

Toxicity study and effect of protein hydrolysates derived from black tilapia (*Oreochromis placidus*) on hypertensive induced mice

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Abstract

Protein hydrolysates are specific protein fragments, produced during enzymatic hydrolysis and can exert various biological functions. The present study investigated the acute toxicity study of protein hydrolysates derived from black tilapia (*Oreochromis placidus*) and their effect on hypertensive-induced mice. Protein hydrolysates were produced from black tilapia protein isolate through enzymatic hydrolysis. A single dose of protein hydrolysates at 2000 mg/kg of body weight was administered and any changes were observed for 14 days. During 14 days, there were no significant changes in liver enzyme content and kidney function content, adverse clinical signs and mortality. The effect of the administration of protein hydrolysates in hypertensive-induced mice was studied. The body weight, blood pressure, full blood count {red blood cell (RBC), white blood cell (WBC) and platelets} were observed. Hypertensive mice (systolic blood pressure; 161±3 mm/Hg) were fed with pellet consisting of protein hydrolysates at different concentrations as well as carvedilol and combination of carvedilol and protein hydrolysates and systolic blood pressure was monitored. After 28 days, there was a significant reduction of blood pressure in the group of carvedilol (107±4 mm/Hg), 5% protein hydrolysate (119±1 mm/Hg), 10% protein hydrolysate (123±1 mm/Hg), 20% protein hydrolysate (115±2 mm/Hg) and 10% protein hydrolysate in combination with carvedilol (103±2 mm/Hg). Red blood cells significantly increased in hypertension mice but no significant changes in white blood cells and platelets after 28 days. Administration of protein hydrolysates reduced red blood cell levels similar to control group. A significant increase in body weight was observed in hypertension mice administered with protein hydrolysates at different concentrations as well as carvedilol and a combination of carvedilol and protein hydrolysates compared to control group and hypertensive group. Studies on any changes in liver enzyme level, kidney function level and angiotensin converting enzyme (ACE) activity on blood serum were carried out. Results showed that treatment with protein hydrolysates on hypertensive-induced mice reduced liver enzyme levels (ALP, ALT and GGT) and kidney function levels (Na⁺, K⁺ and Cl⁻) to normal levels. ACE enzyme activity of hypertensive-induced mice in the serum was also reduced similar to control mice after being treated with protein hydrolysates.

1. Introduction

Hypertension, also known as high or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure. Blood pressure is created by the force of blood pushing against the walls of blood vessels (arteries) as it is pumped by the heart. The higher the pressure, the harder the heart has to pump. Hypertension is a major cause of premature death

worldwide where an estimated 1.13 billion people have hypertension and two-thirds are living in low- and middle-income countries (WHO, 2021). The global prevalence of hypertension accounts for 9.4 million deaths every year and it is expected that up to 1.58 billion adult patients will suffer from hypertension in 2025 (WHO, 2011). In Malaysia, the Institute of Public Health (2011) reported that during the period of 1996 to

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2011 showed a rising trend in the prevalence of hypertension in adults above 30 years old: 32.9% (30 - 35.8%) in 1996 (Lim and Morad, 2004), 42.6% (37.5 - 43.5%) in 2006 (Institute of Public Health, 2008), and 43.5% (40.4 - 46.6%) in 2011 (Institute of Public Health, 2011). Institute of Public Health (2020) reported the overall prevalence of hypertension among adults aged 18 years and above in this survey was 30.0% (28.57 - 31.50%).

Among the mechanisms associated with hypertension, angiotensin I converting enzyme (ACE) plays an important role in the regulation of blood pressure. Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase (EC 3.4.15.1) and is widely distributed in mammalian tissues (Li *et al.*, 2004). In the renin-angiotensin system, ACE converts angiotensin I to angiotensin II, a vasoconstrictor. Meanwhile, in the kinin-kallikrein system, it inactivates bradykinin which is a vasodilator peptide (Bougatef *et al.*, 2010). Further, in the conditions of hypertension, angiotensin II amplifies oxidative stress as it intervenes in many of its cellular functions by stimulating the formation of intracellular reactive radical species (ROS) (Schiffirin and Touyz, 2004).

ACE inhibitors have been shown to be effective antihypertensive agents. Therefore, in addition to blood pressure control, ACE inhibitors have been shown to intensify the antioxidant defense system in animals and humans by inhibiting of angiotensin II (de Cavanagh *et al.*, 2000). Many synthetic ACE inhibitors including Captopril, Enalapril and Lisinopril among others are available for clinical use, however, some undesirable side effects may occur such as cough, loss of taste, renal impairment and angioneurotic oedema (Brown and Vaughan, 1998). Other natural sources of ACE inhibitors, such as antihypertensive agents are needed to substitute synthetic ACE inhibitors.

