

## Propolis increased BDNF expression in the hippocampus of stress-induced rats

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### ABSTRACT

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**Background:** Brain-derived neurotrophic factor (BDNF) is a neurotrophin secreted by dendrite, which plays a role in differentiation, maturation, neuroplasticity, learning, and memory, which the expression decreases under stress conditions. Propolis contains chrysin which has antioxidant and neuroprotectant effects.

**Objective:** This study aimed to examine the effect of propolis on BDNF expression in the hippocampus of stress-induced rats.

**Methods:** Experimental study using posttest only control group design. The subjects were 25 male Sprague-Dawley rats (*Rattus norvegicus*), four months old, weighing 200-300 grams. Rats were randomly divided into five groups: group N, did not receive any treatment; group K, received stress treatment; groups P1, P2, and P3, received stress treatment and followed by administered propolis at doses of 100, 150, and 200 mg/kg/day. Social isolation stress was carried out by putting one rat in one cage. Oral propolis administration used oral gavage. In the end, the rats were terminated and brain tissue was collected. Immunohistochemical staining using anti-BDNF antibodies was performed to make histological slides. Observations were made with a light microscope with 1000x magnification in the CA1 area of the hippocampus.

**Results:** There is a significant difference in BDNF expression in the CA1 area of the hippocampus in all groups ( $p=0.000$ ). The highest BDNF expression was in the P3 group and the lowest in group K.

**Conclusion:** There is an effect of propolis on BDNF expression in the hippocampus of the stress-induced rat. Propolis dose of 100 mg/kgBW/day has increased BDNF expression.

**Latar Belakang:** Brain-derived neurotrophic factor (BDNF) merupakan neurotrophin yang disekresi oleh dendrit, berperan pada diferensiasi, maturasi neuroplastisitas, learning and memory dan ekspresinya menurun pada kondisi stres. Propolis mengandung chrysin yang memiliki efek antioksidan dan neuroprotektan.

**Tujuan:** Penelitian ini bertujuan untuk mengetahui pengaruh pemberian propolis terhadap ekspresi BDNF di hippocampus pada tikus yang diinduksi stres.

**Metode:** Penelitian eksperimental dengan menggunakan rancangan posttest only control group design. Subjek penelitian ini 25 ekor tikus *Rattus norvegicus* jantan, berumur 4 bulan dengan berat badan 200-300 gram dari galur Sprague-Dawley. Tikus dibagi secara random menjadi 5 kelompok, Kelompok N tidak mendapat perlakuan. Kelompok K mendapat perlakuan stres, kelompok P1, P2 dan P3 mendapat perlakuan stres dan pemberian propolis dosis 100, 150 dan 200 mg/kgbb/hari. Perlakuan stres isolasi sosial dan pemberian propolis secara oral menggunakan sonde selama 14 hari, dilanjutkan terminasi dan pengambilan jaringan otak, pembuatan sediaan histologis dengan pewarnaan imunohistokimia menggunakan antibodi

anti BDNF. Pengamatan menggunakan mikroskop cahaya dengan perbesaran 1000 kali pada area CA1 hippocampus.

**Hasil:** Terdapat perbedaan signifikan ekspresi BDNF di area CA1 hippocampus pada semua kelompok ( $p=000$ ). Ekspresi BDNF tertinggi pada kelompok P3. Ekspresi terendah pada kelompok K.

**Kesimpulan:** Terdapat pengaruh pemberian propolis terhadap ekspresi BDNF pada hippocampus tikus yang diinduksi stres. Dosis propolis 100 mg/kgbb/hari sudah mampu meningkatkan ekspresi BDNF.

## INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a neurotrophin secreted by dendrites, which founded in various brain areas, including the olfactory bulb, cortex, hippocampus, hypothalamus, mesencephalon, brain stem, and spinal cord. Brain-derived neurotrophic factor plays a role in the differentiation, maturation, survival of neurons and has a neuroprotective effect in conditions of increased glutamate, cerebral ischemia, hypoglycemia, and exposure to neurotoxic substances.<sup>1</sup> In addition, BDNF plays a role in axon growth and the development of dopaminergic, GABAergic, cholinergic, and serotonergic neurons. Brain-derived neurotrophic factor also plays an important role in neuroplasticity, learning, and memory. Brain-derived neurotrophic factor expression decreases in conditions of stress and depression.<sup>2</sup>

The stress model social isolation for 14 days in rats caused a decrease in the number of neurons in the cornu ammonis (CA) area hippocampus and prefrontal cortex.<sup>3</sup> Neuron atrophy is caused by glutamate excitability, neuronal apoptosis, and decreased BDNF expression. Acute restraint stress increases BDNF mRNA expression, especially in the CA3 area of the hippocampus glucocorticoid receptor-impaired mice (GR-1 mice).<sup>4</sup> Social isolation stress exposure in mice and rats reduces BDNF expression in the midbrain, prefrontal cortex, and hippocampus. Acute restraint stress has different effects on BDNF expression, some increase and some decrease BDNF in various brain areas such as the prefrontal cortex, amygdala, and hippocampus.

