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Total Anthocyanin Extract from Purple Sweet Potato Improves Neurotransmitter and Locomotor Behavior by Altering Brain Corticosterone in Chronic-Stressed Mice

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Abstract

Background: Prolonged stress plays an essential role in depression disorder through brain inflammation and neurotransmitter imbalances. The natural plant antioxidant is promising to resist the negative impact of stress.

Objectives: This study was purposed to analyze the effect of total anthocyanin extracts (ANC) from purple sweet potatoes (PSP) on brain neurotransmitters, inflammation, and locomotor behavior in the chronic-stressed mice model.

Methods: Twenty males of adult BALB/c mice were assigned to control; stress (STR); STR + ANC 10 mg/kgBW; STR + ANC 20 mg/kgBW and STR + ANC 40 mg/kgBW. A restraint stress was applied 2 hours/day for 14 days. The Enzyme-linked immunosorbent assay (ELISA) was performed to measure brain dopamine, gamma-aminobutyric acid (GABA), and corticosterone levels. The Locomotor behavior was analyzed using an open field test at pre- and post-ANC treatment. In silico molecular docking was carried out between ANC and monoamine oxidase-B (MAO-B) enzyme.

Results: Administration of ANC decreased brain corticosterone levels. The neurotransmitter of dopamine decreased in the stress-induced group and then increased following ANC treatment. Increased GABA levels were observed in stressed and treated groups. Locomotor analysis showed tendencies of reduction of total distance movement and velocity after ANC treatment. Molecular prediction showed ANC has the potential to inhibit the MAO-B enzymes.

Conclusion: The ANC from PSP relieved brain inflammation and modified the neurotransmitter of dopamine and GABA thereby affecting the locomotor of chronic stressed-

induced mice. Further, in vivo studies are necessary to evaluate the molecular mechanism of ANC from PSP in chronic stress exposure, particularly on MAO enzyme regulation.

Keywords: Anthocyanin, brain, dopamine, GABA, stress

Introduction

Stress is strongly associated with mental health deterioration including depression (Juliana et al., 2022). Stress activates paraventricular nucleus (PVN) neuron in hypothalamus to secrete a corticotrophin releasing hormone (CRH), then initiates the axis of the hypothalamic-pituitary-adrenal cortex thereby extra corticosterone released into circulation. In parallel, stress triggers the sympathetic nervous system to produce noradrenalin as well as provokes the adrenal medulla to release adrenaline neurotransmitter (Chaves *et al*., 2021). Both hormonal and neural mechanism are objected to maintaining energy requirements against homeostatic disruption during stress (John E Hall, 2016).

Unfortunately, chronic stress blunted physiological responses to stress (Bloomfield *et al*., 2019) and dysregulate brain neurotransmitter of dopamine and gamma-aminobutyric acid (GABA) (Baik, 2020; Lowes *et al*., 2021). Dopamine is an important neurotransmitter that is responsible for the motivation and locomotor function of brain. Chronic stress attenuates

dopamine level in dopaminergic circuit areas and correlates with learning deficit symptoms (Baik, 2020; Jia et al., 2021). In addition, stress induced disruption of GABA in the ventral tegmental area and involved in anhedonia symptom development in depression (Lowes *et al*., 2021).

Antidepressant is playing crucial roles in depression management (Solmi et al., 2021). Recently, the medication of depression is focusing on serotonin as target of neurotransmitter. However, therapeutic limitations are challenging for further improvement due to the recent treatment has long onset, residual symptoms and partial remission (Vahid-Ansari *et al*., 2019). Neurotransmitter systems are proposed as the target of therapy to obtain better therapeutic outcomes (Liu *et al*., 2018). Alternative approaches are suggested from the plant's bioactive compounds. Several plants are categorized as "adaptogen" that promotes recovery from stress condition, such as *Panax gingseng, Rhodiola rosea, Schisandra chinensisc,* and etc. (Panossian et al., 2021). Previous work predicted the antidepressant activity of cyanidin from PSP as an antidepressant by agonist binding to dopamine receptor. In addition, previous research demonstrated the behavioral benefit effects of ANC from PSP (Kurnianingsih *et al*., 2020, 2021).

Purple sweet potatoes are major staple food with high ANC content. Anthocyanin from PSP was demonstrated as antioxidant oppose to oxidative stress on neuronal cells (Zhong et al., 2023) and neuroprotective effect on ischemia stroke model animals (Rahmawati *et al*., 2018).

