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Effects of Nile Tilapia (*Oreochromis niloticus*) Viscera Hydrolyzate on Blood Pressure, TNF-α and IL-6 Expression in Rats (*Rattus norvegicus*) Induced by DOCA-Salt

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ABSTRACT

Background: Hypertension is a disease that has the potential to become the most significant cause of death in the world in 2020. Hypertension caused by an imbalance of the renin angiotensinogen system. Viscera hydrolyzate extract has an anti-hypertension activity. The research aimed to determine tilapia viscera hydrolyzate extract's ability to reduce blood pressure, suppressing the expression of TNF-α and IL-6. Viscera hydrolyzed with alcalase and extracted to obtain viscera bioactive crude extract (VisBC).

Methods: Spontaneously hypertensive rats induced by DOCA-salt, gave VisBC therapy. 15 Wistar rats divided into 5 treatment groups, namely normal control (without induced DOCA-salt), positive control (SHR-Spontaneous Hypertensive Rat = induced by DOCA-salt), comparative control (SHR + Captopril 5 mg/kg), VisBC 1 (SHR + BC 150 mg/kg) and VisBC 2 (SHR + BC 300 mg/kg), where each treatment consisted of 3 replications. Each treatment measured for blood pressure, TNF-α and IL-6 expression. The data represented as mean ± SD and analyzed by ANOVA ($p<0.05$) in any difference, a subsequent Duncan test. Data analyzed by SPSS 20.0 for Windows 10.

Result: The results showed the treatment of VisBC could reduce systole (19.64%) and diastole (29.15%) blood pressure. Also, VisBC inhibited the expression of TNF-α (8.08%) and IL-6 (8.39%). The VisBC of tilapia with a dose of 150 mg/kg could inhibit the expression of TNF-α and IL-6, thereby affected the decrease in blood pressure (systole and diastole) in rats induced by DOCA-salt. This research proved that fish waste could produce bioactive as anti-hypertension.

Key words: Angiotensin, Diastole, Hypertension, Inflammation, Systole.

INTRODUCTION

Production of Tilapia (*Oreochromis niloticus*) in Indonesia over the past five years increased by 18% (Riyadi *et al*., 2019). Augmented tilapia production result in increased fish waste (viscera). One way to add value to viscera that contains protein is to convert it to hydrolyzate (Horn *et al*. 2005). The results of previous studies reported extracts of fish by-product hydrolyzate has anti-hypertensive activity, for example head of salmon chum, skate skin (*Okamejeri kenojei*), salmon skin, gelatin from Thornback Gray skin, viscera smooth-hound (*Mustelus mustelus*), tuna fins, meat red tuna, tuna liver, skipjack egg, head and viscera *Sardinella aurita*, bone *Limanda aspera* (Riyadi, 2018).

Hypertension is a disease that has the potential to become the most significant cause of death in the world in 2020 (Norris and FitzGerald, 2013). Generally, hypertension was divided into primary and secondary hypertension (Choudhary *et al*., 2010). Hypertension caused by an unbalanced renin angiotensinogen system. Renin catalyzes the angiotensinogen substrate into the active compound angiotensin I (ANG I). Furthermore, it is catalyzed by the enzyme ACE (angiotensin-I converting enzyme) to angiotensin II (ANG II). ANG I to ANG II causes increased aldosterone secretion, sympathetic nerve activity, salt retention and vasoconstriction of blood vessels. It caused an increase in blood pressure (Kearney *et al*. 2004).

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ANG I to ANG II caused an increase in blood pressure (Chamarthi *et al*. 2013), TNF-α (Ferreri *et al*. 2017) and IL-6 (Lee *et al*. 2006). Increased blood pressure causes oxidative stress conditions in endothelial cells (Von Känel *et al*. 2004). Conditions of oxidative stress have the effect of inflammatory pathogenesis. TNF-α (Mingli *et al*., 2016) and IL-6 are cytokines as markers of inflammation. In this

study, using rat (*Rattus norvegicus*) induced by DOCA-salt to stimulate spontaneous hypertension. After that, therapy with viscera hydrolyzate extract of tilapia. The research aimed to determine the ability of tilapia viscera hydrolyzate extract in reducing blood pressure, suppressing the expression of TNF-α and IL-6 in rat induced DOCA-salt.

MATERIALS AND METHODS

The experiment was conducted from February to August 2019 at the Department of Biology, Brawijaya University, Indonesia. This study used viscera (intestine, spleen, liver) of tilapia obtained from the freezing industry of Tilapia fillets, PT. Aquafarm Nusantara, Tambak Aji Industrial Park Semarang, Indonesia.

