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Research Article

Modifying Effect of Anthocyanin from Purple Sweet Potatoes on Visceral Fat Tissue Inflammation and Liver Oxidative Stress in Psychological Stress-Induced Mice

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ABSTRACT

Psychological stress generates inflammation and oxidative stress in various tissues. Visceral fat as the target site of inflammation is then correlated with stress-induced obesity. Redox imbalance following inflammation also has prominent impacts on hepatic tissue. Therefore, the development of anti-inflammatory and antioxidant properties from plant bioactive compounds is necessary to be investigated. Anthocyanin (ANC) from various plants is previously described as a powerful anti-inflammatory and antioxidant even though its effect in psychological stress remains underexplored. Purple sweet potatoes (PSP) has ANC as its natural pigment. Previous studies revealed the potential effect of ANC from PSP on behavior and an antidepressant candidate. Thus, this study was purposed to determine the effect of ANC from PSP on inflammation and oxidative stress in visceral fat and hepatic tissue respectively. A total of 25 adult male BALB/c mice were assigned into groups of control, stress, stress+ANC 10 mg/kgBW, stress+ANC 20 mg/kgBW and stress+ANC 40 mg/kgBW. Restraint stress was applied 2 hours/day for 14 days. Enzyme-linked immunoassay (ELISA) was conducted to measure level of IL-6 and IL-10 in visceral fat as well as SOD and MDA from hepatic tissue. The results demonstrated that the supplementation of ANC reduces the level of IL-6 cytokine (p=0.005), tends to increase IL-10 (p=0.612), reduces hepatic SOD (p=0.03), and does not significantly affect the level of hepatic MDA (p=0.432). Both ratios of IL-6/IL-10 and SOD/MDA were reduced following ANC administration. Total ANC extracts are suggested to have a potential role of resisting inflammation and oxidative stress in the psychologically stressed model. Further studies are necessary to evaluate the benefits of ANC from PSP in other peripheral organs under psychological stress exposure.

Keywords: *Anthocyanin, Free radical, Inflammation, Psychological stress*

Introduction

Stress is related to internal perception against environmental source stimuli. Higher stress tension causes a broad range of psychological, biological and social problems [1]. Individual maladaptation to stress elicits imbalance in neuroendocrine responses, resulting in extensive impact on peripheral organs through biomolecular mechanisms [2]. Stress activates both hormonal and neural pathways. The hormonal pathway is initiated by hypothalamic-pituitary-adrenal (HPA) axis stimula-tion, which produces an end hormonal product, cortisol. Cortisol is involved in the catabolism process among tissues, including adipose tissue surrounding the visceral organ. In chronic stress, cortisol induces insulin resistance and shifts food preference into rich fat and sucrose food [3].

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Therefore, greater psychological chronic stress is associated with central adiposity and obesity [4].

However, the correlation between stress and adiposity-related inflammatory biomarkers remain conflicting results [4]. The previous report described that visceral fat depot is mostly marked in psychological stress-induced inflammation due to abundant proinflammatory cytokine interleukin-6 (IL-6). On the other hand, sympathetic neural activation during stress releases epinephrine and norepinephrine hormones that aim at the lipolysis mechanism. This process provokes the enhancement of inflammation on visceral fat [5].

Despite visceral fat inflammation, stress has a prominent impact on other tissue, including hepatic tissue. Epinephrine has been demonstrated to induce hydroxyl radicals in hepatic tissue cells as well as proinflammatory cytokine production. Stress also reduces the liver blood flow, thereby inducing mitochondrial hypoxia and thus stimulating the production of free radicals in hepatic tissue [6]. Redox imbalance between oxidant and prooxidant level in hepatic tissue was aggravated by restraint stress and correlates with the increase of cortisol level [7].