However, the effect of total ANC from PSP on neurotransmitter and locomotor behavior are remaining under-explored. Therefore, the next studies are essential to be developed in animal model of stress to provide intelligible biomechanism of ANC from PSP in improving the behavior through neurotransmitter regulation. Thus, this study aimed to evaluate the effect of ANC from PSP on brain corticosterone, dopamine, GABA level as well as locomotor behaviors in chronic stress model mice. This study also provided computational prediction of ANC in inhibiting neurotransmitter degradation as monoamine oxidase (MAO) inhibitor by molecular docking interaction.

Matrerials and methods

Animals

All animal procedures followed the Declaration of Helsinki for animal welfare. Eight-weeks-old male BALB/c mice were housed in pairs at Animal Experimental Laboratory, Institute of Bioscience, Universitas Brawijaya. The animals were maintained in a controlled temperature and humidity room with 12:12 light-dark photoperiod. The standard laboratory food pellet and drink were provided ad libitum (Ghotbitabar et al., 2022; Nikjooy et al., 2022).

Experimental design

A total of 25 mice were divided into 5 groups, i.e control (CTRL); stress (STR); stress + ANC 10 mg/kgBW (STR+ANC10); stress + ANC 20 mg/kgBW (STR+ANC20) and stress + ANC 40 mg/kgBW (STR+ANC40). Stress was exposed 2 hours/day for a total duration of 14 days. Total anthocyanin extracts were administrated via intragastric tube at a frequency once/day under a duration of stress. Open field test behavioral assessment was conducted as pre- and post-treatment evaluation for distance travelled and velocity of movement. All animal procedures were performed in light period (Kurnianingsih et al., 2023; Resae et al., 2023).

Plant materials

A Local cultivar of PSP (Antin-3 variety) was harvested from Research Centre of Legume and Tuber Plant, East Java Indonesia at 4 months plantation age. Plant tuber root was freshly ground then followed by homogenization and maceration in acidic-methanol (99.9%; Sigma-Aldrich (Darmstadt, Germany) solvent pH 4.5 at room temperature for 24 hours. After the filtration procedure, the homogenates were evaporated at 50-60°C using rotary evaporator. The extracts were stored in 4°C until further application (Dwiwibangga et al., 2022; Kurnianingsih et al., 2020).

Stress exposure

Restraint stress was carried out as a modification of previous methods by immobilizing each animal in a transparent, ventilated and fitted diameter of acrylic cylinder with free

110 breathing. The restraint stress was applied as random schedules to avoid animal habituation (Reyhanditya et al., 2022).

Open-field test

Behavioral assessment of open field test was carried out according to previous studies (Kurnianingsih *et al*., 2020). In brief, after room habituation, each animal was placed in the
115 centre of 40x40x40 cm dimensional of acrylic box. The movement of each animal was recorded for 6 minutes. The video was analysed using EthoVision XT software (Noldus Information Technology b.v., Wageningen, Netherland) to measure the total distance travelled, the velocity of movement, track-line and heatmaps visualization (Iturra-Mena *et al*., 2018; Khodadadeh *et al*., 2020).

120 **Enzyme-linked immunosorbent assay (ELISA)**

After 14 days of the experiment, mice were sacrificed as preveuthanized then followed by brain isolation. After weighting, the whole region of brain tissues was processed for ELISA analysis to measure dopamine, GABA, and corticosterone level. The ELISA procedures were conducted as listed in the protocol of ELISA kit. The catalogue number was EM1603
125 (FineTest,Wuhan Fine Biotech Co., Ltd, China) for mouse-GABA analysis, EM1712 (FineTest,Wuhan Fine Biotech Co., Ltd, China) for mouse-Dopamine analysis and DEV9922 (Demeditec Diagnostics GmbH, Germany) for corticosterone analysis (Xie *et al*., 2013).

Molecular docking prediction

Ligand and protein preparation

130 Ligand of cyanidin (CID 128861), cyanidin-3-O-glucoside (CID 441667), and peonidin-3-O-glucoside (CID 443654) were retrieved from PubChem Database (https://pubchem.ncbi.nlm.nih.gov/). Rasagiline (CID 3052776) as MAO inhibitor drug was used as control ligand. The protein of human monoamine oxidase-B (MAO-B), PDB ID:2BK3 was downloaded from Protein Data Bank database. Active sites of protein were predicted using 135 Molegro virtual Docker 5.0 software with Molecular surface van der Waals no more than five (Bitencourt-Ferreira & de Azevedo, 2019).