Viscera bioactive crude (VisBC)

Tilapia viscera cleaned from fat. Then, viscera hydrolyzed using the enzyme alcalase (Sigma-Aldrich No. 126741) (Riyadi *et al*., 2019). Viscera was homogenized with distilled water (1:1) and heated (85°C, 20 min) to inactivate endogenous enzymes. Samples centrifuged (10°C, 20 min, 5.800 rpm) to separate fat and protein. The protein-rich residue extracted three times with distilled water (1:1 w/v) to obtain VisBC. VisBC sprayed with a spray drier to reduce water content (Labplant SD05, LP Technologies, UK). VisBC used as therapy in rat induced DOCA-salt.

Animal subjects preparations

Experimental animals consisted of 15 male rats (Ethical clearance No.1064-KEP-UB, Brawijaya University, Indonesia). Rats with 200 - 210 g adapted for 14 days, controlled at 24°C ± 2°C, humidity 50-60% and 12 hours dark/light cycles). Rats gave a standard diet (maximum water content of 12%, crude protein at least 15%, crude fat 3-7%, crude fiber up to 6%, maximum ash 7%, calcium 0.9-1.1%, phosphorus 0.6-0.9%) and drinking water. Stimulation of spontaneous hypertension, rats induced DOCA-salt orally 10 times for 5 weeks (2 times a week). Rats were induced DOCA-Salt at a dose of 20 mg/kg (as much as 5 times), then a dose of 10 mg/kg (as much as 5 times). After that, continued with VisBC and captopril therapy for 8 days orally. The therapy was given after all rats had hypertension (blood pressure 140/100).

Experimental design

15 Wistar rats divided into 5 treatment groups, namely normal control (without induced DOCA-salt), positive control (SHR-Spontaneous Hypertensive Rat = induced by DOCA-salt), comparative control (SHR + Captopril 5 mg/kg), VisBC 1 (SHR + BC 150 mg/kg) and VisBC 2 (SHR + BC 300 mg/kg), where each treatment consisted of 3 replications (Nurmahdi *et al*., 2017). Each treatment measured for blood pressure, TNF-α and IL-6 expression. The data represented as mean ± SD and analyzed by ANOVA ($p<0.05$) in the case of any difference, a subsequent Duncan test. Data analyzed by SPSS 20.0 for Windows 10.

Blood pressure

The day before surgery, rats measured by the Tail Cuff Method using a Blood Pressure Analyzer (Prahalathan *et al*., 2012).

TNF-α and IL-6 expression

(Riyadi *et al*., 2019): Rats anesthetized with ketamine and dissected to collect the spleen. The spleen crushed and homogenized. Homogenates were placed in propylene tubes, then added PBS and centrifuged (1500 rpm, 10°C, 5 min). The pellet part was taken and resuspended with PBS until homogeneous. After that, the pellet was stained with FITC-conjugated CD68; Bioss: MBS438131 and incubated (20 min, 4°C). The previously stained splenocytes were fixed and permeabilized using the cytofix/cytoperm kit (BD-Biosciences, Pharmingen). The pellets were stained with PE anti-mouse/rat TNF-α (Bioss: TN3-19.12) and IL-6 (Bioss: bs-0379R), then incubated. The staining combination used $CD68^{+}TNF\text{-}\alpha^{+}IL\text{-}6^{+}$. The final suspension was analyzed by Flow Cytometry (BD Cell Quest program, San Jose, CA).

RESULTS AND DISCUSSION

Blood pressure

The Fig 1 showed DOCA-salt induction increased systole and diastole blood pressure in the SHR ($p<0.05$) compared to normal rats. Induction of DOCA-salt caused an increase

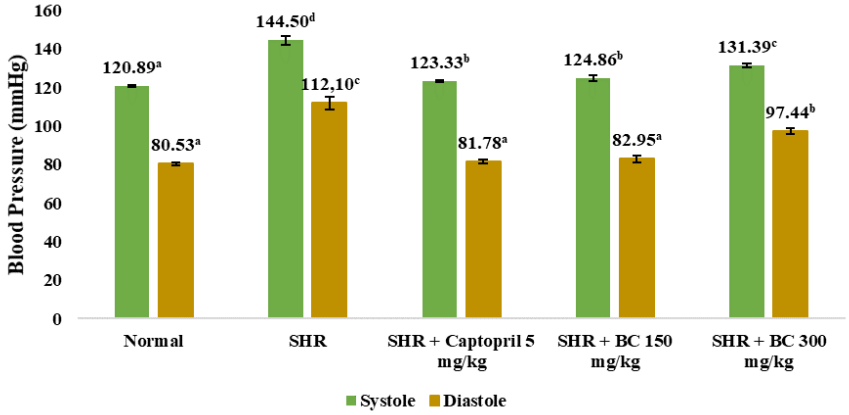


Fig 1: Effects of Tilapia VisBC on blood pressure (mmHg) of rats induced by DOCA-salt.