The present study was to investigate an acute toxicity study of protein hydrolysates derived from black tilapia (*Oreochromis placidus*) and the effect of administration of protein hydrolysates on the body weight, blood pressure, full blood count (red blood cell, white blood cell and platelets), liver enzyme level, kidney function level and angiotensin converting enzyme (ACE) activity of hypertensive induced mice.

2. Materials and methods

2.1 Materials

Black tilapia was supplied by the retailer. All chemicals used in this study were of analytical grade unless otherwise specified. Sodium tetraborate decahydrate, sodium chloride, papain, ethyl acetate,

DPPH (1,1-diphenyl-2-picrylhydrazyl), angiotensin converting enzyme (ACE) from rabbit lung and hippuryl histidine leucine (HHL) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium acetate trihydrate, Anhydrous Ferric (III) chloride, Ferrous heptahydrate were purchased from Nacalai Tesque, Japan, 2,4,6-Tri(2-pyridyl)-s-triazine was purchased from Acros Organics, Belgium. Ascorbic acid, hydrochloric acid (HCl), glacial acetic acid, sodium acetate, methanol, sodium chloride, carvedilol, and tris-HCl were purchased from Sigma (USA).

2.2 Extraction of protein from black tilapia fish meat

The proteins were extracted by grinding the black tilapia flesh in a mixer (IKA, A10 Basic, Willmington, NC). Protein was extracted by suspending the minced muscle in water at 1:10 ratio (w/v, minced muscle: water) and homogenised with Ultra Turrax homogeniser, IKA, Germany at high speed for 1 min at 25°C. The solution was gradually added with 0.5 M NaOH until the pH was constant at pH 9. Soluble proteins present in the solution were separated by centrifugation at 12,000 g for 30 min at 4°C in a Sorvall RC-5B Superspeed Refrigerated centrifuge (DuPont Instruments-Sorvall, Canada). The supernatant was adjusted to pH 5.2 with 2 N HCl enabling the precipitation of protein. The precipitate was separated by centrifuging at 12,000×g for 30 mins in a Sorvall RC-5B Superspeed refrigerated centrifuge (DuPont Instruments-Sorvall, Canada) at 4°C and the supernatant was discarded. The precipitate was washed with water and re-solubilized in deionized water and the final pH was adjusted to pH 7.0 with 2 N NaOH, freeze dried and stored at -20°C for further use. The extracted protein is called black tilapia protein isolate.

2.3 Preparation of protein hydrolysate

The hydrolysis was performed according to Madzlan *et al.* (2006). An amount of 10 g of freeze-dried fish protein was dispersed in 500 mL distilled water to make 2% substrate. The solution was heated at 95°C for 5 mins and then cooled immediately to room temperature. The enzyme was added (enzyme:substrate ratio of 2:100 on a w/w basis) to hydrolyze the extracted protein at pH 6 and temperature 60°C. L-cysteine (free base) was added at a concentration of 0.01 M as a papain activator. The hydrolysate solution was incubated for 4 hrs at 200 rpm. After 4 hrs, the solutions were heated at 95°C for 10 mins to inactivate the enzyme. The hydrolysates were then centrifuged at 7800×g in a Sorvall RC-5B Superspeed Refrigerated centrifuge (DuPont Instruments -Sorvall, Canada) at 4°C for 30 mins followed by filtering through filter paper (Whatman No. 1) to remove free lipids. The filtrates were then freeze-dried and stored at -20°C until further use.

2.4 Animal study design on the effect of protein hydrolysates in hypertension-induced mice

2.4.1 Experimental animals

Healthy C57BL/6 mice (25-30 g) were used and maintained at standard laboratory conditions. The mice were procured from the Laboratory Animal Facility and Management Faculty of Pharmacy (LAFAM), UiTM Puncak Alam, Selangor. The mice were fed with a commercial pellet diet and water ad libitum for an acclimatization period of one week before proceeding to experiment for 28 days. The protocol was approved by the Committee on Animal Research and Ethics of the Universiti Teknologi MARA (UiTM). The laboratory animals were handled and managed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.4.2 Acute oral toxicity study

Acute oral toxicity tests were carried out according to OECD guidelines 423. Twelve healthy C57BL/6 mice (25-30 g) multi-gender equally were divided into two groups: control group and the protein hydrolysates treatment group. The mice were left for overnight fasting (8-10 h) and then the treatment group was given a single dose of protein hydrolysates (2000 mg/kg of body weight) on day one by oral administration (force feed) whereas the control mice received only distilled water. All animals were observed for clinical signs including mortality and any adverse reactions twice daily for 14 days and sacrificed to obtain the blood and serum. Alanine aminotransferase (ALT), alkaline phosphatase (ALP) gamma-glutamyl transferase (GGT), creatinine, urea, Na⁺, and K⁺ and Cl⁻ from the serum were then analysed.