Docking simulation

Molegro virtual docker software was used to interact protein and ligand with specific grid (X=54.30; Y=147.7; Y=22.01; radius 16). The docking parameter of Molegro virtual docker were 140 Function Moldock Score [Grid]; grid resolution 0.30; algorithm MolDock SE; Number of Runs 10, Max iteration 1500; maximum population size 50; pose generation energy threshold 100, tries 10 – 30; simplex evolution maximum steps 300; neighbor distance factor 1.00; multiple pose number of pose 5; energy threshold 0.00; and cluster similar poses RMSD threshold 1 (Bitencourt-Ferreira & de Azevedo, 2019)

145 **Molecular docking analyses**

Docking complex from Molegro virtual docking versi 5 software was superimposed with PyMol versi 2.2. software. Visualization of complex interaction was displayed both 3D and 2D view using Discovery Studio version 21.1.1 programme (Bitencourt-Ferreira & de Azevedo, 2019).

Statistical analysis

The results were expressed as mean ± SEM. The differences between treatment were assessed using two-way ANOVA followed by LSD multiple comparison using GraphPad Prism 9.0.0 software. The significance was set as $p < 0.05$ (Kurnianingsih et al., 2023).

Results

Chronic stress exposure for 14 days reduced brain weight (0.40±0.004 g) significantly compared to control (0.43±0.003 g). The ANC from PSP increased brain weight into 0.42±0.002 g and 0.42±0.001 g in a dose of 20 and 40 mg/kgBW respectively (Figure 1A). Stress dramatically increased brain corticosterone level (141.80±2.44 ng/ml) higher than the control (82.63±4.51 ng/ml). Treatment of ANC reduced this corticosterone level following a dose-dependent manner that is 9.45%, 13.08% and 27.23% for 10,20 and 40 mg/kgBW respectively (Figure 1B). Brain neurotransmitter of GABA increased among stress and ANC-treated groups (p=0.004) (Figure 1C). Meanwhile, brain dopamine level in stress-exposed mice (10.54±0.19

ng/ml) were lower than control (12.95±0.17 ng/ml). Contrarily, ANC administration increased brain dopamine higher than control for dose of 10 mg/kgBW (14.59±0.45) and 20 mg/kgBW (13.42±0.24) (Figure 1D).

Locomotor analysis revealed the decline in distance travelled and movement velocity after stress exposure. Stressed mice have 495.37 cm shorter distance movement than control. The ANC reduced the delta of travelled distance by about 306.61 cm, 248.82 cm, and 231.06 cm in a dose dependent manner at the post-test evaluation (Figure 2A). Stress significantly declined the velocity of movement. Despite ANC did not increase velocity movement, the post-test evaluation showed that the velocity among ANC treated groups was higher than post-stressed only group (Figure 2B).

