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Neuronal cell death in the amygdala and cerebral cortex of mice (*Mus musculus*) induced by bee (*Apis mellifera*) venom

Rian Oktiansyah[1*], Ummi Hiras Habisukan[1], Anggun Wicaksono[1], Yustina Hapida[1], Fahmy Armanda[2]
[1]Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Raden Fatah
Jl. Pangeran Ratu, 5 Ulu, Seberang Ulu I, Palembang, South Sumatera, Indonesia. 30267
[2]Study Program of Biology Education, Faculty of Teacher Training and Education, Universitas Samudra
Jl. Prof. Syarief Thayeb, Meurandeh, Langsa, Aceh, Indonesia. 24415
*Email: rianoktiansyah@radenfatah.ac.id

ABSTRACT. Brain is an organ to control our activities, such as human consciousness, emotional, and movement. It was controlled by amygdala and cerebral cortex as the parts of the brain. Many things that can bring over it. Bee venom (BV) is known as traditional medicine and probably can sway the brain. The objective of this study was to determine the dose of BV that causes excessive neuronal cells death, especially in the amygdala and cerebral cortex. About 15 white male mice Deutsch Denken Yoken (DDY) strain were divided into control group and the treatment group. BV was administrated intraperitoneally for two weeks with multilevel doses, that was 1.88 mg/kg, 3.76 mg/kg, 5.6 mg/kg, and 7.48 mg/kg. Brain tissue isolation was performed three days of the last administration by using perfusion method. Morphological sectioned of brain tissue (amygdala and brain cortex) was stained by hematoxylin-eosin (HE). The results indicated that the BV inclined to affect neuronal cells death in the amygdala and cerebral cortex. Based on the study, the highest doses (7.48 mg/kg) of BV caused the highest neuronal cell death.

Keywords: Bee venom; cortical cerebral; *Mus musculus*; neuronal cells death; perfusion method

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INTRODUCTION

The brain is a vital organ that is responsible for many things. It plays a role for everything we do (Hayden & Kidd, 2015). It controls our movement (Armstrong *et al*., 2018; Cebolla & Cheron, 2019; Teka *et al*., 2017) and regulates unexpected activities, such as breathing and heart rate (Bordoni *et al*., 2018; Breit *et al*., 2018; Fink *et al*., 2018; Satsangi & Brugnoli, 2018). It also has functions as the center of human consciousness (Kotchoubey, 2018), stores the memories (Camina & Güell, 2017), allows us to feel emotions, and gives us personality (Gu *et al*., 2019; Montag & Panksepp, 2017; Riess, 2017; Scarpelli *et al*., 2019; Shackman & Wager, 2019; Tyng *et al*., 2017). The cerebral cortex and amygdala of the brain, which are responsible for our ability to feel emotions and regulate movement.

The amygdala and brain cortex function in emotional and motor responses so that they can integrate various stimuli and responses to it (Diano *et al*., 2017; Palomero-Gallagher & Amunts, 2021; Šimić *et al*., 2021). This part is a component in the limbic system. The cells in this section are very supportive for the system (Cai, 2018; Woalder, 2017). Therefore, the resistance of cells in this region is very necessary to keep the system running, by using bee venom (BV).

In Indonesia, *Apis mellifera* produces honey from domesticated bees and woodlands. In 2020, Java Island produced the most honey in the country. Its production reaches 81.06% of the total nationally (Badan Pusat Statistik, 2021). In addition to producing honey, *A. mellifera* is also known to produce BV, which is one of the most known and often used natural medicines for treatment (apitherapy). It has a very diverse chemical content, such as peptides (melittin, apamin, adolapin, MCD peptide), enzymes (phospholipase A2 (PLA2), hyaluronidase, acid phosphomonoesterase, lysophospholipase, and amines (histamine, dopamine, norepinephrine) (Kim *et al*., 2019; Korošec *et al*., 2019; Pucca *et al*., 2019; Carpena *et al*., 2020; Darwish *et al*., 2021). They act as an antioxidant and anti-inflammatory, especially neuroinflammation (Azam *et al*., 2019; Barnes *et al*., 2021; Kader

et al., 2019; Szabat *et al*., 2019; Wehbe *et al*., 2019; Zarrinnahad *et al*., 2018). Many substances or secondary metabolites from plants or endophytic fungi have antioxidant activity and it is suspected that they can prevent nerve cell death (Elfita *et al*., 2022, Oktiansyah *et al.*, 2023). The objective of this study was to determine the dose of BV that causes excessive neuronal cells death, especially in the amygdala and cerebral cortex. Based on the content and its characteristic as neuroinflammatory, BV is suggested to enhance the resistance of neurons. Hence, the study about neural resistance induced by BV is necessary.