2.4.3 Induction of hypertension

The mice were fed with cookies containing 6% NaCl (w/w) for two weeks. The hypertension state was confirmed by measuring blood pressure (CODA, Kent Scientific). Control and hypertension mice were divided into the following groups consisting of six animals each. Within 28 days of treatment, the mice were administered orally with 1% NaCl (w/v) followed by the treatments. The body weight was measured every week for four weeks.

Group I – Control.

Group II – Hypertension.

Group III – Hypertension mice administered with Carvedilol (CV, 0.15 mg/kg).

Group IV – Hypertension mice feed with 5% protein hydrolysates.

Group V – Hypertension mice feed with 10% protein

hydrolysates.

Group VI – Hypertension mice feed with 20% protein hydrolysates.

Group VII – Hypertension mice feed with 10% protein hydrolysates in combination with CV (0.15 mg/kg).

2.4.4 Blood samples test

Blood samples were collected at the end of the experiment and run for full blood count (red blood cell (RBC), white blood cell (WBC) and platelets followed by centrifugation at 2000 rpm to obtain the serum. Serum was then used to measure angiotensin converting enzyme (ACE) activity, liver enzymes (ALT, ALP and GGT) and kidney function (Na⁺, K⁺, Cl⁻, urea and creatinine levels).

2.4.5 Angiotensin converting enzyme activity analysis on serum

Standard and serum were added into each well followed by incubation for 2 hrs at 37°C. Subsequently, the plate was incubated with Biotin-antibody and HRP-avidin for another 1 hour and washed. The substrate was then added into the well (15-30 min) in the dark and the reaction was stopped with a stop solution. The plate was read using a Microplate Reader within 5 mins.

2.5 Statistical analysis

All experiments were performed in three replicates. Statistical analysis was performed by using SPSS ver. 20. The results were first checked for normality before proceeding to one-way ANOVA, two-way ANOVA and paired Sample T-test analysis. The data is considered statistically significant at $p \leq 0.05$ or $p \leq 0.001$.

3. Results and discussion

3.1 Acute oral toxicity study

During 14 days of acute toxicity study, there were no significant changes or adverse clinical signs and mortality. The result showed the protein hydrolysates are safe as there were neither toxic signs nor mortality observed after administration of a single high dose (2000 mg/kg BW). Figure 1 shows liver enzyme level and kidney function level after administration of protein hydrolysates at high single dose (2000 mg/kg BW). No significant differences were observed in control and protein hydrolysates group after administration of maximum dose of protein hydrolysates for liver enzyme level (ALP, ALT, GGT) and kidney function level (creatinine, urea, Na⁺, and K⁺ and Cl⁻ ($p > 0.05$)). Barkia et al. (2020) reported that oral administration of microalgal protein hydrolysates at three doses (D1, 100 mg kg⁻¹ BW; D2, 400 mg kg⁻¹ BW; and D3,

2000 mg kg⁻¹ BW) to male Wistar rats did not induce any adverse effects or mortality up to 13 days of treatment.

3.2 Effect of black tilapia protein hydrolysates on

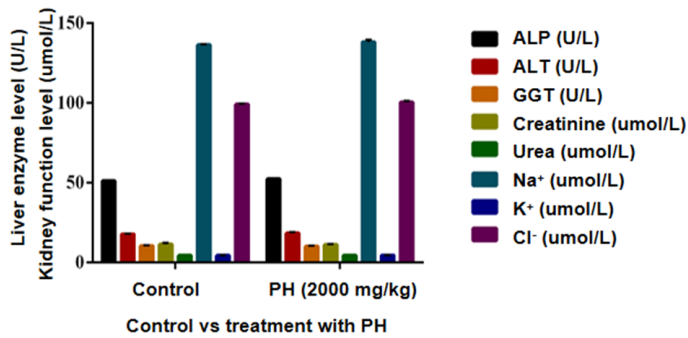


Figure 1. Liver enzyme level (ALP, ALT and GGT) and kidney function level (creatinine, urea, Na⁺, and K⁺ and Cl⁻) in acute toxicity test of protein hydrolysates (PH) when administered at maximum dosage (2000 mg/kg body weight) after 14 days of experiment. Results represent mean±SD of three independent experiments.

