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The Behavioral Effect of Anthocyanin from Purple Sweet Potatoes on Prenatally Stressed Offspring Mice

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ABSTRACT

Prenatal stress is major risk factor for long-lasting psychopathological development on offspring including depression. The objectives of this study were to investigate the effect of anthocyanin from purple sweet potatoes on behavioral performances of prenatally stressed offspring as well as the prediction of mechanism using in silico analysis. The pregnant mice were physically restrained during the half late pregnancy for 2 hours each day. The adolescent offspring were then treat using total anthocyanin extracts from purple sweet potatoes. The open field test and tail suspension test were performed for behavioral analysis. The results were demonstrated that the administration of anthocyanin significantly increases the immobility time. Conversely, track visualization was shown more explorative movement on mice. Virtual molecular interaction was demonstrated that anthocyanins of delphinidin and malvidin have similar interaction with GABA and baclofen as $GABA_B$ receptor agonist. Recent results are gaining a necessary of further exploration with more complex behavioral test procedures and the determination of anthocyanin on GABAergic transmission in animal model of depression.

Keywords: Anthocyanin, behavior, neurotransmitter, pregnancy stress

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INTRODUCTION

Depression is a mental health disorder which are characterized with depressed mood, diminished of pleasure, sleep disturbances, body weight changes, appetite alteration, fatigue, worthlessness, decrease of concentration, psychomotor agitation/retardation and thought of death/suicide (1). Depression is highly prevalent in population and become a major contributor of disability, health loss and suicide globally (2). One of the strong risk factor for depression is maternal stress during pregnancy (3). Prenatal stress is defined as broad range of perceive stress, anxiety and depression during pregnancy (4). Prenatal stress is being emphasized because of the long-term impacts on pregnancy outcomes, child-adolescence behaviors and psychopathological development on adulthood (5)(6)(7). The susceptibility of foetal neuronal development to maternal stress hormones is widely accepted as proposed mechanism on behavioral/emotional problems of offspring (8).

Previous studies were summarized an ameliorate effect of prenatal stress on brain areas connectivity. Prenatal stress impaired the activation of prefrontal cortex thus resulting an inappropriate trajectories of gamma-aminobutyric acid on ventral tegmental area of brain limbic system (9). Gamma-aminobutyric acid (GABA) is a major synaptic inhibitory neurotransmitter in mammalian brain (10). The deficit and disfunction of GABA neurotransmission have been identified in both human and animal depression studies (11). Despite there are two types of GABA receptors, $GABA_B$ receptor have been proposed as new target of antidepressant action (12). Nevertheless, the modulation of $GABA_B$ receptor by its ligands were shown a convergence results on depressive behavior (13). Meanwhile, commonly prescribed antidepressant has long onset acting limitation, thus new rapid agent of antidepressant on GABA target transmission offering greater efficacy for this mood disorder (14). For the present, the drugs that targeted GABA systems widely used for anxiolytic and sedative purposes as well as the development of herbal medication on GABA target were act as anti-insomnia (15).

Flavonoid, a plant natural compound has been investigated as antidepressant in preclinical studies by modulating the GABA systems, such as the content of hyperoside from *Apocynum venetum L* plant (16). Anthocyanin is one of the plant flavonoids that previously reported as protective agent against oxidative stress induced by psychological stress (17). Anthocyanins as red-purple plant pigment are also contain in purple sweet potatoes (PSP) (18). Anthocyanin from PSP previously reported as neuroprotective effect on animal model of brain ischemia and spatial memory enhancer on diabetic model rats (19)(20). This memory function effect was assumed related to the capacity of anthocyanin to inhibit the neuronal apoptosis particularly at hippocampus (21). As far our knowledge, the effect of anthocyanin from PSP on prenatal stressed induced depression is still lacking and need further exploration. This study aimed to investigate the effect of anthocyanin from PSP on behavioral performances of prenatally stressed offspring as well as determination the function of anthocyanin on $GABA_B$ receptor using virtual molecular docking interaction. This study would be supporting the development of anthocyanin from PSP as potential antidepressant as well as the potential mechanism of action on $GABA_B$ receptor.