Unpredictable stress (repeated foot shock) increases or decreases BDNF expression in the hippocampus.<sup>5</sup>

Neuroprotective drugs are needed to inhibit the decrease in BDNF expression due to stress exposure, one of which is propolis, a flavonoid from honey. The content of flavonoids in propolis is useful as an antioxidant, anti-inflammatory, antimicrobial and anticancer. Several compounds in propolis have been identified, including phenolic acids, flavonoids, esters, diterpenes, sesquiterpenes, lignans, aromatic aldehydes, alcohols, amino acids, fatty acids, vitamins, and minerals. The phenolic acid content of propolis mainly consists of chrysin, galangin, pinostrobin, pinobanksin, and pinocembrin. Propolis and its derivatives prevent oxidative stress in brain tissue by inhibiting the formation of lipid peroxidation and increasing the activity of antioxidant enzymes and inhibiting the formation of free radicals. Pinocembrin and chrysin contained in propolis have antioxidant effects and inhibit neuronal apoptosis. In addition to its antioxidant effect, propolis has an anti-inflammatory effect through the mechanism of cyclooxygenase (COX) inhibition and prostaglandin biosynthesis inhibition, free radical scavenging, inhibition of nitric oxide synthesis, decreased concentrations of pro-inflammatory cytokines, and immunosuppressive activity.<sup>6</sup>

Previous studies showed the neuroprotective effect of propolis. Propolis doses of 100 and 200 mg/kgBW/day administered to rats induced by social isolation stress for 14 days inhibit decreasing the number of neurons in the CA1 area of the hippocampus and prefrontal cortex. Moreover, propolis administration at doses of 100 and 200 mg/kgBW/day inhibits Bax expression and decreases the number of neurons in the CA1 area of the hippocampus rat induced by sodium nitrite for 60 days.<sup>7</sup> Administration of propolis inhibits oxidative stress and prevents the death of dopaminergic neurons in the substantia nigra in parkinsonian rats.<sup>8</sup> This study aimed to examine the neuroprotective effect of propolis on BDNF expression in the hippocampus of

rats induced stress. Differ from previous study that demonstrated the neuroprotective effect of propolis on the neuronal count and Bax expression in the hippocampus.

## METHODS

This experimental study was used a post-test-only control group design and ethically approved by the Study Ethics Committee of the Faculty of Medicine, Universitas Islam Indonesia, with the number: 30/Ka.Kom.Et/70/KE/I/2020.

### Study Subject

The subjects of this study were male Sprague-Dawley rats (*Rattus norvegicus*), four months old, weighing 200-300 grams. They were randomly divided into five groups, with five rats (n=5) each, as follows: group N, did not receive treatment; group K, received stress treatment; groups P1, P2, and P3 received stress treatment followed by administered propolis doses of 100, 150, and 200 mg/kgBW/day.

### Time and Place of Study

This study was carried out for six months, from December 2019 to May 2020, at the Study Laboratory of the Faculty of Medicine, Universitas Islam Indonesia.

### Stress Treatment and Administration of Propolis

The social isolation stress model was carried out by putting one rat in one cage to prevent interaction (no body contact with other rats). This situation causes psychological stress because rats live in groups naturally.<sup>9</sup> In groups P1, P2, and P3 were given propolis at doses of 100, 150, and 200 mg/kgBW/day orally using a oral gavage. Groups K and N were given aquadest. Social isolation stress and propolis administration were carried out for 14 days. On day 15, termination, perfusion, and brain tissue extraction were performed. The histological slide was made with immunohistochemical staining using anti-BDNF antibodies.

### 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/day) followed by transcardial perfusion using NaCl solution with 100-200 ml volume until the perfusion fluid was clear. After that, the perfusion was continued with 200 ml of PBS buffered formalin. Then, the brain was carefully dissected, fixed with formalin buffered PBS solution for 24 hours.