hypertension-induced mice

3.2.1 Effect of black tilapia protein hydrolysates on body weight

Figure 2 shows the effect of protein hydrolysates on body weight. A hypertensive mouse (systolic blood pressure; 161±3 mm/Hg) was fed with a pellet consisting of protein hydrolysates at different concentrations as well as carvedilol and combination of carvedilol and protein hydrolysates and monitored for 28 days. The body weight of the control group hypertensive group and treatment group was compared. Results showed no significant differences were observed in the control mice group throughout the experiment ($p > 0.05$). There was a significant reduction of body weight in hypertension mice on week 2 (23 ± 0.3333 g, $p = 0.017$), week 3 (22 ± 0.3122 g, $p = 0.030$ and week 4 (21 ± 0.3487 g, $p = 0.000$) in comparison to week 1 (25 ± 0.3333 g). Significant reduction of body weight was observed in the hypertension mice group on week 4 as compared to control group ($p < 0.05$). The reduction of body weight is usually considered an indicator of a toxic substance

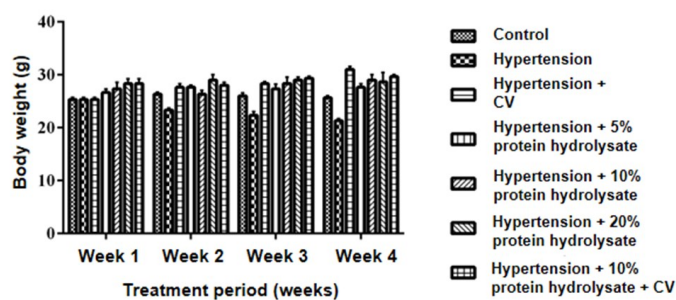


Figure 2. Effect of carvedilol, protein hydrolysates and combination of carvedilol and protein hydrolysates on body weight of hypertension-induced mice treated for 28 days and compared with control and hypertensive mice. Results represent mean±SD of three independent experiments.

(Thanabhorn et al., 2006).

A significant increase in body weight was observed in hypertension mice administered with protein hydrolysates at different concentrations as well as carvedilol and a combination of carvedilol and protein hydrolysates compared to the control group and hypertensive group throughout the experiment ($p < 0.05$). No significant difference was observed in the 5%, 10% and 20% protein hydrolysates group ($p > 0.05$). Results showed that the body weight of hypertension-induced mice reduced as a function of experiment period from 1 to 4 weeks. Treatment with protein hydrolysates, as well as carvedilol and a combination of carvedilol and protein hydrolysates, improved the body weight of mice. Teo et al. (2002) and Hilaly et al. (2004) reported that abnormal body weight changes were used to predict any adverse effects of chemicals and drugs. A positive body weight increment ($p < 0.05$) indicates non-toxicity effects and health improvement.

3.2.2 Effect of black tilapia protein hydrolysates on blood pressure

The effect of black tilapia protein hydrolysates on blood pressure is shown in Figure 3. A hypertensive mice (systolic blood pressure; 161 mm/Hg±3) was fed with pellet consisting of protein hydrolysates at different concentrations as well as carvedilol and a combination of carvedilol and protein hydrolysates and compared with control group. For control and hypertensive mice, no significant differences were observed in blood pressure throughout the experiment ($p > 0.05$). The systolic blood pressure for control group was maintained at around 100 mm/Hg and the hypertension group at around 170 mm/Hg. In comparison to week 1, there was a significant reduction of blood pressure in the group of carvedilol (107 ± 4 mm/Hg, $p = 0.001$), 5% protein hydrolysates (119 ± 1 mm/Hg, $p = 0.000$), 10% protein hydrolysates (123 ± 1 mm/Hg, $p = 0.008$), 20% protein hydrolysates (115 ± 2 mm/Hg, $p = 0.002$) and combination of 10% protein hydrolysates with carvedilol (103 ± 2 mm/Hg, $p = 0.000$) on week 4 respectively. No significant differences were observed in the hypertension group treated with protein hydrolysates or in combination with carvedilol compared to control. The results showed protein hydrolysates exhibited similar properties as carvedilol, a commercial drug for reducing blood pressure.

Treatment with other antihypertensive proteins and peptides isolated from fish products such as yellowfin sole, cuttlefish, chum salmon and sardine has revealed a reduction in systolic blood pressure in spontaneously hypertensive rats (SHR) (Jung et al., 2006; Otani et al., 2009). The antihypertensive effect of surimi from olive flounder peptide in SHR has also been studied by Oh et

al. (2020) who suggested that surimi consumption was effective at decreasing systolic blood pressure. Alashi *et al.* (2014) reported the blood pressure lowering effects of Australian canola oil protein hydrolysates in SHR where alcalase hydrolysate produces the greatest and fastest decrease in systolic blood pressure.