MATERIALS AND METHODS

Animal

All animal procedures were approved by Research Ethic Committee of Universitas Brawijaya (No:1193-KEP-UB). The BALB/c mice were obtained from animal laboratory breeding of Bioscience Institute, Universitas Brawijaya. All

mice were housed at animal experimental laboratory of Bioscience Institute, Universitas Brawijaya under controlled of temperature, humidity and 12:12 hours light-dark cycle (22). All the animal experimental procedures were performed on the light phase. The standard laboratory food and water were provided *ad libitum* (23).

The extraction of total anthocyanin

The purple sweet potatoes variety of Antin-3 were harvested from Legume and Tuber Plant Research Centre, East Java Indonesia. The fresh grinded tuber roots were macerated using acidic methanol solvent pH 4.5 at room temperature. After 24 hours the homogenates were filtrated using Whatman filter paper (0.45µm) and evaporated using vacuum rotary evaporator at 50-60°C. The extracts were then stored at 4°C until further utilization (24)(25).

Mating

The adult females were stimulated for oestrus by Lee-Boot and Whitten method as previously described (26). The oestrous phase was identified by behavioral observation and the presence of vaginal opening and relative tumescence of external genitalia. Oestrus females were demonstrated sexual receptivity behavior, i.e. lordosis, jumping, running around and ear wiggling (27)(28). The oestrus females were mated with sexually experience male with mating ratio 1:1. The presence of vaginal plug were assigned to gestational day 0 (GD 0)(29).

Experimental design

The fertilized females were then placed in individual cage. The pregnant mice randomly assigned into non-stressed (NS) group and prenatal stressed (PS) group. The restraint stress was given from GD 10 until the end of gestation, meanwhile the mother in NS group remained undisturbed in their home cage (30)(31). All the mother was remained with their pups until weaning period. The young offspring were removed from their home cage on PND 21 and placed individually. After new home cage habituation, young offspring were carried out for behavioral analysis sequentially on post-natal day (PND) 30-34. The offspring from both group of mothers were randomly assigned into 4 group of treatment respectively, i.e. anthocyanin (-), anthocyanin 10 mg/BW; anthocyanin 20 mg/BW and anthocyanin 40 mg/BW. The anthocyanin (ACN) extract was administrated to young offspring at PND 35. This mid-adolescence period was chosen according to the importance of the
period as explained before (32). The ACN extract were given once a day for duration of 7 weeks (20). Furthermore, second behavioral analysis was conducted before the surgery procedure (Figure 1).

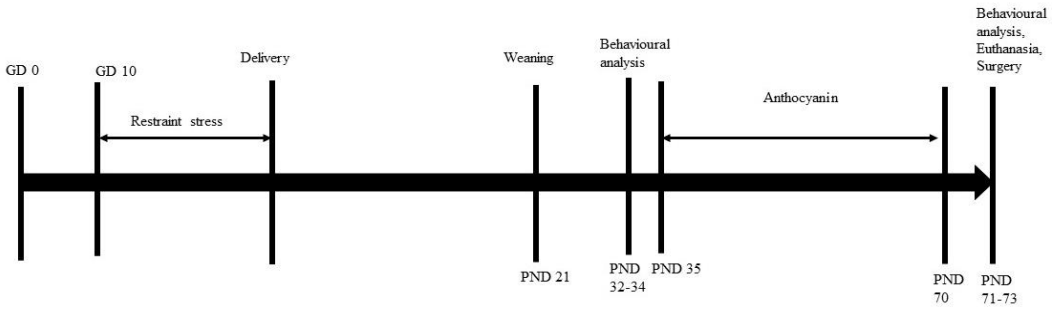

Figure 1: The scheme of the experimental design. Prenatal restraint stress was applied on half late pregnancy period. The behavioral analysis was performed both before and after the anthocyanin administration. Anthocyanin was given from period of adolescence until adulthood.

Prenatal stress exposure

The stress exposure was applied according to previous studies with minor modification. Briefly, the mother from PS group were restraint in a well ventilated cylindrical acrylic with long and diameter fitted close to the body for 2 hours with randomly schedule each day (30)(33).

Behavioral analysis

Open field test

The open filed test procedure was conducted according to Salari, et al (34) with minor modification. The mice were habituated at testing room for an hour prior the observation. Mice were placed individually at the center of acrylic box with dimension of 40×40×40cm, followed by video recording for 6 minutes of duration. The apparatus was cleaned with ethanol between animal tests. Afterwards, the videos were analysed using EthoVision XT (Noldus Information Technology b.v., Wageningen, Netherland) to measure the total distance, velocity, track and heatmaps of tracking visualization (35).