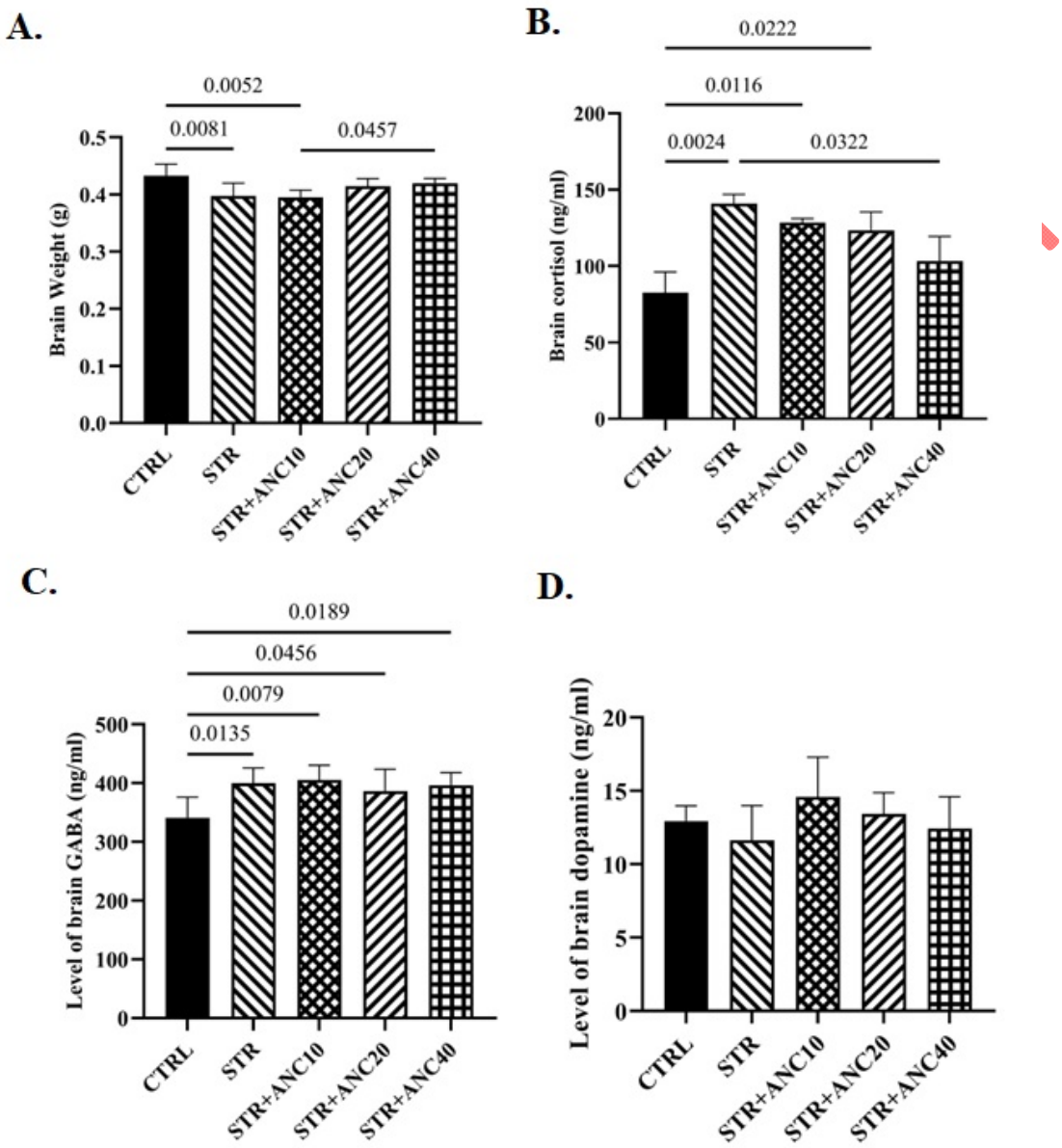


Figure 1. (A) The enhancement of brain weight following ANC treatment (B). Brain corticosterone level increased after stress application; the administration of ANC observed

reduce the cortisol level. (C). Upregulation of brain dopamine level after ANC treatment among stressed mice. (D). The incline of brain GABA level both in stressed and ANC treated mice. Data presented as mean ± SEM. Significant p-value between groups denoted above each bar of groups.

The movement of animals visualized as heatmap and track line. Lack of movement was shown after stress exposure. Reduce movement in central zone demonstrated among stress and ANC treated animal. At pre-test evaluation exhibited more heatmap spot that visualize as green-yellow-red colour. The color of heatmap represent as longer time spent at point of track lines. Post-test evaluations qualitatively showed decline of green-yellow-red colour spot in heatmap observation (Figure 2C).