Considering the interplay effect of psychological stress, inflammation, and oxidative stress among tissues [8][9], exploration of plant metabolite compounds has been emphasized to find anti-inflammatory and antioxidant sources in order to reduce the systemic effect of stress [10]. Anthocyanin is a plant flavonoid derivatives from red-purple colour plants that is well-established as both anti-inflammatory and antioxidant [11]. Components of anthocyanin such as cyanidin-3-O-glucoside and delphinidin-3-O-glucoside were reported to prevent cell death by inhibiting caspase-3 protein [12]. However, investigation of the benefit of anthocyanin on psychological stress was limited to the improvement effect of anthocyanin to brain dopamine level and lipid peroxidation [13]. Subsequently, our investigation demonstrated that anthocyanin from our local cultivar PSP improves the behaviour of offspring from prenatal stress mothers [14]. Our previous work identified cyanidin as major anthocyanin content in a local cultivar of PSP. Cyanidin is predicted to act as a dopamine D2 agonist that has the potential for antidepressant activity through molecular docking study [15]. However, the molecular mechanism pathway of anthocyanin on stress behaviour remains underexplored. Thus, this study aimed to investigate the effect of anthocyanin from PSP on inflammatory and oxidative stress in visceral fat and hepatic tissue, respectively using an animal model of psychological stress. This study would support the fundamental development of anthocyanin from PSP as pharmacological support in stress-induced inflammation and oxidative stress-related diseases.

Material and Methods

Animals

Adult males of BALB/c mice at age of 7-8 weeks were housed under controlled temperature and humidity in animal experimental facilities of Bioscience Institute, Universitas Brawijaya, Indonesia. Period of the room light/dark cycle was set at 12:12 hours. Animals had free access to water and food. All experimental procedures were previously approved by the Research Ethic Committee, Universitas Brawijaya, Indonesia (No:029-KEP-UB-2022) [14].

Total anthocyanin extraction

Purple sweet potatoes of Antin-3 was obtained from the Research Centre of Legume and Tuber Plant, East Java, Indonesia. Total anthocyanin extraction was conducted by grinding the fresh tuber root, both skin and flesh, using maceration in acidified methanol pH 4.5 for 24 hours at room temperature. The filtrate of homogenates was then evaporated at 50-60°C [14].

Experimental design

A total of 25 mice were randomly assigned into 5 experimental groups (n = 5) consisting of control, stress (STR), STR+ANC 10 mg/kgBW, STR+ANC 20 mg/kgBW and STR+ANC 40 mg/kgBW. Stress was exposed for 2 hours each day for a duration of 14 days in a random schedule to avoid animal habituation [16]. Total anthocyanin extract was administered via intragastric route at a frequency once per day following the stress period [14].

Stress exposure

Stress was conducted as a restraint stress method. Mice were immobilized individually in a ventilated transparent cylinder. The diameter of the cylinder was fitted close to the animal body [16]. Restraint stress was performed to produce both physical and psychological stress among animals [17].

Sample collection and analysis

Visceral fat tissue was collected from the abdominal fat depot as previously described [18]. The level of IL-6 and IL-10 were measured using a commercial enzyme-linked immunoassay (ELISA) kit i.e., Elabscience Biotechnology Inc (Texas, USA) ELISA kit (cat. no: E-EL-M0044 for IL-6; E-EL-M0046 for IL-10), Wuhan Fine Biotech Co., Ltd., (Wuhan, China) ELISA kit (Cat. No: EM0419 for superoxide dismutase (SOD) and cat. no: EM1723-1 for malondialdehyde (MDA). The hepatic SOD enzyme and MDA level were determined using ELISA according to the manufacturer's protocols [19, 20].

Statistical analysis

The data were statistically analyzed using GraphPad Prism 9 software. One-way ANOVA was used to determine the mean difference between groups. The significant p-value was set at $p < 0.05$ [14].

