonds produces gelatin (Hosseni *et al*., 2014).

Collagen is the main material of extracellular matrix for connective tissues (O'Keeffe *et al.,* 2017). Collagen peptides, the subunit of collagen, will take part of formation for collagens and figure the connective tissues. Additionally, collagen peptides have the smaller size and easily can be digested into smaller molecules easily than collagen. What's more, collagen di-peptides and tri-peptides can be absorbed directly (Freeman, 2015) and in human body, collagen tri-peptides are absorbed more efficiently (Yamamoto et al., 2016). Two peptides from bovine collagen are confirmed having antihypertensive activity by *in vivo* testing (Yu *et al.,* 2016).

The Effectiveness Test of Bovine's Bone Gelatin Peptides

Antihypertensive activity testing was carried out *in vivo* and *in vitro*. The potential bioconversion of ACE inhibitor peptides will react to oral administration, because the maximum absorption process depends on the target organ (Herregods *et al*., 2011; Noviani & Vitrinurilawaty, 2017; Sarbon *et al*., 2019). *In vivo* experiments are typically performed on spontaneously hypertensive rats (SHR). The treatment was administered orally, and blood pressure was measured by the tail-cuff method and blood pressure monitoring (Fitzgerald & Meisel, 2000; Herregods *et al*., 2011), shown in Figure 3 (Wang *et al*., 2017).

Treatment trials on rats were also conducted by giving a high salt diet using the post-test only control group design method. Male and female Wistar rats were treated with a high-salt diet of 3 ml 8% NaCl solution for 4 wk (Lailani *et al*., 2013). The measurement of blood pressure is done by intraperitoneal anesthetizing using 60 mg of pentobarbital-Na. The rats' hair on the anterior neck was shaved and then operated to attach the trachea with a cannula. The carotid arteries are cannulated with a cannula on the transducer connected to a computer for blood pressure monitoring (Armenia *et al*., 2004).



Source: Wang *et al.* (2017)

Figure 3. Schematic illustration of tail-cuff treatment on SHR

The *in vivo* antihypertensive test of bovine gelatin had been carried out using various types of peptides. It has also been known that short peptides derived from gelatin hydrolysate (e.g. Pro-Hyp) can get through the intestinal barrier and enter the blood stream by human (Iwai *et al.,* 2005) or mice (Taga *et al.,* 2016). The ACE inhibitor Leu-Arg-Pro test in SHR injected intravenously with 30 mg/kg bovine hide gelatin peptide can reduce blood pressure in SHR by 15 mmHg (Kim *et al*., 2001). Tail-cuff test was carried out for 12 h on SHR with 10 mg/kg and 30 mg/kg of RGL and RGM bovine bone gelatin hydrolysate. Through this test, maximum systolic blood

pressure reduction was obtained by RGL-(Hyp)-GL and RGM-(Hyp)-GF at 30 mg/kg of 31.3 mmHg and 38.6 mmHg respectively (Cao *et al*., 2020). Other experiments on 30–50 kDa (permeate P1) of bovine and pork collagen and 1–2 kDa (permeate P3) of hydrolyzed pig collagen, reduced blood pressure by 22 mmHg and 21.33 mmHg (Faria *et al*., 2008). Herregods *et al.* (2011), done the research for reducing blood pressure by tail-cuff method in SHR for 6 h using bovine hide, found that giving the single dose of 300 mg/kg could reduce systolic blood pressure at 17 mmHg. Thus, the antihypertensive effects reported in some studies conclude that the natural animal collagen, especially bovine bone collagen, has bioactive peptides for blood pressure-lowering properties.

Antihypertensive findings on bovine bone collagen are still limited. There are many forms of peptide protein in bovine bone collagen that have not been studied.

CONCLUSION

Bovine bone contains natural-source collagen peptides that are highly digested and safe for consumption. Several studies have been conducted to prove that collagen peptides have other functions as antihypertensive agents for lowering blood pressure with less adverse effects. Bovine collagen contained unique peptides which are structurally similar to those contained in synthetic drugs. It also performs antihypertensive activity and acts as ACE inhibitor in lowering blood pressure. It is supported with the large amount of Gly (27%), Pro (17.6%), and Hyp (14.4%), and has a hydrophobic C-terminal region with an amino acid sequence of G-X′-Y′ or Gly-X-Y, which potent for ACE inhibitor. These results indicate that bioactive peptides of bovine bone collagen have the same potential as ACE inhibitor agents. The use of bovine bone gelatin is needed to be studied further to determine other ACE inhibitory peptides as antihypertensive agents.

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