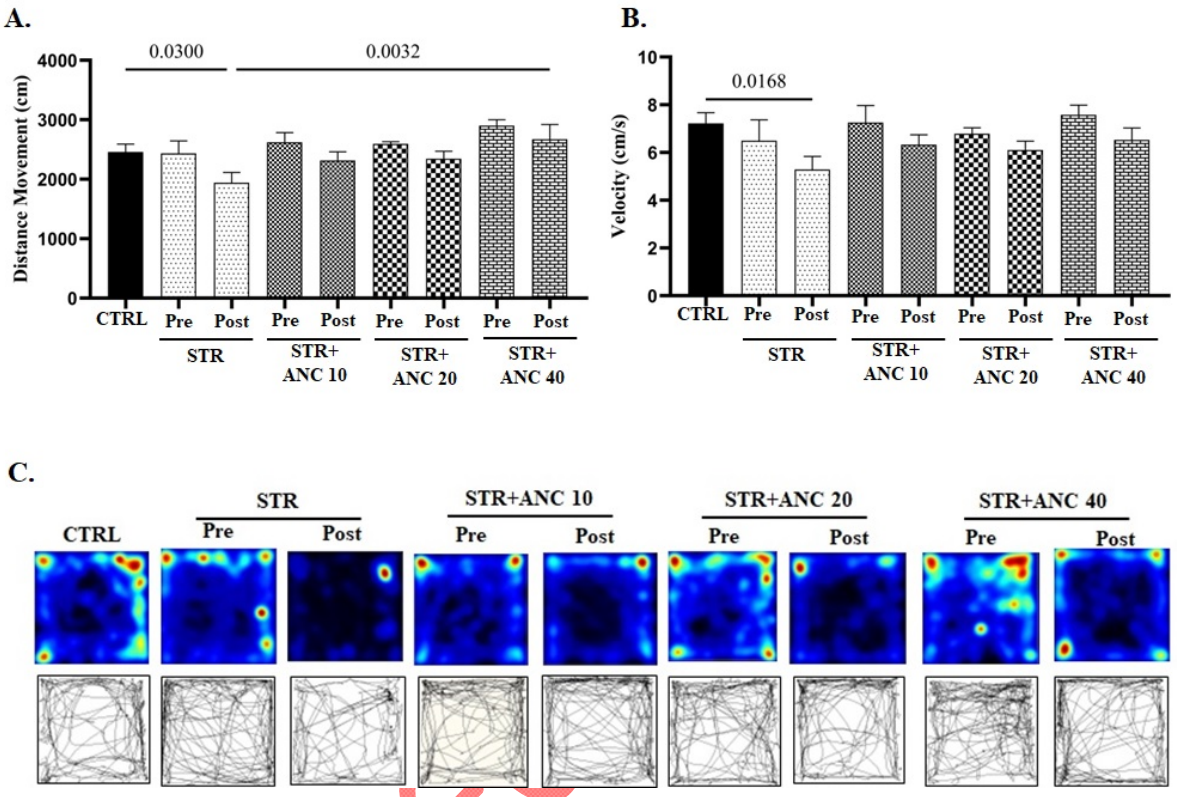
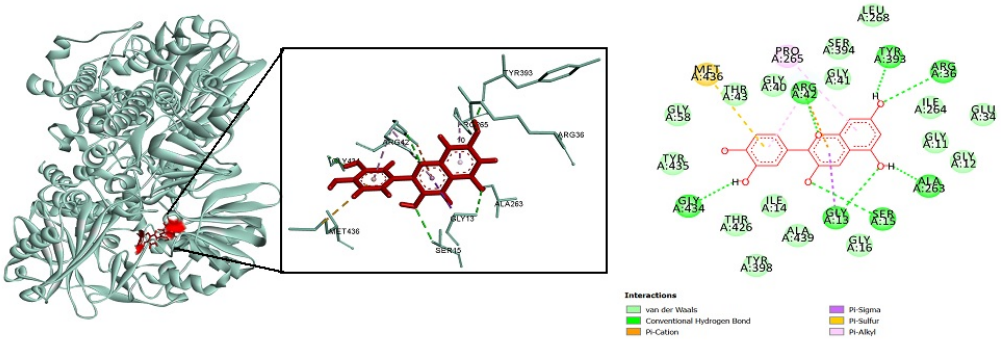


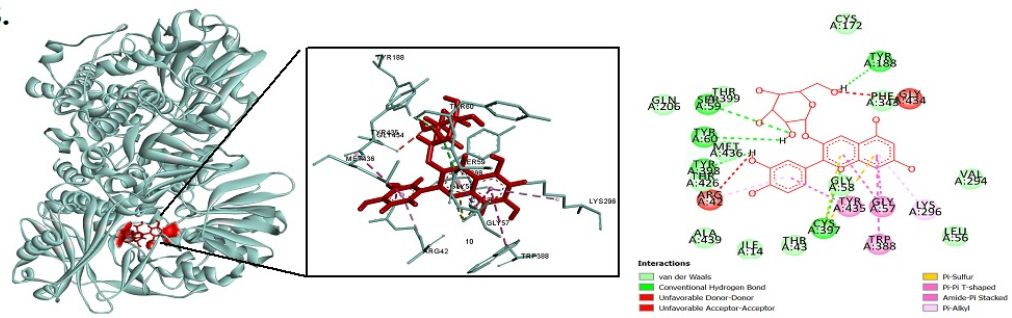
Figure 2. Locomotor assessment of ANC treated mice. (A) Stress reduced the distance movement of mice. The decline of distance movement among ANC treated mice are lower than stressed-only mice. (B). The velocity of stressed mice lower than control. Total anthocyanin extracts treatment in stressed mice tend to reduce the velocity of movement. (C) The differences of heatmap pattern and track of movement animals among groups visualized with EthoVision XT software (Noldus Information Technology, Netherlands). Data presented as mean ± SEM. Significant p-value between groups denoted above each bar of groups.

To evaluate the prediction of ANC roles in dopamine neurotransmitter regulation, molecular docking was performed to interact monoamine oxidase-B (MAO-B) with selected ANC. Molecular docking demonstrated cyanidin, cyanidin-3-O-glucoside and peonidin-3-O-glucoside have similar interaction site with control ligand (Rasagiline) (Figure 3). Amino residues of ALA263 and PRO265 from MAO showed interaction with cyanidin. Meanwhile, peonidin-3-O-glucoside bind at amino residue of ALA439 and ILE14. All anthocyanins have interaction on residue ARG42. The interaction of MAO and cyanidin resulted the most positive energy binding that is -370 kJ/mol. Furthermore, energy binding of MAO with cyanidin-3-O-glucoside and peonidin-3-O-glucoside was -492 kJ/mol and -498.4 kJ/mol respectively. In comparison to anthocyanins, Rasagiline as control ligand has energy binding 264.8 kJ/mol that indicated more positive value than anthocyanins (Table 1).