Tail suspension test

Tail suspension test was performed as minor modification of previous work (36)(37). The tail of mice was suspended above the floor using adhesive tape. The observation was recorded as video for 6 minutes. The completely motionless was defined as immobility time. The measurement of immobility duration was conducted manually by two double blind trained observers.

In silico analysis

Ligand and protein preparation

The ligand of cyanidin (CID: 128861), delphinidin (CID: 128853), malvidin (CID: 159287), pelargonidin (CID: 440832), peonidin (CID: 441773), petunidin (CID: 441774), and baclofen (CID: 2284) were elected from PubChem National Centre for Biotechnology Information (NCBI) database. The ligands were converted into pdb file using PyRx 0.8 software (38). The protein of GABA receptor (PDB: 4ms4) was retrieved from Protein Data Bank (PDB) (http://rcsb.org). The water molecule and previous ligand associated with protein were removed using Discovery Studio Visualizer v19.1.0.18287 program (http://3dsbiovia.com/products/)(39).

Molecular docking

The molecular docking interaction between ligand and GABA receptor was performed using PyRx 0.8 software as well as the binding affinity calculation. The visualization of the interaction was conducted using Discovery Studio

Visualizer v19.1.0.18287 program (http://3dsbiovia.com/products/) (38)(39).

Statistical analysis

The results are 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 $p< 0.05$.

RESULTS

The effect of restraint stress on maternal and pregnancy outcome

Our study was revealed that PS mothers tend to have a lower weight gain along the pregnancy as well as the food intake and water intake compared to NS mothers, even though these differences were not statistically significant. The pregnancy outcomes were showed that gestational length was similar between two groups. However, the NS mothers had a greater number of pups than PS mothers. Female pups were more prevalence than male pups in both groups. The PS mothers had higher percentage of preweaning mortality of pups than NS mothers (Figure 2).

The effect of total anthocyanin extracts on behavioral analysis

Total distance movement and velocity were observed to evaluate the locomotor activity by open field test (Figure 3a-b). The test was performed both before and after the administration of total anthocyanin extract. We were found that anthocyanin administration was not statistically significantly affect the locomotor in all dose. Other behavior such as the depressive-like behavior was evaluated by tail suspension test. Interestingly, anthocyanin significantly increases the immobilization time in almost doses both NS group and PS group (Figure 3c). However, according to track visualization and heat mapping, the mice on dose of 20mg tend to be more explorative after anthocyanin treatment in both groups of mothers. The mouse has more spot areas of exploration (Figure 3d). We suggest that it is an interesting finding of this study.