MATERIALS AND METHODS

This study was approved by the ethics committee of IPB University, Indonesia (Number: 6-2016 RSHP FKH-IPB). About 15, three months old male mice strain Deutche Denken Yoken used for this study were divided into control (aquadest) and treatments group (doses of BV). Mice were acclimated for a week before treatments. Bee (*Apis mellifera*) venom was obtained from an apitherapy center in West Java, Indonesia. BV was administrated intraperitoneally for two weeks with doses 1.88 mg/kg (BV1), 3.76 mg/kg (BV2), 5.6 mg/kg (BV3), and 7.48 mg/kg (BV4) (Bogdanov, 2012). Brain was isolated after the treatments.

Brain tissue isolation was performed three days of the last administration by using perfusion method (Di Giovanna *et al*., 2018; Omatsu-Kanbe *et al*., 2018). Morphological sectioned of brain tissue was stained by hematoxylin-eosin (HE). Parameters observed were the number of dead neuron in the amygdala and brain cortex (outer and deep layer) (Chi *et al*., 2018). The number of neuronal cell death was counted using a counter.

Data analysis. Data were analyzed by using one-way ANOVA in SPSS ver. 26 for the assumption test data at a p-value of 0.05.

RESULTS AND DISCUSSION

Photomicrograph revealed that there are normal neurons and dead neurons in the amygdala and cerebral cortex (Fig. 1). The characteristics of normal neurons are larger size, purple nuclei, and bright cell cytoplasm, while dead neurons are irregularly shaped, shrinkage as well as darkened cytoplasm (Llorens-Martín *et al*., 2016; Oktiansyah *et al*., 2018). Analysis of neuronal cells death density in the amygdala exhibited no significant differences between treatment groups (one-way ANOVA; $p > 0.05$). Nevertheless, it was significantly differences in cerebral cortex (one-way ANOVA; $p < 0.05$). Statistically, neuronal cells death in amygdala has no effect, yet based on the data (Fig. 2), amount of neuronal cells death was different. These results indicated that the administration of BV tend to affect neuronal cells death in the amygdala and cerebral cortex. Cell death is important to do as a mechanism to maintain continuity of the neurogenesis process and maintain cell mass in the tissue (Andreotti *et al*., 2020; László *et al*., 2020; Lin *et al*., 2020). It occurs continuously. However, excessive or too low cell death can cause the diseases (Priante *et al*., 2019; Xu *et al*., 2019; Singh *et al*., 2020).

Injection of antigen can inhibit or induce neuronal cell death in the brain (Javidi & Magnus, 2019; Yanuck, 2019; Carpanini *et al*., 2020; Dinet *et al*., 2021). In this study, inhibition of the process of neuronal cell death in the amygdala and cerebral cortex at low doses (BV1) interpreted that BV had neuroprotective effect. However, the highest dose (BV4) elucidated that BV has the potential to expedite cell death in the brain, especially in the amygdala and cerebral cortex. Neuroprotective effects are important in cases of neurodegenerative diseases such as stroke, Alzheimer, and Parkinson so the surviving neurons functionally able to regulate metabolism such as memory, emotional control, and motion activity (Rehman *et al*., 2018; Castillo *et al*., 2019; Gómez-Gómez & Zapico, 2019; Guzman-Martinez *et al*., 2019; Barua *et al*., 2021; Bulck *et al*., 2021).

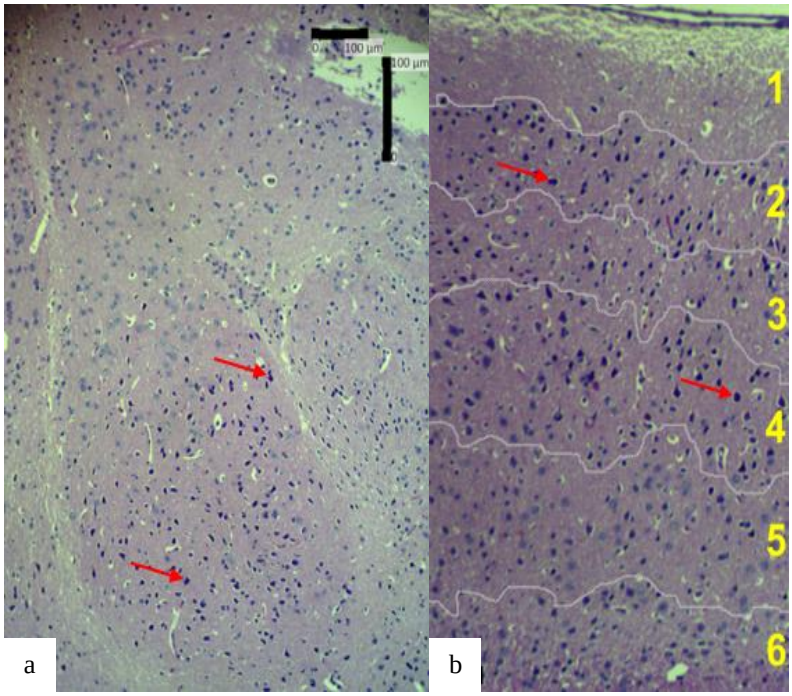

Fig. 1. Representative image of neuronal cells death (red arrow): a. amygdala; b. cerebral cortex (1-4): outer layer, 5-6: (deep layer) in 100× magnification

