Propolis Increases Brain Derived Neurotrophic Factor Expression in the Prefrontal Cortex of Rat Stress Model

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Abstract. Stress causes an increase in glucocorticoid hormones and glutamat release in the central nervous system. This condition can increase calcium influx in the neuron, mitochondrial membrane permeability and triggers apoptosis, thereby reducing the number of neurons. In the prefrontal cortex, stress exposure increase bax expression and decrease in the number of neurons. Stress causes decrease in the expression of brain derived neurotrophic factor (BDNF). To determine neuroprotective effect of propolis on BDNF expression in the prefrontal cortex of rat stress model. Experimental study with male Spraque-Dawley rats, aged 2–4 months and weighing 200–300 g. Rats were randomly divided into 4 groups, each group consisted of 5 rats (n = 5). Group K is the stress group, Groups P1, P2 and P3 are the stress group and propolis therapy with doses of 100, 150 and 200 and mg/kgbb, respectively. Stress treatment and administration of propolis for 14 days. On the 15th day, rats was decapitation and brain tissue taken. Brain tissue was made histological preparations with immunohistochemical staining using bdnf antibody. Observations using a light microscope with a magnification of 1000 times. Statistical data analysis using one way Anova. Group K showed the lowest bdnf expression. The highest bdnf expression was in the P2 group. There was a significant difference in groups K and P2 (p. 0.020). Propolis 150 mg/kgbw increase bdnf expression in the prefrontal cortex of rat stress model..

Keywords: Propolis · Bdnf · Prefrontal Cortex · Stress

1 Introduction

Stress causes an increase in glucocorticoid hormones and release glutamate in the central nervous system. This condition results in an increase calcium influx in cells, mitochondrial membrane permeability and triggers apoptosis, thereby reducing the number of cells [1, 2]. In the prefrontal cortex, stress exposure results in an increase bax expression and a decrease in the number of neurons [3, 4].

The prefrontal cortex plays an important role in working memory, self-regulatory and goal-directed behavior. The prefrontal cortex displays structural and functional plasticity over the life of an individual. Stress affects changes in the structure and function of

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the prefrontal cortex. Stress causes changes in the structure of dendrites and synapses in various areas of the brain, including the hippocampus, amygdala and prefrontal cortex. This affects cognitive and emotional function and self-regulatory behavior. The Prefrontal cortex is associated with various disorders in the brain such as attention deficit disorder, schizophrenia, depression, and post traumatic stress disorder (PTSD). The prefrontal cortex, amygdala and hippocampus are interconnected and influence each other via direct and indirect neural activity [5].

Stress affects the expression of brain derived neurotrophic factor (BDNF) in the hippocampus, prefrontal cortex and amygdala. Several studies show that stress has a different effect on BDNF expression, some show a decrease in BDNF expression. On the other hand, BDNF can improve cognitive function due to stress exposure [6]. BDNF is a neurotrophin that plays an important role in the proliferation, differentiation and growth of neurons. BDNF also plays an important role in maintaining neuronal survival, synapse formation and neuroplasticity. BDNF has antiapoptotic, antioxidant and suppressing autophagy effects [7]. Stress increases the glucocorticoid hormone that enters cells and regulates the expression of the BDNF gene. Chronic restraint stress increases the expression of BDNF mRNA and BDNF protein in the basolateral amygdala area, but decreases BDNF expression in the CA3 area of the hippocampus. This condition is accompanied by an increase or decrease in the density of dendritic branches [8].

Neuroprotective drug therapy is needed to inhibit the effects of stress on impaired cognitive function and changes in brain structure. One of the neuroprotective drugs from natural medicine is propolis. Administration of propolis at doses of 50, 100 and 200 mg/bw for 7 days can reduce apoptosis and increase BDNF expression in the brain of Rattus norvegicus traumatic model [9]. This study aims to examine the effect of propolis on BDNF expression in the prefrontal cortex of rat stress model.

2 Method

2.1 Research Subjects

This research is an experimental study using a posttest only control group design. This research has received approval from the Research Ethics Committee of the Faculty of Medicine, Islamic University of Indonesia with the number: 50/Ka.Kom.Et/70/KE/XI/2018. The subjects of this study were male Rattus norvegicus rats, 4 months old, weighing 200–300 g from the Spraque-Dawley strain. Rats were randomly divided into 5 groups, each group consisted of 5 rats ($n = 5$). The groups were divided as follows: Group K received stress treatment, groups P1, P2 and P3 received stress treatment and were given propolis doses of 100, 150 and 200 mg/kgbw/day. Stress treatment and propolis administration for 14 days.

2.2 Stress Treatment and Administration of Propolis

This study use a social isolation stress. Social isolation stress was carried out by placing one rat in a cage so that it could not interact with other rats (no body contact with other rats). This situation causes psychological stress because in general rats live in groups [10]. In groups P1, P2 and P3 were given propolis at a dose of 100, 150 and 200 mg/kgbw orally using a probe. Social isolation stress and propolis given for propolis for 14 days.