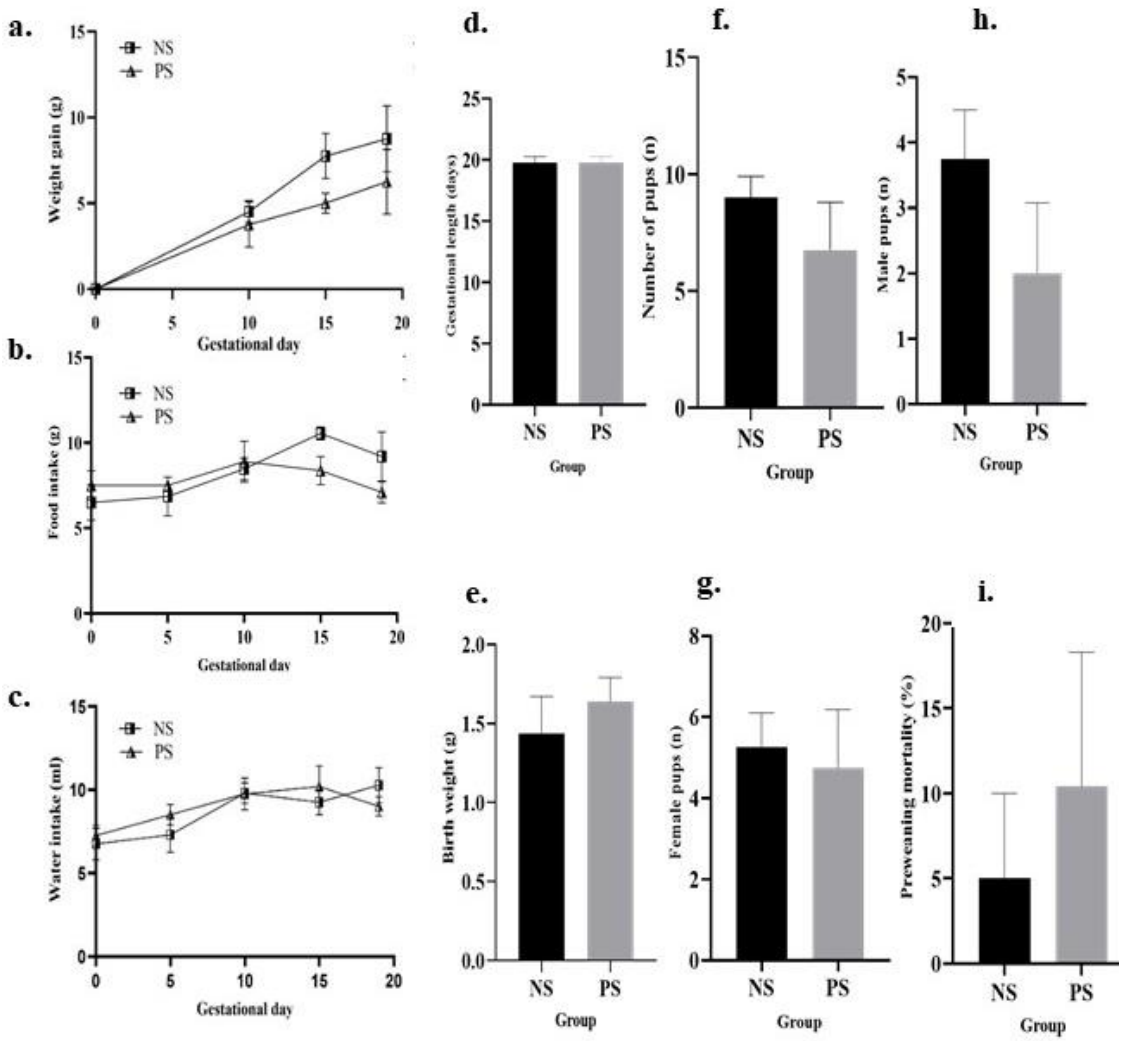

Figure 2: The maternal characteristics of NS and PS mothers (a) The profile of weight gain tends to reduce in prenatal stressed mother as well as the food intake (b) and (c) water intake. The profiles of pregnancy outcomes were demonstrated the similar gestational length (d). The PS mother have a slightly heavier birth weight of pups (e) and lower number of pups (f). The composition of female and male pups was shown in (g-h). Preweaning mortality of pups was greater in PS mothers (i).

In-silico analysis

To evaluate the predictive mechanism of immobility increment after anthocyanin treatment, we were conducted a molecular docking analysis between six anthocyanin and GABA receptor. Our analysis was showed that all anthocyanins have more negative binding affinity than GABA and drug of baclofen. Delphinidin has the most negative binding affinity followed by petunidin, pelargonidin, malvidin, cyanidin and peonidin.