in systole 23.61% and diastole 31.57%. Hypertension blood pressure, which is systole above 140 mmHg and diastole above 100 mmHg (Badyal *et al*., 2003). DOCA-salt increased in reabsorption of sodium ions and water from renal distal nephron epithelial cells. It affected blood volume and causing an increase in blood pressure.

Fig 1 showed captopril and VisBC (150 mg/kg) therapy could reduce blood pressure in the SHR. VisBC could reduce systole 19.64% and diastole 29.15%, while captopril reduces systole 21.17% and diastole 30.17%. VisBC 150 mg/kg could reduce blood pressure almost the same as captopril as a positive control. From literature studies, it was suspected that VisBC contains some bioactive peptides which act as anti-hypertensive agents (Wu *et al*., 2006). *In vivo* studies of bioactive peptides from viscera as an inhibitor of angiotensin-converting enzyme (Fahmi *et al*., 2004). Riyadi *et al*., (2019) characterized amino acids contained in tilapia visceral protein hydrolysates. Amino acids are the smallest unit that forms bioactive peptides.

TNF-α expression

TNF-α is a Pro-inflammatory cytokine synthesized by monocytes or macrophages (Tasgin and Haliloglu, 2018). The results with flow cytometry showed the relative amount of TNF-α increased in SHR induced by DOCA-salt compared with normal rats ($P<0.05$). Whereas, rats with captopril and VisBC (150 mg/kg) therapy could reduce the relative amount of TNF-α ($P<0.05$) (Fig 2).