Retardation of neuronal cells death in the amygdala actually renders a negative leverage. It has an important role in maintaining the limbic system. This inhibition can inflict someone will always remember the entire memory, especially memory of fear that can affect emotions. In addition, the enhancement of neuronal cells death in cerebral cortex can involve lapse of motor function. Each layer in the cortical has kind of cells with different function. Outer layers, II, III, and IV, receive sensory information (as the first perception of external stimuli) and coordinate each other to translate the contextual information. Layer V and VI, as deep layer, receive signals from outer layer to broadcast the contents to other location within the brain (Li *et al*., 2019; Scala *et al*., 2019). Cell death in the tissues or organs can upset the body functionally. The excessive of neuronal cells death in brain can cause the neurodegenerative diseases, such as Parkinson, Alzheimer, Huntington (Alonso *et al*., 2019; Cankaya *et al*., 2019; Franco *et al*., 2019; Guo *et al*., 2019; Ikram *et al*., 2020; Jones *et al*., 2019; Vasic *et al*., 2019; Cai & Jeong, 2020). The result suggested that BV could cause the neuronal cells death in amygdala and brain cortex which contributed to the poor performance in controlling emotion and movement. However, BV is recommended to therapeutic of neurodegenerative diseases in moderation dose.

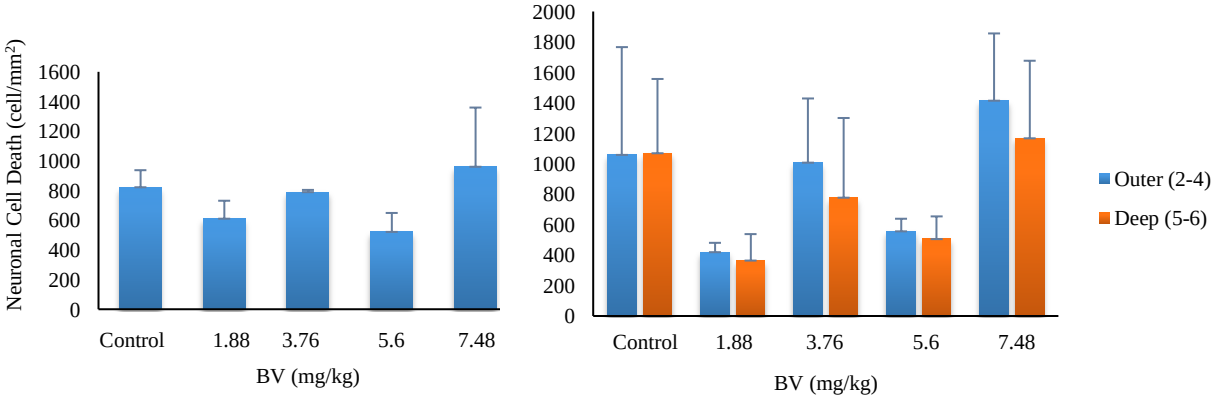

Fig. 2. Average of the number of neuronal cells death: a. amygdala; b. cerebral cortex

Analysis of one-way ANOVA disclosed that bee venom had not significant effect to neuronal cells death in amygdala. Nonetheless, the number of neuronal cells death in the amygdala and cortical fluctuated in each treatment after injection (Fig. 2). BV1 evinced the reduction of neuronal cells death compared to control. This alteration was caused by the inhibition of cells death due to neuroprotective effect of bee venom. Several previous studies had shown the role of apamin and phospholipase A2 preserved the neuron (Kim *et al*., 2019; Wehbe *et al*., 2019). Prior studies have also shown that apamin and phospholipase A2 are the compounds which produced by *A. mellifera* (Kim *et al*., 2019; Pucca *et al*., 2019). The oppressiveness of neuronal cell death ssignified the replenishment in the doses of BV2, BV3, and BV4 after injection. It is presumedly caused by the neuronal homeostasis process after the production of many new young neurons. in other words, neurogenesis occurs in amygdala and cerebral cortex due to injection of BV2, BV3, and BV4. The process of homeostasis to neurogenesis is carried out by apoptosis of pre-existing neurons in dentate gyrus, it does not rule out this also occurs in the amygdala and brain cortex.

CONCLUSION

Bee venom caused neuronal cell death in amygdala and cortical cerebral. BV4 was the dose which caused the highest neuronal cell death. BV1 was a dose that can increase neuronal cell survival because it could reduce nerve cell death in the amygdala and cortex. This research can be used as a reference for therapy using bees as the subject.

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