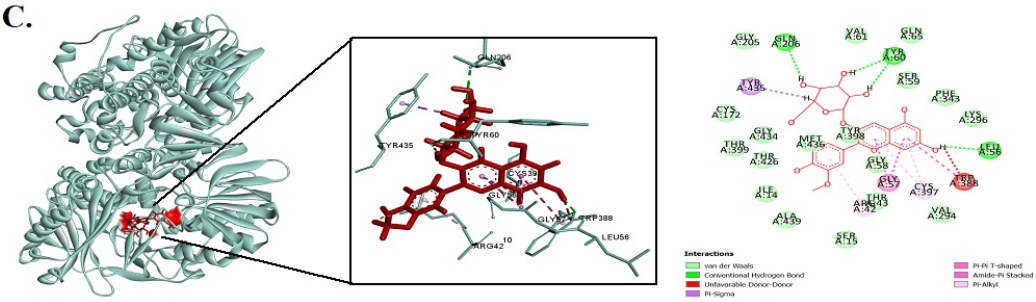
A.


Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Sulfur
Pi-Alkyl

B.


Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Unfavorable Acceptor-Acceptor
Pi-Sulfur
Pi-Pi T-shaped
Amide-Pi Stacked
Pi-Alkyl

C.


Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Pi-Sigma
Pi-Pi T-shaped
Amide-Pi Stacked
Pi-Alkyl

D.

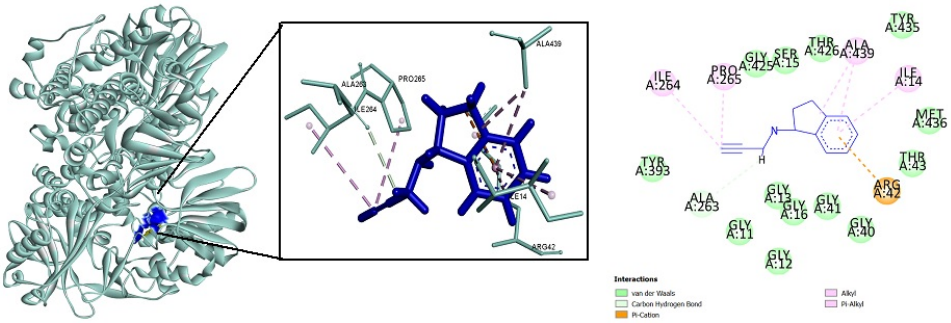

Interactions
van der Waals
Carbon Hydrogen Bond
Pi-Cation
Alkyl
Pi-Alkyl

Figure 3. Molecular docking interaction prediction of anthocyanins and Rasagiline as control ligand to monoamine oxidase (MAO). A) Cyanidin-MAO complex. B) Cyanidin-3-O-glucoside-MAO complex. C) Peonidin-3-O-glucoside-MAO complex. D) Rasagiline-MAO complex. Left panels show 3D structure of interaction between ligand and MAO protein. Central panels show ligand interaction that visualize as type of interactions in right panel.

Tabel 1. Interaction between anthocyanins and Rasagiline on human monoamine oxidase-B (MAO-B)

Ligands	Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types
Cyanidin	-370	A:GLY13:N - :10:O3	2,98682	Hydrogen Bond	Conventional Hydrogen Bond
		A:SER15:N - :10:O2	3,07293	Hydrogen Bond	Conventional Hydrogen Bond
		A:ARG36:NH1 - :10:O4	3,13127	Hydrogen Bond	Conventional Hydrogen Bond
		A:ARG42:N - :10:O1	3,03441	Hydrogen Bond	Conventional Hydrogen Bond
		A:ARG42:NE - :10:O1	3,15469	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H8 - A:ALA263:O	1,61827	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H9 - A:TYR393:O	1,73229	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H10 - A:GLY434:O	2,3035	Hydrogen Bond	Conventional Hydrogen Bond
		A:ARG42:NH2 - :10	4,29851	Electrostatic	Pi-Cation
		A:GLY13:CA - :10	3,71408	Hydrophobic	Pi-Sigma