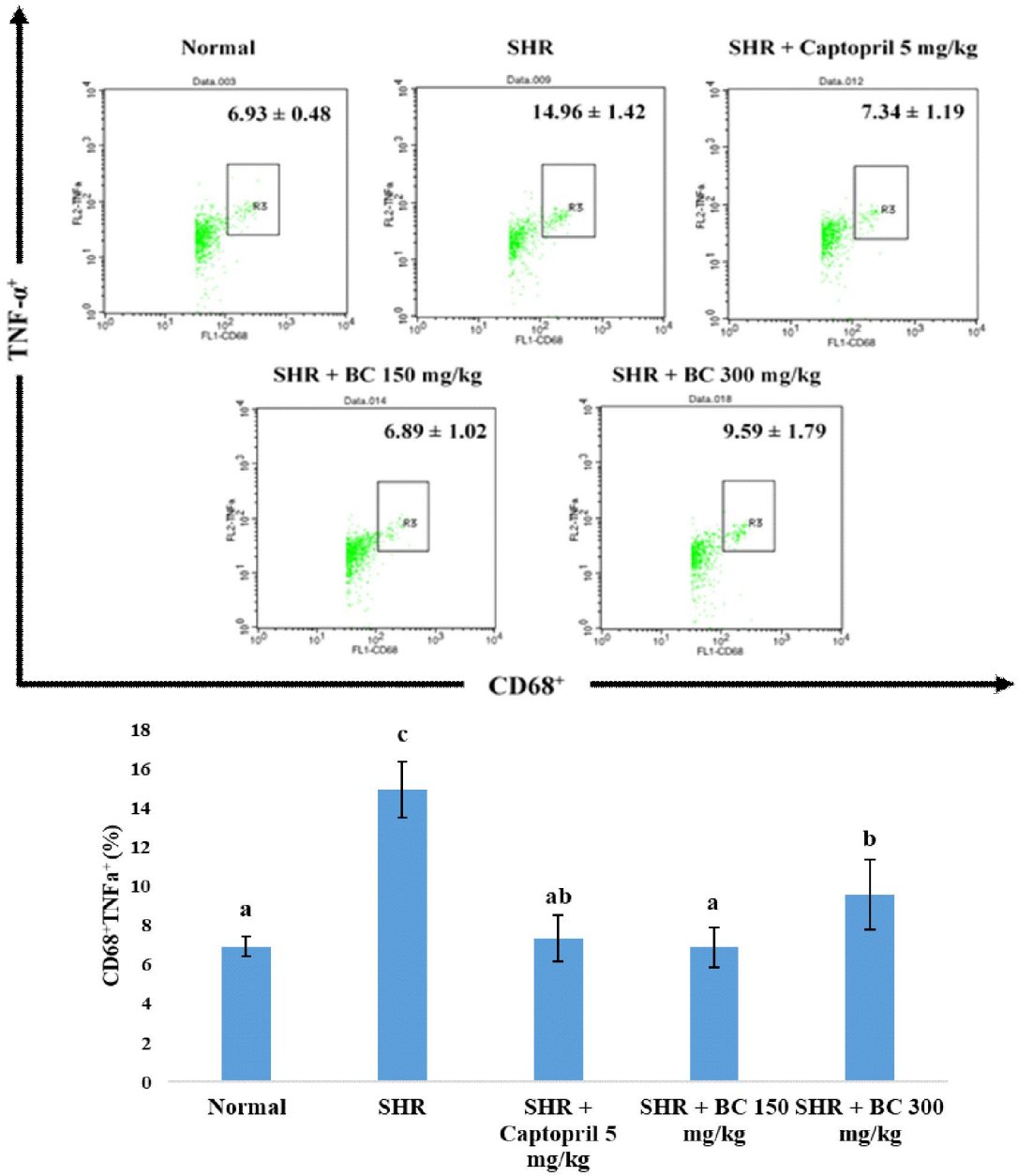


Fig 2: Effects of Tilapia VisBC on TNF-α (%) of rats induced by DOCA-salt.

TNF-α expression increased in response to the condition of spontaneous hypertension due to DOCA-Salt (Elmarakby *et al*. 2008). The role of TNF-α in regulating blood pressure has been studied using a rat model (Alexander *et al*., 2002 and Bae *et al*., 2010). TNF-α could support an increase in blood pressure mediated by ANG II (Ramseyer and Garviscera, 2013). The inflammation caused by increased TNF-α (Venegas-pont *et al*., 2010). Suppressing TNF-α could inhibit the development of hypertension (Chatziantoniou *et al*., 2004). Elmarakby *et al*., (2008) showed suppression of TNF-α could reduce renal inflammation in hypertensive models. Inhibition of TNF-α can reduce most of the inflammatory index and cause the effect of decreasing blood pressure.

Fig 2 showed VisBC with dose 150 mg/kg able to inhibit the TNF-α (8.08%) increase, which has implications for a decrease in blood pressure. This decrease is related to hypertension mediated by ANG II (Ramseyer and Garviscera, 2013). TNF-α caused vasodilation, increased vascular permeability and platelet activated (Navarro and Mora-ferna, 2006). The action mechanism of VisBC suspected by binding to TNF-α and prevented it from interacting with TNF-α receptors (Goffe and Cather, 2003). These data indicate that TNF-α involved in the inflammatory response and related hypertension cause by DOCA-salt. Therefore, by inhibiting TNF-α can be a potential therapy for lowering blood pressure and reducing inflammation in the blood vessels caused by hypertension.