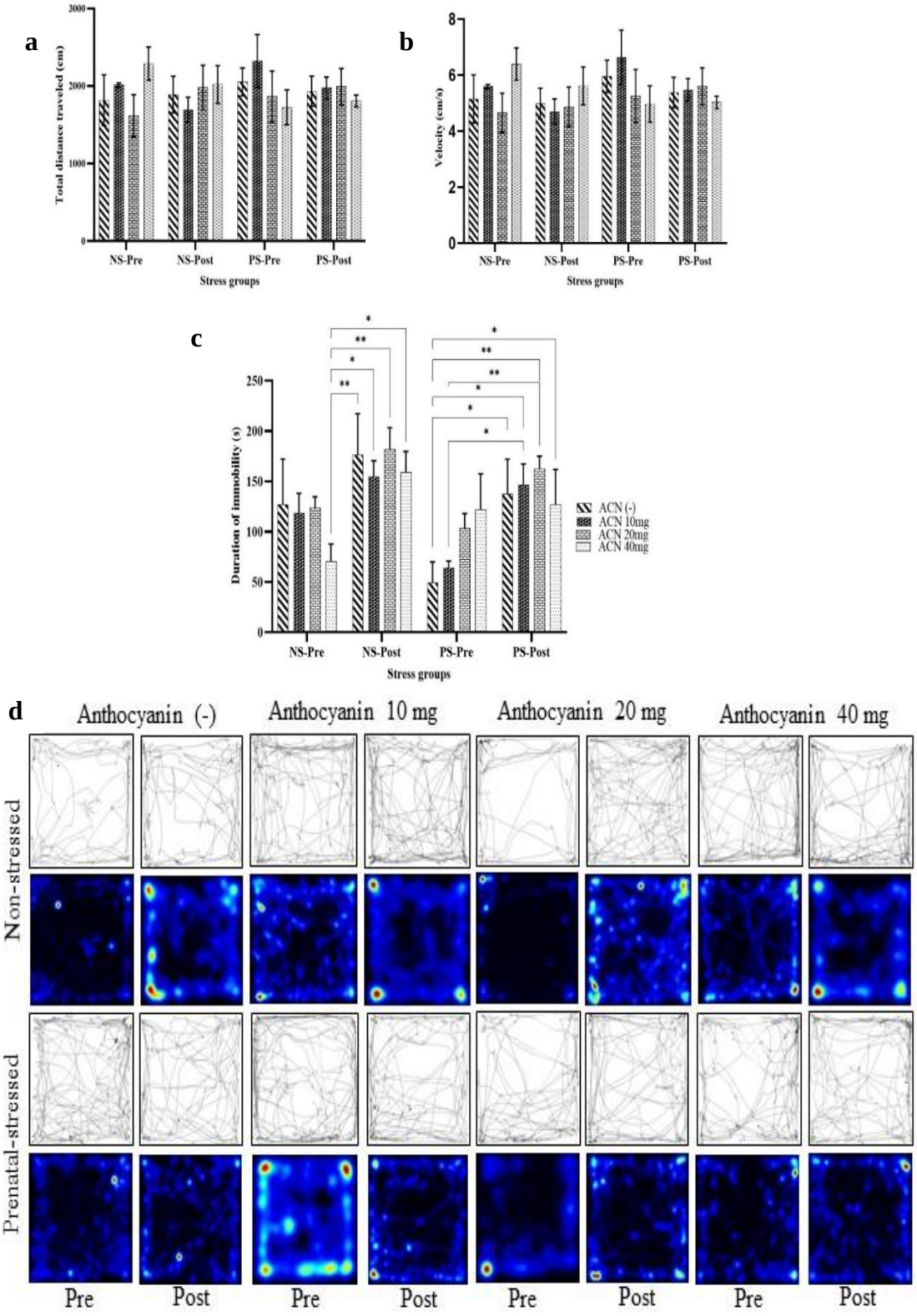

Figure 3: (a-b) The total distance movement and velocity of offspring from NS and PS group were not significantly different after anthocyanin treatment. (c) The immobility duration of offspring was demonstrated significantly increases on almost doses in both group of mothers. The data was shown as mean ± SEM. *p<0.03, **p<0.002. (d) The tracking and heatmaps

visualization of offspring movement was analysed using EthoVision XT software (Noldus Information Technology, Netherlands).