Ligands	Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types
		A:MET436:SD - :10	5,98818	Other	Pi-Sulfur
		:10 - A:ARG42	4,87147	Hydrophobic	Pi-Alkyl
		:10 - A:PRO265	5,37395	Hydrophobic	Pi-Alkyl
		:10 - A:ARG42	4,29901	Hydrophobic	Pi-Alkyl
Cyanidin-3-O-glucoside	-492	A:SER59:N - :10:O5	3,3335	Hydrogen Bond	Conventional Hydrogen Bond
		A:CYS397:SG - :10:O7	3,4316	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H10 - A:TYR60:O	2,60322	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H11 - A:TYR188:OH	1,84323	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H20 - A:TYR398:O	2,15947	Hydrogen Bond	Conventional Hydrogen Bond
		A:CYS397:SG - :10	4,68116	Other	Pi-Sulfur
		A:CYS397:SG - :10	4,53842	Other	Pi-Sulfur
		A:TRP388 - :10	5,30088	Hydrophobic	Pi-Pi T-shaped
		A:GLY57:C,O;GLY58:N - :10	3,74136	Hydrophobic	Amide-Pi Stacked
		A:GLY57:C,O;GLY58:N - :10	3,70119	Hydrophobic	Amide-Pi Stacked
		A:TYR435:C,O;MET436:N - :10	4,82201	Hydrophobic	Amide-Pi Stacked
		:10 - A:LYS296	5,02574	Hydrophobic	Pi-Alkyl
		:10 - A:ARG42	4,11246	Hydropho	Pi-Alkyl

Ligands	Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types
				bic	
		A:ARG42:NH1 - :10:H20	2,38389	Unfavorable	Unfavorable Donor-Donor
		A:GLY434:O - :10:O6	2,73868	Unfavorable	Unfavorable Acceptor-Acceptor
Peonidin-3-O-glucoside	-498,4	:10:H8 - A:TYR60:O	2,25123	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H9 - A:GLN206:OE1	2,58876	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H10 - A:TYR60:O	2,26974	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H19 - A:LEU56:O	2,3069	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H21 - :10:O11	2,38268	Hydrogen Bond	Carbon Hydrogen Bond
		:10:H4 - A:TYR435	2,5646	Hydrophobic	Pi-Sigma
		A:TRP388 - :10	5,55153	Hydrophobic	Pi-Pi T-shaped
		A:GLY57:C,O;GLY58:N - :10	4,15828	Hydrophobic	Amide-Pi Stacked
		A:GLY57:C,O;GLY58:N - :10	3,54895	Hydrophobic	Amide-Pi Stacked
		A:ALA439 - :10:C22	3,65013	Hydrophobic	Alkyl
		:10:C22 - A:ILE14	3,3547	Hydrophobic	Alkyl
		:10:C22 - A:MET436	4,51516	Hydrophobic	Alkyl
		:10 - A:CYS397	4,94376	Hydrophobic	Pi-Alkyl
		:10 - A:CYS397	4,43016	Hydropho	Pi-Alkyl

Ligands	Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types
				bic	
		:10 - A:ARG42	4,0933	Hydrophobic	Pi-Alkyl
		A:TRP388:NE1 - :10:H19	2,55109	Unfavorable	Unfavorable Donor-Donor
Rasagiline	-264,8	:10:H10 - A:ALA263:O	3,03662	Hydrogen Bond	Carbon Hydrogen Bond
		A:ARG42:NH2 - :10	4,71293	Electrostatic	Pi-Cation
		A:ALA439 - :10	4,09578	Hydrophobic	Alkyl
		:10:C12 - A:ILE264	5,17272	Hydrophobic	Alkyl
		:10:C12 - A:PRO265	5,37402	Hydrophobic	Alkyl
		:10 - A:ILE14	4,99427	Hydrophobic	Pi-Alkyl
		:10 - A:ARG42	4,93827	Hydrophobic	Pi-Alkyl
		:10 - A:ALA439	4,46751	Hydrophobic	Pi-Alkyl

Discussions

Systemic effect of stress is mediated by ultradian corticosterone release from the adrenal cortex. This stress hormone quietly across the blood brain barrier to bind on glucocorticoid receptors. The broad site of glucocorticoid receptor activation by corticosterone then affects neuronal activities including neurotransmitter signalling (Joëls, 2018). High corticosterone

concentration previously used as severe stress mimicked on basolateral amygdala cells culture. Neuronal firing enhancement is well documented because of higher corticosterone stimulation. The combination between high dose corticosterone and β-adrenoceptor agonist isoproterenol long-lasting the enhancement of post synaptic current (Karst & Joëls, 2016). Therefore, in this study, the escalation of brain corticosterone in stress exposed groups strongly suggests as significant responses towards restraint stress procedure.

Total anthocyanin extract from PSP demonstrated favourable effect on reducing brain corticosterone level. Previous animal study reported similar result that anthocyanin-supplemented food limited the corticosterone level both in rest and activities (Casagrande et *al*., 2020) . A study on lipopolysaccharide induced-inflammation in BV-2 murine microglial cells showed anthocyanin fractions reduced inflammation by declining cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNFα), and nuclear factor κB (NF-κB)(Poulose *et al*., 2012).