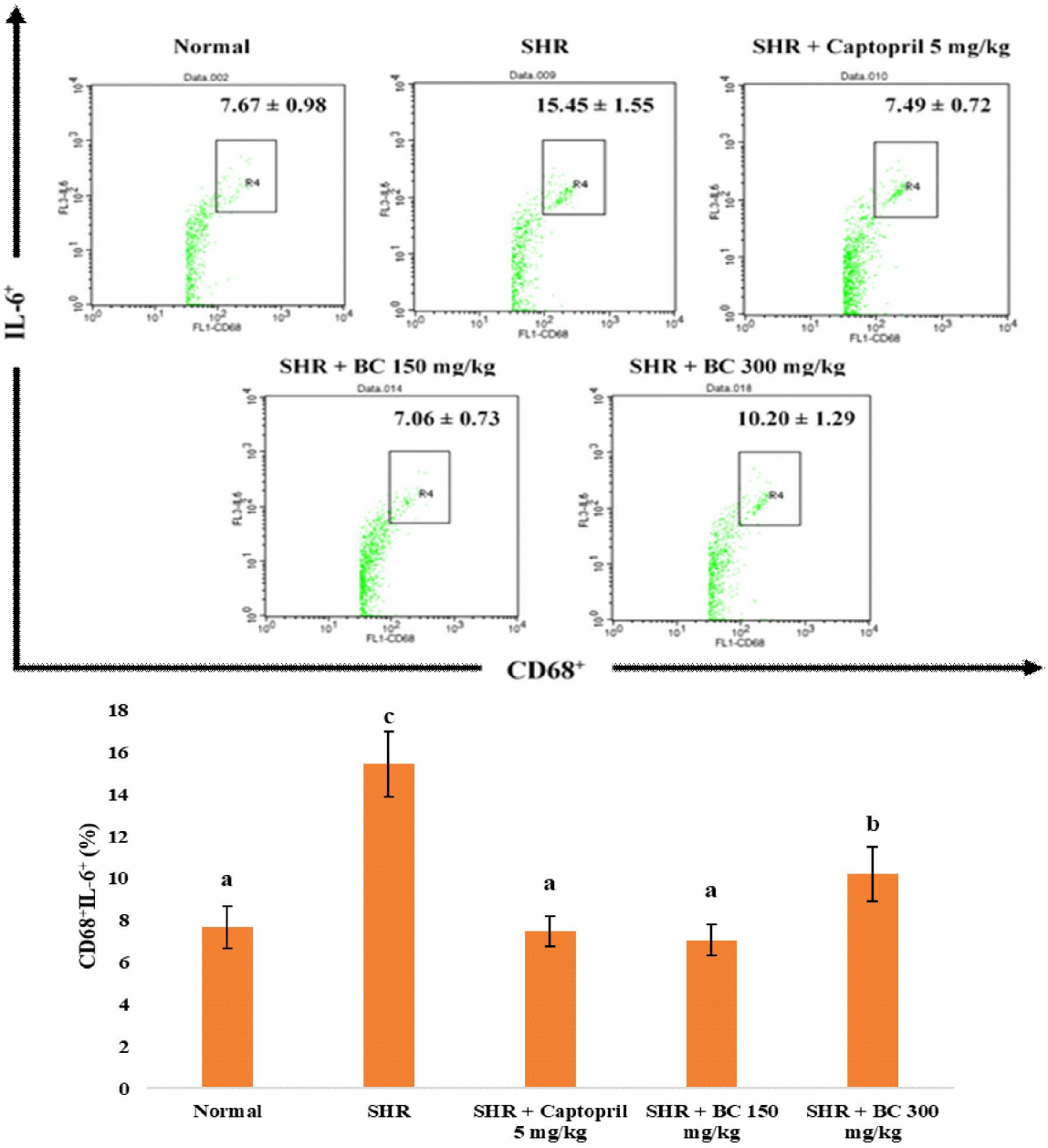


Fig 3: Effects of Tilapia VisBC on IL-6 (%) of rats induced by DOCA-salt.

IL-6 expression

IL-6 is a pro-inflammatory cytokine and showed to be associated with an increased risk of myocardial infarction (Ridker *et al*., 2000), prognostic markers of coronary risk and mortality with acute coronary syndrome (Tan *et al*., 2009). IL-6 plays a major role in animal model hypertension (Coles *et al*., 2007).

The results with flow cytometry showed the relative amount of IL-6 increased in SHR due to DOCA-Salt compared to normal rats ($P<0.05$). The SHR group, with the therapy of captopril and VisBC with dose 150 mg/kg, could reduce the relative amount of IL-6 by 8.39% ($P<0.05$) (Fig 3). Hypertension condition due to DOCA-salt can cause an increase in IL-6, TNF-α and ROS (Wang and Wang, 2009). IL-6 suppression can prevent the diastole dysfunction and is thought to reduce myocardial fibrosis.

IL-6 plays a role in the pathogenesis of hypertension mediated by ANG II. By suppressing IL-6 levels, it could reduce the increase in blood pressure mediated by ANG II (Brands *et al*., 2010). The condition of hypertension also increases chronic inflammation (Bautista *et al*., 2005). The Renin-angiotensin system (RAS) acts as a pro-inflammatory mediator and could cause organ damage due to hypertension (Mehta and Griendling, 2007). IL-6 was released from blood vessel tissue in response to ANG II (Funakoshi *et al*., 1999). VisBc with a dose of 150 mg/kg was thought to block the effects of ANG II and prevent the release of IL-6. VisBC with a dose of 150 mg/kg demonstrates the ability to suppress the amount of TNF-α and IL-6, to reduce the increase in systole and diastole in spontaneously hypertensive rats induced by DOCA salts.

CONCLUSION

It concluded that VisBC of viscera tilapia with dose 150 mg/kg could inhibit the expression of TNF-α and IL-6, thus affected the decrease in blood pressure (systole and diastole) in rats induced by DOCA-salt. This research proved that fish waste could produce bioactive as anti-hypertension.

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