### Preparation of histological slide and immunohistochemistry staining

The brain part containing the hippocampus was made of paraffin blocks. They were cut with a thickness of 4 micrometers. Immunohistochemistry staining was performed with anti-BDNF antibodies (FineTest: FNab10014).

### Observation of immunohistochemical staining results

The stained histological preparations were observed using a light microscope connected to an optilab camera with a magnification of 1000x. Observations were made on the CA1 area of the hippocampus 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%). The proportion calculation is the number of positive cells divided by the total number of cells. Intensity scores include 0, 1, 2, and 3 (negative, weak, moderate, and strong intensity). The total score is the sum of the proportion and intensity scores.<sup>10</sup>

### Data Analysis

To compare the mean Alred score of BDNF expression between groups, statistical analysis used One-Way ANOVA followed by a post hoc test.

**RESULTS**

BDNF expression in the CA1 area of the hippocampus using immunohistochemistry staining shown in Figure 1 and using the Alred

score presented in Table 1. Group K that received stress treatment had the lowest mean Alred score. Analysis using One-Way ANOVA showed significant results with a p value of 0.000.

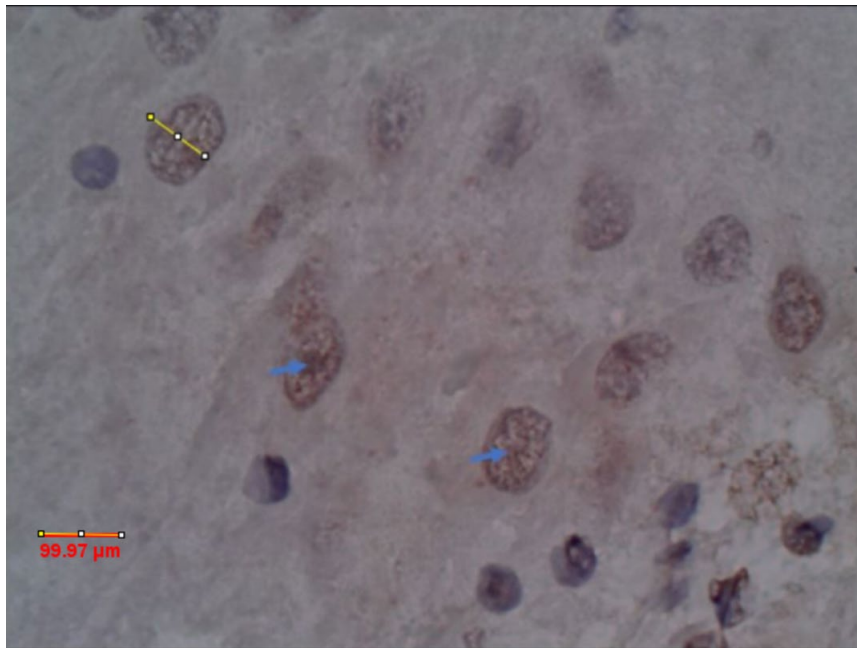


Figure 1. BDNF expression in the CA1 area hippocampus. Immunohistochemistry staining, viewed with a light microscope with a magnification of 1000x. Blue arrows are positive BDNF expression cells, brown cells appear.

Table 1. BDNF expression in the CA1 area of the hippocampus

Group	Mean	p value
K	2,00	0.000
N	4,33	
P1	6,33	
P2	6,33	
P3	6,50	

Group K: stress group, N: non-stress, P1, P2, P3: stress + propolis 100, 150 and 200 mg/kgBW/day.

The results of the post hoc LSD analysis (Table 2) showed significant differences between the stress group (K), the non-stress group (N), and stress + propolis 100, 150, and 200 mg/kgBW/day (P1, P2, P3). In addition, there was

a significant difference between group N and the P1, P2, and P3 groups but no significant difference between groups P1, P2, and P3.

Table 2. LSD post hoc analysis

BDNF expression	Mean different	p-value
N	2.33	0.002
K	P1	4.33
	P2	4.33
	P3	4.50
N	P1	2.0
	P2	2.0
	P3	2.16
P1	P2	0.00
	P3	0.17
P2	P3	0.17

Group K: stress group, N: non-stress, P1, P2, P3: stress + propolis 100, 150 and 200 mg/kgBW/day.