In the present study, the reduction of corticosterone level was followed by increase of dopamine neurotransmitter among anthocyanin treated group. Corticosterone as glucocorticoids hormone increases blood glucose level to enhance metabolism during stress that is accompanied by the release of reactive oxygen species (ROS) as free radical. Therefore, chronic glucocorticoid exposure during stress-induced oxidative state affect the neurogenesis (Spiers *et al*., 2015). An additional study showed an elevation of brain dopamine in

psychological stress-induced oxidative stress mice. Pre-treatment with anthocyanin increase dopamine level in several brain areas including cerebellum. As the motor coordination function of cerebellum, the increase of dopamine might accelerate motor skills and movement (Rahman *et al*., 2008). However, this present study analysed whole brain tissue, so the certain specific function of the dopamine-related areas are required further evaluation.

Therefore, locomotor behavior of mice was explored following anthocyanin treatment. Oppositely, the present study showed a decline in locomotor among stress and ANC-treated mice. The GABA level was examined to analyse the potential of inhibition effect of ANC. Accordingly, GABA level of stress and ANC treated mice simultaneously higher than control. As correlated with our previous study showed the potential binding of cyanidin as a major anthocyanin component from PSP as agonist towards $GABA_B$ receptor (Kurnianingsih *et al*., 2020). Anthocyanins is absorbed into intestinal epithelial cells then modulates the synthesis of GABA neurotransmitter via intestinal microbiota pathway. Anthocyanins promote the growth of neurotransmitter-producing bacteria such as *Lactobacillus* and *Bifidobacterium* species. The bacteria transforms short chain fatty acid to neurotransmitter of GABA, serotonin, and glutamate. Phenol katabolic products from anthocyanins act as source of short chain fatty acid production. This indicates the involvement of anthocyanins in brain behavior functions (Zhong et al., 2023).

Despite the potential of ANC on dopamine and GABA neurotransmitter, molecular docking prediction showed the potential of anthocyanins from PSP to inhibit the MAO. Monoamine oxidases enzymes are functionated to degrade biogenic amine neurotransmitter in neuronal cells. Inhibition of the MAO enzyme increases the concentration of monoamine neurotransmitter in synaptic cleft including dopamine (Patetas & Gartner, 2006). Our previous study revealed cyanidin, cyanidin-3-O-glucoside and peonidin-3-O-glucoside as major anthocyanin from purple sweet potatoes from our local cultivar (Kurnianingsih *et al*., 2021). This study also demonstrated the prediction of anthocyanins to bind with MAO protein in similar site with Rasagiline as an inhibitor. Anthocyanins are then assumed as MAO inhibitor that potential to inhibit monoamine degradation neurotransmitter.

Certain animal behaviors were influenced by the modulation of brain specific receptors, anti-inflammatory agents and specific protein treatment. Spatial memory observation showed that the inhibition of orexin 1 receptor in nucleus accumbens (NAc) impairs the consolidation of spatial memory in rats. Other treatment agents such as anti-inflammatory drugs inhibited seizures behavior in rat model of acute epilepsy by intracerebroventricular injection. Per nostril application of Prolactoliberin (PrRP) decreased time spent in open arms at elevated plus maze test thus indicated increased of anxiety behavior in rats (Fazlelahi et al., 2023; Ghazi Ghanim et al., 2023; Zokaei & Akbari, 2023). The modulation of plant extract on neurotransmitter system

275 and organ weight indicated the important of future research in animal models (Chukwu et al., 2022; Nikjooy et al., 2022). Therefore, further evaluations are necessary to reveal the molecular mechanism of ANC from PSP to inhibit the adverse effect of stress.

280 Conclusion

This study conclude that ANC from PSP reduces brain inflammation thus enhancing the dopamine and GABA neurotransmitter, followed by a decrease of movements of chronic restraint-stress induced mice. In addition, molecular prediction showed anthocyanins from PSP potential to interact with MAO enzyme as an inhibitor.

285 Ethical consideration

Compliance with ethical guidelines

All experimental procedures were previously approved by Research Ethic Committee of Universitas Brawijaya with ethical clearance certificate No:029-KEP-UB-2022.

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Author contribution

Research conceptualization and design: NK, RR; Supervision: NK, NTH, AFF, RR; Data acquisition: ARH, DF, AFF; Data analysis: NK; Manuscript drafting: NK. All authors revising it critically for intellectual content.

Conflict of interest

Authors declare no conflict of interest.

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