2.3 Termination, Transcardial Perfusion and Removal of Brain Tissue

On day 15, termination and transcardial perfusion were performed. Rats were anesthetized by intramuscular injection of ketamine (100 mg/kgbw). After being anesthetized, transcardial perfusion was performed using NaCl solution with a volume of 100–200 ml until the perfusion fluid that came out was clear. After that, the perfusion was continued with PBS buffered formalin with a volume of 200 ml. After perfusion, the brain was carefully dissected, fixed with formalin buffered PBs solution for 24 h.

2.4 Preparation of Histological Slide and Immunohistochemistry Staining

The part of the brain containing the prefrontal cortex was made of paraffin blocks, then cut with a thickness of 4 μm. One rat brain was made 1 piece as a sample. Next, immunohistomic staining was performed with anti-BDNF antibodies (FineTest: FNab10014).

2.5 Observation of Immunohistochemical Staining Results

The stained histological preparations were observed using a light microscope connected to an optilab camera with a magnification of 1000 X. Observations were made on the prefrontal cortex and interpretation of BDNF expression using the Alred score. The Alred score assesses the proportion (score 0–5) and intensity (score 0–3) in BDNF-expressing neurons. The proportion scores include (-) (0%), (+) (<1%), (+ +) (1–10%), (+ + +) (11–33%), (+ + + +) (34–66%), and + + + + + (67–100%). Intensity scores include values 0.1,2 and 3 (negative, weak, moderate and strong intensity) [11].

2.6 Data Analysis

To compare the mean Alred score of BDNF expression between group K, group P1, P2 and P3 statistical analysis was used One Way Anova, then followed by a post hoc test.

3 Result and Discussion

BDNF expression in the prefrontal cortex can be seen in Fig. 1. Alred score for BDNF expression in the prefrontal cortex are presented in Table 1.

Group K who received stress treatment had the lowest Alred score. Analysis using one way ANOVA showed significant difference (p. 0.000).

The results of the post hoc bonferroni analysis (Table 2) showed a significant difference between the stress group (K) and the stress group +150 mg/kgbw propolis (P1). There was no significant difference between group K and groups P1 and P3.

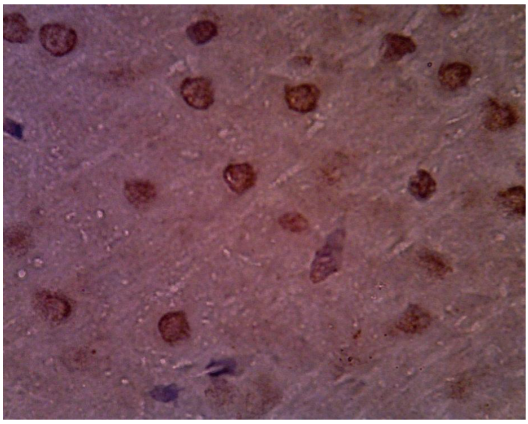

Fig. 1. BDNF Expression in the prefrontal cortex

Table 1. BDNF Expression In The Prefrontal Cortex

Groups	***Alred score***	***P value***
K	2.6	0.000
P1	3.2	
P2	6.4	
P3	6.0	

K: stress group, P1, P2, P3: Stress group + propolis doses 100, 150 and 200 mg.kgbw.

Table 2. Bonferonni Post Hoc Analysis

BDNF expression		***Mean difference***	***P value***
K	P1	0.6	1.000
	P2	3.8	0.020[a]
	P3	3.4	0.080
P1	P2	3.2	0.009
	P3	2.8	0.301
P2	P3	0.4	1.000

K: stress group, P1, P2, P3: Stress group + propolis doses 100, 150 and 200 mg.kgbw. [a] Significant diference.

4 Discussion

The results of this study showed that BDNF expression in the stress group was the lowest compared to the stress group given propolis. The results of this study are in accordance with Nakagawa et al. (2019) which stated that social isolation induced anxiety-like behavior, decreased BDNF protein in the prefrontal cortex and hippocampus. Social isolation decreases mRNA BDNF mRNA in the hippocampus, prefrontal cortex and amygdala. The levels of BDNF protein in blood and saliva in the stress group were lower than in the control group [12]. Niknazar et al. (2015) used a chronic mild repeated stress (CMRS) model for 21 days on male and female rats. The results showed that female rats had higher levels of corticosterone and anxiety than male rats. BDNF gene methylation was higher in females, BDNF protein decreased in male and female groups compared to the control group. BDNF protein in females are lower than males [13]. Qiu et al. (2014) research on rats treated with chronic unpredicted stress. The results showed that stress increased corticosterone levels in the blood and decreased BDNF mRNA in the hippocampus [14]. Zhao et al. (2020) used an inescapable foot shock (IFS) model to induce chronic and traumatic stress. The results showed that (IFS) as a model of stress induces post-traumatic stress disorder (PTSD) in rats, thereby inhibiting the dendritic branches and shortening of dendrites in the CA1 area of the hippocampus and prefrontal cortex. In addition, IFS decreased BDNF expression in the hippocampus and prefrontal cortex [15]. Makhathini et al. (2017) used a repetitive restrain stress (RRS) model 6 h per day for 28 days. The results showed that stress increased the hormone corticosterone. Stress decreased levels of BDNF mRNA BDNF and BDNF,protein, glucocorticoid receptors and mineralocorticoid receptors in the hippocampus [16].