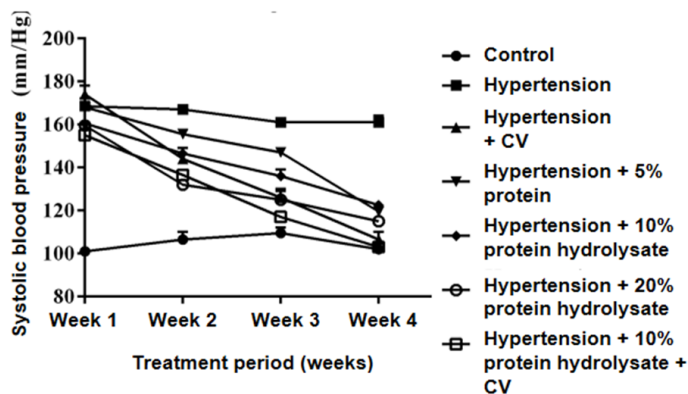


Figure 3. Systolic blood pressure of hypertension-induced mice treated with carvedilol, protein hydrolysates and combination of carvedilol and protein hydrolysates for 28 days compared to control and hypertensive mice. Results represent mean \pm SD of three independent experiments. Values with $p < 0.05$ were considered significant.

3.2.3 Effect of hypertension on red blood cells, white blood cells and platelets

At the end of the experiment after 28 days of treatment, the blood samples of all groups of mice were collected and were run for red blood cell (RBC), white blood cell (WBC) and platelets analysis. The effect of hypertension on red blood cells (RBC), white blood cells (WBC) and platelets is shown in Figure 4. There was a significant increase in RBC in the hypertension group compared to control. However, the administration of carvedilol, 5%, 10% and 20% protein hydrolysates reduced the RBC similar to the control group. Meanwhile, for WBC and platelets, there were no significant changes were observed for all groups of mice ($p > 0.05$).

Vedernikov *et al.* (1998) studied the effect of red blood cells and haemoglobin on SHR and normotensive

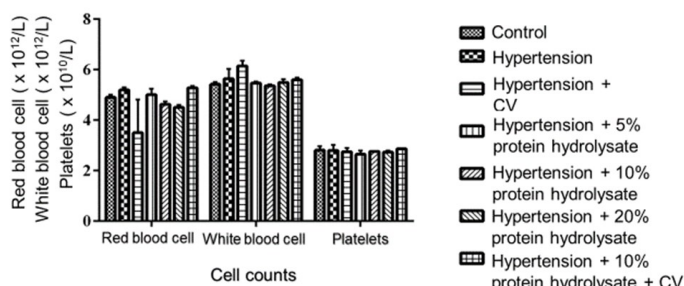


Figure 4. Effect of hypertension on red blood cell (RBC), white blood cell (WBC) and platelets. Results represent mean \pm SD of three independent experiments.

rat aortas. Experiments were designed to compare the contractile effect of red blood cells (RBC) on aortic rings with and without endothelium from normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. The results found that red blood cell breakdown products, namely free haemoglobin might be considered as vasoconstricting factors in the development and maintenance of hypertension. Red blood cells are able to increase the tension of the isolated aorta of SHR at the prehypertensive stage and more pronounced at the hypertension stage. Removal of the endothelium increased the contractile effect of red blood cells, more in SHR where haemoglobin increased the tension at the hypertension stage (16-week-old SHR). Haemoglobin causes vasoconstriction both by inhibiting endothelium derived relaxing factor (EDRF) and promoting the production of eicosanoids by endothelial and smooth muscle cells. Another study (Atsma *et al.*, 2012; Xuan *et al.*, 2018) reported that there was an association between haemoglobin level and systolic blood pressure and diastolic blood pressure in healthy persons.

3.2.4 Effect of black tilapia protein hydrolysates on liver enzyme level in hypertension-induced mice

The serum was collected after centrifugation of the blood samples. The serum was used to measure liver enzymes (ALT, ALP and GGT levels), kidney function (Na^+ , K^+ , Cl^- , urea and creatinine levels) and angiotensin converting enzyme (ACE) activity.