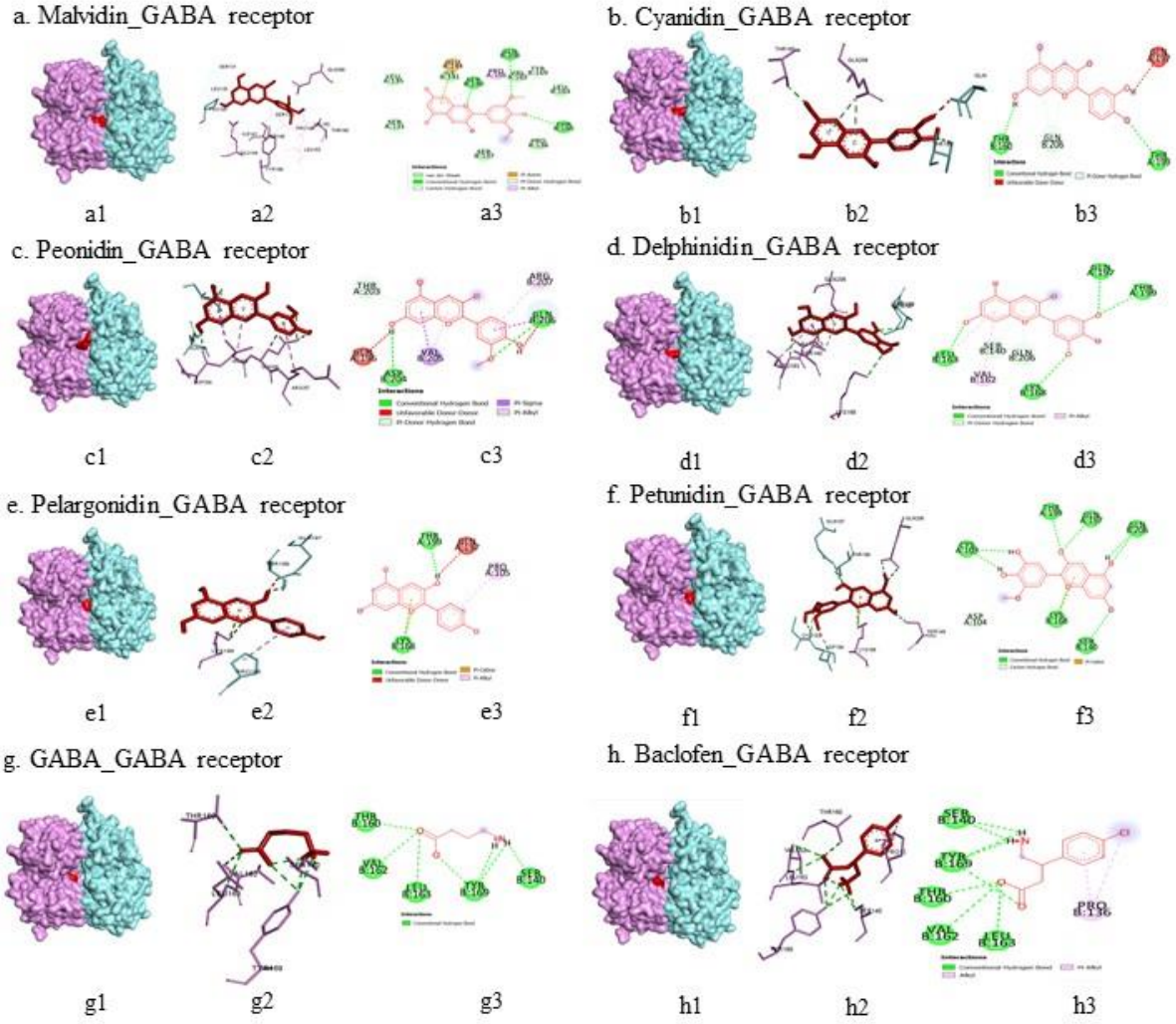

Figure 4: The 3D structure of interaction between anthocyanin (red) with GABA receptor (purple – light blue). Code number 1 and 2 are visualized the 3D structure of the interaction, while code number 3 are 2D interaction and category bond of the complex. The molecular docking was performed using Pyrx 8.0.0 software and visualization was analyzed by Discovery Studio Visualizer v19.1.0.18287 program.

Table 1. Molecular docking score and interaction of anthocyanin ligand against $GABA_B$ receptor analysed using Pyrx 8.0.0 software and Discovery studio software.

Compound	**Docking score (kcal/mol)**	**Hydrogen bonds interaction**		**Compound**	**Docking score (kcal/mol)**	**Hydrogen bonds interaction**	
		Amino residues (interaction type)	Distance (A°)			Amino residues (interaction type)	Distance (A°)
Malvidin	-7.2	Ser140(Conventional)	3.24	**Peonidin**	-6.4	Asp204(Conventional)	2.62
		Thr160(Conventional)	3.03			Gln206(Conventional)	3.07
		Gln206(Conventional)	3.18			Thr203(Pi-Donor)	3.82
		Tyr169(Carbon)	3.45	**Cyanidin**	-6.7	Thr160(Conventional)	2.20
		Ser140(Pi-Donor)	4.15			Thr199(Conventional)	2.99
Delphinidin	-7.9	Gln197(Conventional)	2.96			Gln206(Pi donor)	4.03
		Thr199(Conventional)	2.88			Gln206(Pi donor)	4.19