The results of this study are different from research by Moravcova et al. (2020) which states that social defeat stress increases corticosterone levels in the blood. Stress increase BDNF expression in the prefrontal cortex, hippocampi, pineal glands, olfactory bulbs, cerebella, and pituitary glands [17]. Chiba et al. (2012) study on Chronic restrain stress rats, 6 h per day for 28 days. Stress causes weight loss, decreased food intake, increased anxiety and corticosteroid levels. BDNF expression in CPF was not significantly different from the control group [18]. Research Kaptan et al. (2019) predictable chronic stress increases BDNF expression in the hippocampus. Stress causes MDA levels to increase in the hippocampus, nNOS increases in the CA1, CA3 hippocampus and dentate gyrus areas. Protein carbonyl was decreased in the right hippocampus. SOD levels were not significantly different in the stress and non-stress groups [19].

The results of this study showed propolis at a dose of 150 mg/kgbw/day significantly increased BDNF expression in rats social isolation stress model. The results of this study are in accordance with research by Kasai et al. (2011) in wistar rats treated with spinal cord injury and given propolis therapy at doses of 0.2 mg/kg, 1 and 5 mg/kgbw intraperitoneally immediately after injury. Propolis administration was continued every 24 h for 3 weeks. The results showed an improvement in locomotor function in the group that received propolis doses of 1mg/kgbw and 5 mg/kgbw. Propolis reduces inducible nitric oxide (NO) synthase (iNOS) mRNA, thereby reducing nitric oxide production. This shows that propolis has an antioxidant effect. Propolis increased the expression of BDNF and neurotrophin-3 mRNAs. Propolis had no significant effect on Glial-derived neurotrophic factor (gdnf) mRNA. Propolis accelerates wound healing at the site of

spinal cord injury. This indicates that propolis has an anti-inflammatory effect [20]. Rahman et al. (2020) study on rat model of focal cerebral ischemia (ischemic reperfusion) given propolis at doses 50 and 100 mg/kgbw. The results showed improvement in neurological deficits, locomotor function and motor coordination. Propolis decreases malondialdehyde levels and increases levels of the antioxidants glutathione, superoxide dismutase, glutathione peroxidase, catalase, BDNF and dopamine levels in the brain homogenates of ischemic reperfusion rats. Propolis reduces the infarct area in the brain and increases the number of healthy cells in the cortex and hippocampus [21]. In vitro study on cytotoxicity-induced SHSY5Y neuroblastoma cell culture using H2O2. Administration of propolis increased cell viability, inhibited the production of reactive oxygen species (ROS) and increased BDNF mRNA expression [22].

Propolis contains Caffeic acid phenethyl ester (CAPE) and Chrysin. In cultured dopaminergic neurons induced by interferone γ or lipopolisaccharide, CAPE inhibited NO production, inhibited NF-kB and inhibited iNOS expression. CAPE has a neuroprotective effect on dopaminergic neuron culture. CAPE increases BDNF expression in cultured dopaminergic neurons. In mice induced by neurodegeneration using LPS 3 g intranigral injection, CAPE doses of 10 and 30 mg/kgbw 30 min before LPS induction continued every day for 3 days, can increase the expression of BDNF proteins [23]. Chrysin is a flavonoid found in bee propolis, honey and various plants. Research on mice model Parkinson's using 6-hydroxydopamine (6-OHDA) and given chrysin therapy at a dose of 10 mg/kg body weight/day for 28 days. Chrysin inhibits inflammation and has anti-oxidant effects. Chrysin increases neurotrophic factors such as BDNF, glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) [24]. Fabbro et al. (2019) reported study on mice Parkinson model. The administration of chrysin at a dose of 10 mg/kgbw/day for 28 days gave the effect of improving cognitive function, behavior and neurochemical parameters. Chrysin inhibits the increase in TNF-α and IL-1β in the striatum [25]. A review by angelopoulou et al. (2020) stated that chrysin has antioxidant effects on dopaminergic neurons. Chrysin inhibited caspase, Bax and increases the anti-apoptotic protein Bcl 2. Chrysin also increases the production of neurotrophic factors that play a role in neuronal survival, including BDNF and nerve growth factor (NGF) [26].

The results of this study different with Kudo et al. (2015) study in vitro using dental pulp cells culture. The results showed that the administration of propolis on the culture medium increased (NGF) mRNA expression. Propolis had no effect on BDNF mRNA expression. Propolis inhibits ROS production and increases cell culture viability. In addition, propolis increases neurite growth [27].

5 Conclusion

Propolis at dose 150 mg/kgbw increase bdnf expression in the prefrontal cortex of rat stress model.

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