The effect of black tilapia protein hydrolysates on liver enzyme levels in hypertension-induced mice is shown in Figure 5. It was found that liver enzyme levels in hypertension-induced mice increased compared to control group. A significant increase in ALP level in the hypertension group (121 ± 2.082 U/L, $p = 0.000$) compared to control (69 ± 0.882 U/L) (A). ALT level also increases significantly in hypertension group (109 ± 1.856 U/L, $p = 0.000$) compared to control (61 ± 0.881 U/L) (B). Meanwhile, in comparison to control (12.6 ± 0.882 U/L), GGT level significantly increases in the hypertension group (19 ± 0.577 U/L, $p = 0.000$) (C). Rahman *et al.* (2020) reported that the prevalence of elevated liver enzymes was higher in hypertensive individuals where increased serum ALT and GGT activities were positively associated with hypertension. A similar result was also reported with a high prevalence of elevated ALT in the hypertensive group compared to the normotensive group (Hong *et al.*, 2016).

The biological mechanism underlying the relationships between hepatic enzymes and hypertension remains unclear. The postulated mechanism could be that increased blood pressure activates pro-inflammatory responses such as $\text{TNF-}\alpha$ and interleukin adiponectin and

leptin that contribute to hepatotoxicity (Musso *et al.*, 2008). Study evidence suggests a link of non-alcoholic fatty liver disease (NAFLD) with cardiovascular disease (CVD) (Bonnet *et al.*, 2017). Some cross-sectional studies showed a higher incidence of NAFLD in hypertensive individuals, as compared with those with normal blood pressure (López-Suárez, 2011; Aneni *et al.*, 2015). On the other hand, a potential mechanism for the link between GGT and hypertension might be related to oxidative stress and the role of cellular GGT in the catabolism of extracellular antioxidant glutathione (Shankar and Li, 2007; Bonnet *et al.*, 2017). It has also been reported that cellular GGT may be related to reactive oxygen species production in the presence of transition metals (Lee *et al.*, 2004). In parallel, oxidative stress is documented to be associated with hypertension (Touyz, 2004) and antioxidant enzyme genes polymorphisms, including a few of the glutathione-S-transferase genes, have been reported to be correlated with the risk of hypertension in general adults (Mansego *et al.*, 2011; Eslami and Sahebkar, 2014).

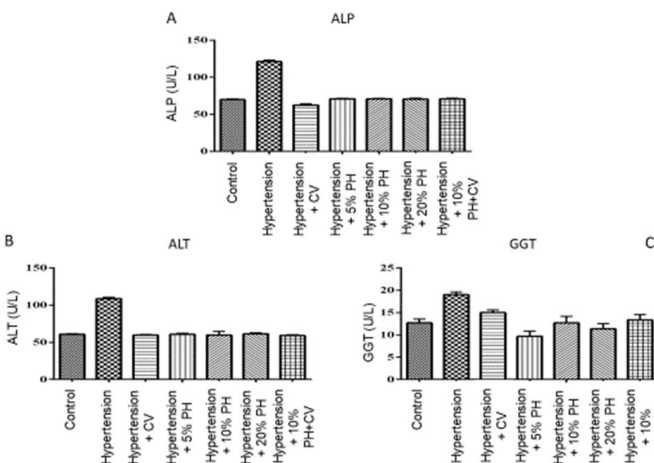


Figure 5. Liver enzyme level in the serum of hypertension-induced mice after treatment with carvedilol, protein hydrolysates (PH) and combination of carvedilol and protein hydrolysates for 28 days in comparison with control and hypertensive mice. Results represent mean±SD of three independent experiments.

An administration of carvedilol, protein hydrolysates and a combination of carvedilol and protein hydrolysates on hypertension-induced mice reduced the ALP, ALT and GGT content similar to the control group. No significant difference was observed in ALP, ALT and GGT levels of all groups of hypertension mice administered with carvedilol, protein hydrolysates and a combination of carvedilol and protein hydrolysates ($p > 0.05$) compared to control. Liu *et al.* (2011) studied the hepatoprotective and antioxidant effects of porcine plasma protein hydrolysates on carbon tetrachloride-induced liver damage in rats. The results found that low molecular weight peptides (MW<3kDa) could significantly lower ($P < 0.01$) the serum levels of hepatic enzyme markers (aspartate transaminase and alanine

transaminase). Similar findings, Cai *et al.* (2017) studied the effects of enzymatic hydrolysate from *Schizochytrium* sp. on acute alcohol-Induced liver Injury in mice, and found that a low molecular weight peptide (MW<3 kDa) significantly decreased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Chen *et al.* (2019) reported that a peptide YGDEY from tilapia fish skin gelatin hydrolysates decreased the expression of gamma-glutamyltransferase (GGT) in HepG2 cells from alcohol-induced damage in HepG2 Cells.