## DISCUSSION

In the current study, rats exposed to social isolation stress for 14 days showed the lowest BDNF expression. This result is in accordance with a previous experimental study, expression of BDNF in the brain was reduced social stress. In addition, social stress causes behavioral changes that lead to anxiety disorders and depression.<sup>11</sup> Social isolation stress in rats resulted in learning and memory disorders, anxiety disorders, and depression. This condition is associated with neuropathological changes, including cell apoptosis, decreased synaptic protein, and myelination failure in the hippocampus and prefrontal cortex.<sup>12</sup> Stress exposure to rats decreases memory function, increases corticosteroid and epinephrine hormones, and decreases the neurotransmitter acetylcholine.<sup>13</sup> The result of this study is different from the previous study, where acute restraint stress increased BDNF mRNA expression, especially in the CA3 area of the rat hippocampus.<sup>5</sup>

Hippocampus is an area in the brain that plays a role in memory formation, emotion regulation, fear, anxiety, and stress response. The hippocampus is often used as an example of a neuroplasticity study model. Neuroplasticity is the ability to adapt and regulate structure or function in response to internal or external

stimuli. Neuroplasticity occurs at the cellular, tissue, and behavioral levels. High hippocampal neuroplasticity is accompanied by hippocampus susceptibility to conditions of ischemia, epilepsy, neuroinflammation, chronic stress, and aging. The CA1 area of the hippocampus is more susceptible to damage because the CA1 area of the hippocampus has more N-methyl-D-aspartate (NMDA) receptors, resulting in greater glutamate- and calcium-mediated excitotoxicity.<sup>14</sup> Administration of NMDA in Japanese quail resulted in neurotoxicity in the hippocampus and decreased spatial memory function.<sup>15</sup> In this study, the administration of propolis at doses of 100, 150, and 200 mg/kgBW/day increased the expression of BDNF in the hippocampus. In accordance with a previous study reported in traumatic model rats, administration of propolis increased BDNF expression and inhibited apoptosis. The doses of propolis used were 50, 100, and 200 mg/kgBW/day, but the dose of 50 mg/kgBW/day was less effective in increasing BDNF expression. The most effective dose of propolis is 200 mg/kgBW/day in increasing BDNF expression and inhibiting apoptosis.<sup>16</sup> Water extract of propolis with the main polyphenols content increases BDNF expression, increasing the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and superoxide dismutase (SOD),

and reducing levels of malondialdehyde (MDA) which cause oxidative stress in neuronal cells.<sup>8</sup> Propolis contains the flavonoid chrysin, which has antioxidant and neuroprotective effects. In aging rats, administration of chrysin at a dose of 10 mg/kgBW improves memory function, inhibits free radicals, and increases BDNF expression in the hippocampus and prefrontal cortex.<sup>17</sup> Furthermore, 500 mg/kgBW of propolis showed increased cell death characterized by increased expression of proapoptotic Bax and reduced anti-apoptotic Bcl-2 expression.<sup>18</sup>

This study adds to the scientific evidence neuroprotective effects of propolis. Previous studies have shown that administering propolis doses of 50, 100, and 200 mg/kgBW/day in rats with brain injury models reduces inducible nitric oxide synthase (iNOS) expression and inhibits the free radicals formation, which is characterized by a decrease in MDA. iNOS is an important inflammatory mediator produced by immune cells in the brain.<sup>19</sup> Administration of Propolis in brain injury model mice inhibited neuronal apoptosis as seen by immunohistochemistry of the tunnel assay. In addition, propolis at doses of 50, 100, and 200 mg/kgBW/day increased the expression of Bcl-2 in neurons and neuroglia.<sup>16</sup> Propolis has a better anxiolytic effect than classical anxiolytics. This result is shown in the behavioral changes of experimental animals observed using the neurobehaviour test. In addition, propolis has an antidepressant effect.<sup>20</sup> The benefits of propolis in humans have been studied in patients with type 2 diabetes mellitus who were given Brazilian green propolis and Chinese propolis showing increased levels of the antioxidant enzymes glutathione and lactate dehydrogenase.<sup>21,22</sup> Although the results of the current study show the benefits of propolis, especially in increasing BDNF expression in rats exposed to stress for 14 days, there are still shortcomings. Weaknesses of this study include observing the expression of BDNF in one slice only. In addition, this study was not investigated aspects of functional decline due to exposure to stress and functional improvement in the group given propolis. Further studies on neurobehavior tests are needed to investigate

functional aspects and increase the number of slices through stereological methods.

## CONCLUSION

Based on the study results, it can be concluded that propolis at the dose of 100 mg/kgBW/day was able to increase the expression of BDNF in the hippocampus of rats induced by stress.

## CONFLICT OF INTEREST

There is no conflict of interest.

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