Results and Discussion

This study demonstrates that restraint stress increases IL-6 cytokine in visceral fat tissue. The administration of total anthocyanin extracts concurrent with restraint stress application significantly reduces the level of IL-6 cytokine. Even though the level of IL-6 among anthocyanin groups has no significant difference, the highest dose of anthocyanin tends to have higher IL-6 levels than other lower doses (Figure 1A). Previous work was showed a similar increment of IL-6 level following acute stress exposure in model mice [21]. Stress activates the sympathetic nervous system, and initiating IL-6 in visceral fat tissue invokes gluconeogenesis as a fuel for flight and fight physiology mechanisms [21]. The IL-6 cytokine is essential for immune regulation; however, overproduction of IL-6 leads to the generation of inflammation and diseases [22]. The benefits of anthocyanin against the inflammatory process were documented in a review study [23]. Cyanidin and peonidin, as major anthocyanin components, reduce the expression of IL-6, monocyte chemoattractant protein-1 (MCP-1) and cyclooxygenase-2 (COX-2) [24]. Sugata *et al*. reported the inhibitory effects of anthocyanin from purple-fleshed potatoes in lipopolysaccharide-induced inflammation of RAW-264.7 cells by reducing nuclear factor kappa B (NF-Kb), tumor necrosis factor alpha (TNF-α) and IL-6 following a dose-dependent manner [25]. Correlates with recent results, the supplementation of local purple sweet potatoes reduces the level of IL-6 as well as TNF-α in high-fat diet mice [26].

Nevertheless, IL-10 cytokine is essential in the anti-inflammation pathway [27]. We found tendencies of lower IL-10 in the stress group and anthocyanins-treated groups (Figure 1B). The opposite finding was proposed by Li *et al*. about improving the anti-inflammatory effect of anthocyanin in brain tissue of neuroinflammation model mice by increasing IL-10 and reducing IL-6 level [28]. Both IL-6 and IL-10 have potential roles in the inflammation process, a ratio of IL-6 to IL-10 represents the compensation phase of homeostatic restoration. A higher ratio of IL-6/IL-10 was correlated with multiple organ dysfunction [27]. The production of IL-6 in adipose tissue related to inflammation-induced obesity [29]. Anthocyanin from purple sweet potatoes was established to

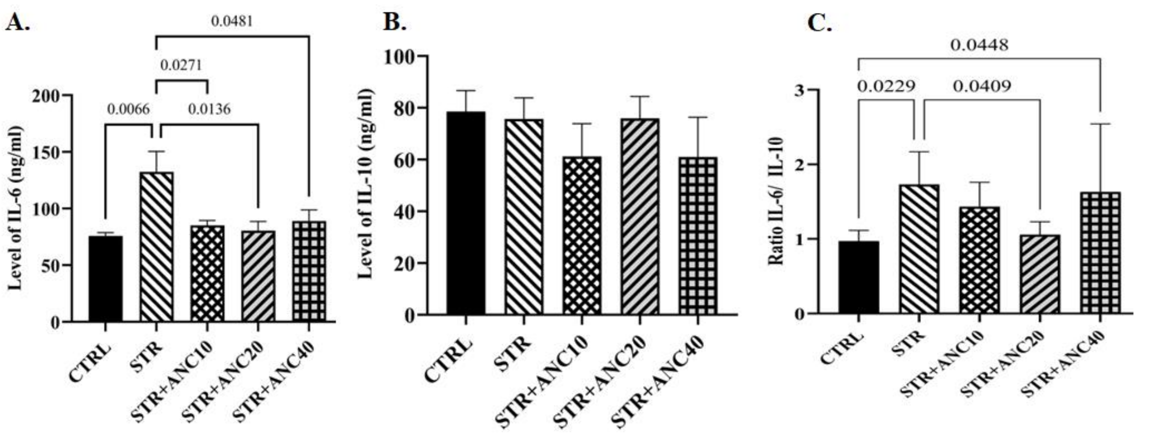

Figure 1. Level of IL-6 (A), IL-10 (B), and ratio of IL-6/IL-10 (C) from visceral fat tissue following anthocyanin administration.