3.2.5 Effect of black tilapia protein hydrolysates on kidney function level in hypertension-induced mice

The effect of black tilapia protein hydrolysates, carvedilol and a combination of carvedilol and protein hydrolysates on kidney function levels in hypertension-induced mice is shown in Figure 6. The results showed no significant changes of creatinine (A) and urea (B) levels in all groups ($p > 0.05$). However, a significant increase of Na^+ (C), K^+ (D) and Cl^- (E) level were observed only in hypertension group ($p \leq 0.05$) compared to the control group. Hypertension is the major leading

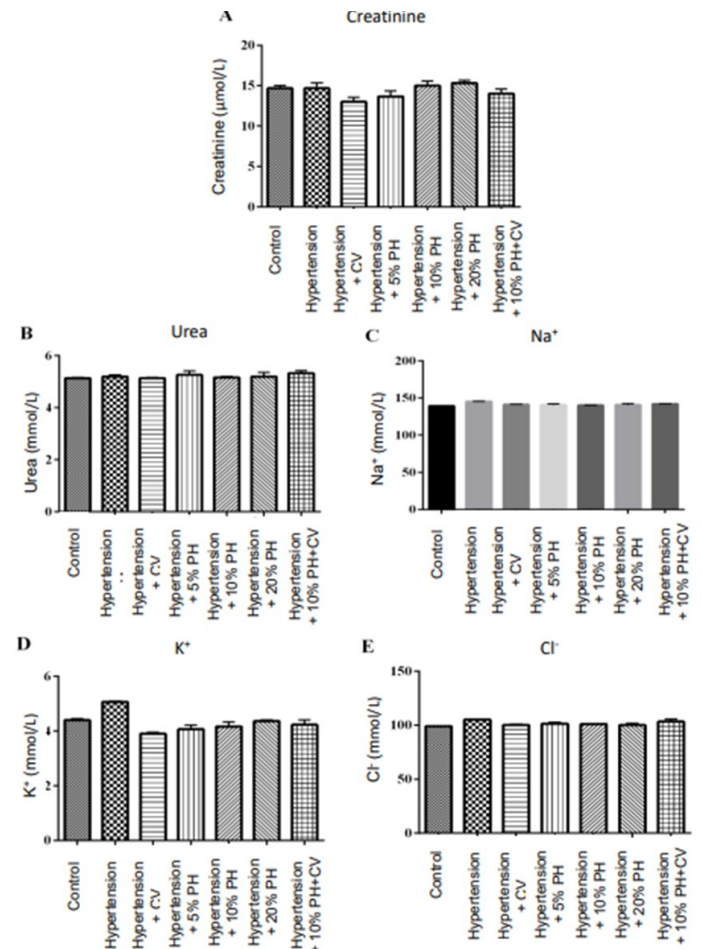


Figure 6. Kidney function level in the serum of hypertension-induced mice after treatment with carvedilol, protein hydrolysates (PH) and combination of carvedilol and protein hydrolysates for 28 days in comparison with control and hypertensive mice. Results represent mean±SD of three independent experiments.

risk factor for atherosclerosis and several diseases, especially renal and cardiovascular disorders, including myocardial infarction, stroke, and heart failure (Rahimi *et al.*, 2015). Sodium is an important mineral which, besides its functions in fluid balance, action potential generation, digestive secretions and absorption of many nutrients, also plays an important role in blood pressure regulation with a reduced sodium intake being associated with a reduction in systolic and diastolic blood pressure (Rust and Ekmekcioglu, 2017). A high NaCl intake convincingly contributes to elevated arterial pressure in a number of animal models of genetic and acquired hypertension (Kotchen and McCarron, 1998). It has recently been demonstrated that the addition of NaCl (5, 10, and then 15 g/d) to the chimpanzee's usual diet (a fruit-and-vegetable diet low in NaCl and high in potassium) over 20 months results in significant and progressive elevations of blood pressure (Denton *et al.*, 1995). The rise in arterial pressure and other harmful cardiovascular effects of dietary salt are related to the primary inadequacy of the kidney to excrete sodium (de Wardener, 1990).

Early studies suggest that chloride has an independent effect on blood pressure (McCallum *et al.*, 2015). It was shown that replacing chloride with bicarbonate, citrate or phosphate as the anion for sodium did not lead to increases in blood pressure in rats or humans compared to sodium chloride (Kurtz and Morris, 1983; Kurtz *et al.*, 1987; Shore *et al.*, 1988). Similar studies on Dahl salt-sensitive rats (Kotchen *et al.*, 1983) showed that hypertension occurred within several weeks when animals were fed on a high NaCl diet, but not when the animals were fed an identical Na⁺ load provided as sodium bicarbonate or other non-chloride salts of Na⁺.