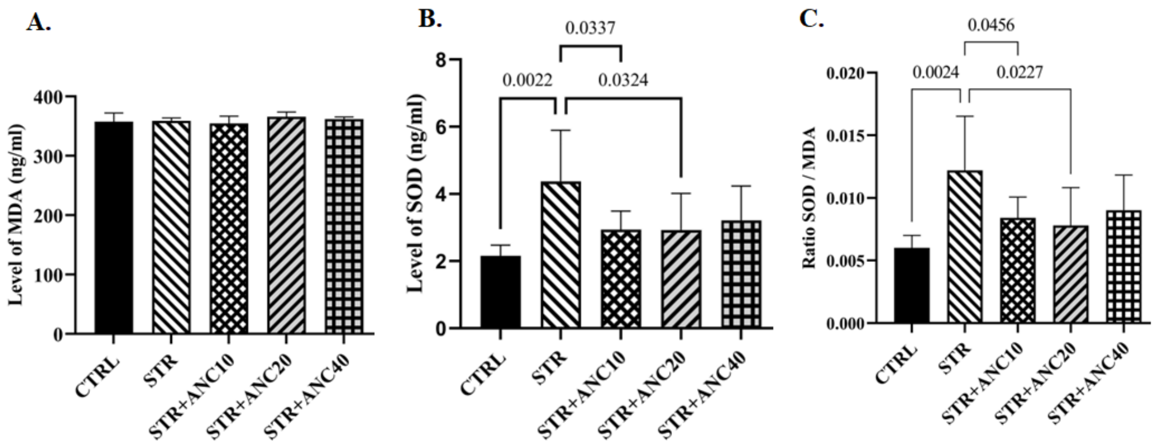

Figure 2. The level of hepatic MDA (A), SOD (B), and ratio of SOD/MDA (C) following anthocyanin administration.

resist inflammation in obesity [30]. Accordingly, anthocyanin from purple sweet potatoes is proposed to inhibit the restraint stress-induced inflammation in visceral fat tissue by reducing the IL-6 and IL-10 elevation, thus reducing the ratio of IL-6/IL-10 (Figure 1C).

Furthermore, restraint stress could induce inflammation as well as oxidative stress in hepatic tissue. The dramatic decrease of antioxidant levels in the liver accompanied by elevation of MDA level as a pro-oxidant molecule in hepatic tissue was reported in previous work [10]. Contrarily, this study shows the rise of SOD as an antioxidant enzyme in hepatic tissue (Figure 2B) with no differences in MDA levels (Figure 2A). The type and duration of stress application are assumed as the causation of these discrepancies. Dachanidze *et al*., revealed a combination of unpredictable stress in 20 days sharply increased the SOD enzyme level. However, a longer duration of stress then significantly reduces the SOD level [31]. The enhancement of SOD expression during a shorter duration of restraint stress is similarly proposed by Chen *et al*. that revealed extended stress sharply reduced the SOD level [7]. Thus, we propose the increment of SOD following stress exposure in this study is a mechanism of compensation to resist oxidative stress, resulting in a similar MDA level between control and stress-treated groups. Previous work demonstrated different MDA level results following stress exposure [32]. The responsivity of each organ towards stress determines the MDA alteration after stress exposure. The elevation of MDA during stress is more prominent in the brain as stress-sensitive tissue than liver and kidney as less stress-sensitive tissues [32]. Anthocyanins from purple sweet potatoes have more powerful antioxidant activity than other plants such as elderberry, purple corn, and red cabbage [33]. Previously, anthocyanin from local purple sweet potatoes induced the elevation of SOD in atherogenic rats [34]. Further relevant studies are necessary to evaluate both the inflammation and antioxidant effect of anthocyanin from purple sweet potatoes in broad stress-animals tissue.

Conclusion

We conclude that anthocyanin from PSP reduces inflammation by reducing IL-6 and IL-10 elevation in visceral fat tissue. Anthocyanin from PSP also reduces the SOD level without significantly affecting the MDA level in hepatic tissue.

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