Treatment with black tilapia protein hydrolysates, carvedilol and a combination of carvedilol and protein hydrolysates reduced the Na⁺, K⁺ and Cl⁻ level equivalent to control group. No significant differences in Na⁺, K⁺ and Cl⁻ levels ($p \geq 0.05$) between control group and protein hydrolysates, carvedilol and combination of carvedilol and protein hydrolysates treatment group. Costa *et al.* (2005) studied the acute effect of the intraperitoneal (*ip*) administration of a whey protein hydrolysate (WPH) on systolic arterial blood pressure (SBP) and renal sodium handling by conscious spontaneously hypertensive rats (SHR). The study demonstrated that the *ip* administration of WPH caused a marked decrease in SBP and glomerular filtration rate. The *ip* administration of 1.0 g WPH/kg decreased fractional sodium excretion compared to 0.15 M NaCl and captopril-treated rats, respectively. Similarly, the fractional potassium excretion in WPH-treated rats was

significantly lower than in 0.15 M NaCl and captopril-treated rats, respectively. Many factors have been proposed as being important for the renal excretion of potassium including blood pH, potential across the luminal membrane, sodium delivery to the distal tubule, and urinary flow rate (Wright and Giebisch, (1992).

3.2.6 Effect of black tilapia protein hydrolysates on ACE activity in serum of hypertension-induced mice

Figure 7 shows the effect of black tilapia protein hydrolysates on ACE activity in the serum of hypertension-induced mice. The effect of antihypertension is measured based on the ability of protein hydrolysates to inhibit ACE activity in serum. After 28 days of the experiment, it was observed that in comparison to control ($41 \pm 0.577\%$), there was a significant increase in ACE activity in the hypertension group ($79 \pm 0.882\%$, $p = 0.000$). Few studies reported that ACE activity in serum was increased in hypertension conditions (Overlack *et al.*, 1983; Forrester *et al.*, 1997). However, administration of carvedilol, 5%, 10%, 20% protein hydrolysates and combination of carvedilol and 10% protein hydrolysates reduced the ACE activity. There was a significant reduction of ACE activity in carvedilol, 5%, 10%, 20% protein hydrolysates and 10% protein hydrolysates in combination to carvedilol group compared to hypertension group. The results showed that protein hydrolysates are able to inhibit ACE activity comparable to carvedilol. Chen *et al.* (2017) studied on effect of foxtail millet protein hydrolysates on lowering blood pressure in spontaneously hypertensive rats and found that the serum ACE activity and angiotensin II levels in the treatment groups were significantly lower than that of the control.

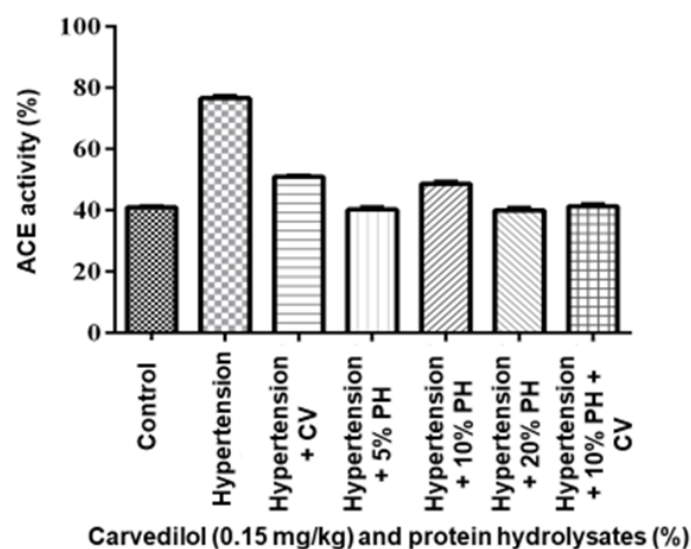


Figure 7. ACE activity in the serum of hypertension-induced mice after treatment with carvedilol, protein hydrolysates (PH) and combination of carvedilol and protein hydrolysates for 28 days in comparison with control and hypertensive mice. Results represent mean \pm SD of three independent experiments.

4. Conclusion

In vivo, a hypertensive mouse fed with pellet consisting of protein hydrolysates showed a significant reduction of blood pressure comparable to carvedilol, a commercial drug for reducing blood pressure. Effect of black tilapia protein hydrolysates on liver enzymes (ALT, ALP and GGT levels), kidney function (Na⁺, K⁺, Cl⁻, urea and creatinine levels) and angiotensin converting enzyme (ACE) activity of hypertension-induced mice showed that protein hydrolysates were able to reduce liver enzymes level, kidney function level (Na⁺, K⁺ and Cl⁻) and angiotensin converting enzyme (ACE) activity of hypertension-induced mice comparable to carvedilol.

Conflict of interest

The authors declare no conflict of interest.

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