		Leu163(Conventional)	3.24	**Baclofen**	-5.9	Ser140(Conventional)	2.88
		Lys168(Conventional)	3.15			Tyr169(Conventional)	2.51
		Ser140(Pi donor)	3.66			Ser140(Conventional)	2.38
		Ser140(Pi donor)	3.90			Tyr169(Conventional)	2.92
		Gln206(Pi donor)	3.67			Thr160(Conventional)	2.94
Pelargonidin	-7.2	Thr199(Conventional)	2.14			Val162(Conventional)	3.12
		Lys168(Conventional)	3.14			Leu163(Conventional)	3.02
		Lys168(Electrostatic)	3.69			Tyr169(Conventional)	2.89
Petunidin	-7.4	Cys103(Conventional)	2.61	**GABA**	-3.8	Ser140(Conventional)	1.91
		Cys103(Conventional)	2.80			Tyr169(Conventional)	2.89
		Gln206(Conventional)	2.85			Tyr169(Conventional)	2.92
		Gln197(Conventional)	2.95			Tyr169(Conventional)	2.92
		Thr199(Conventional)	2.78			Thr160(Conventional)	2.92
		Ser140(Conventional)	2.98			Val162(Conventional)	3.16
		Lys168(Conventional)	3.12			Leu163(Conventional)	2.99
		Gln206(Conventional)	3.12				
		Asp104(Carbon)	3.56				

DISCUSSION

Our study found that mortality of pups from PS mother approximately double than NS group. Maternal infanticide is generally supposed as death causal of the new-born offspring (40). As described in previous review, the maternal infanticide behavior is associated with two principle factors. First factor related to the viability of the young offspring. The weak or moribund offspring are unable to demonstrate the healthy behavior such as loud vocalization, wriggle and warm. Whereas those behavior are very important for originating a signal inhibition for mother not to ingest their new-born (41). The second factor is associated with the maternal condition. Infanticidal behavior is suggested as consequences of external stress stimuli during pregnancy. Earlier study was showed that unpredictable stress exposure during half late pregnancy associated with maternal depressive-like behavior and decrease the survival of pups (42). Despite the mortality of pups, our PS mother was showed the tendency of lower weight gain during pregnancy. In line with previous report, lower maternal weight gain was correlated with prenatal restraint stress application (30). According to those parameters, we assumed that our restraint stress protocol was generated stress in gestational mice.

The maternal condition on pregnancy affect the perinatal outcomes (43). Stress during pregnancy increases the maternal cortisol level. Cortisol is a major hormone that mediates the effect of maternal stress on foetal development. Simultaneously, stress reduces the enzyme of 11 β-hydroxysteroid dehydrogenase type-2 (11β-HSD-2) which has a function to inactivate those excess of cortisol. Accordingly, maternal cortisol straightforward across the placental barrier and reach the foetal circulation. This cortisol subsequently interferes the hypothalamic-pituitary-adrenal (HPA) axis and modify the set point of negative feedback mechanism and resulting a long-term permanent change in HPA axis activity until postnatal life (44). The exposure to glucocorticoid in foetal phase impact the neurodevelopmental outcomes and behavior. Furthermore, prenatal stress has been linked to increase the vulnerability of neuropsychiatric and mood disorders for instance schizophrenia, autism spectrum disorder, attention deficit hyperactivity disorder, depression and anxiety (45)(46)(47).

The impact of prenatal stress on depressive-like behavior previously normalized by the administration of genipin as plant monoterpenoid compound at age 40 PND of offspring (22). Several plant flavonoid compound has been elucidated as interesting candidates for new antidepressant treatment (16). We were investigated the anthocyanin as flavonoid derivate that previously suggested as treatment of oxidative stress induced by psychological stress as well as ameliorates dopamine level in brain (17). The anthocyanin extract was administrated orally at PND 35 of young offspring. This mid-adolescence is very crucial period in mice, characterized with elevation of impulsivity and risk- taking behavior in accordance with dopaminergic system development. Therefore, this period is appropriate to be utilized as animal model of behavioral abnormalities (32).

Our subsequent analysis was demonstrated that anthocyanin administration significantly increases the immobility time in adult offspring as observed by tail suspension test. This finding is controverting with previous research that reported anthocyanin rich extract from maqui berry decreases the immobility time in mouse model of post-stroke depression (48). Similarly, anthocyanin from purple cauliflower were reported decline the immobility time in animal model of depression. Meanwhile, the longer duration of immobility time in tail suspension test are considered as sign of depressive-like behavior in rodent models (49). To confirm this finding, we were performed open field test procedure to evaluate the locomotor activities. We found no significant difference of total distance movement and velocity among mice after the anthocyanin treatment. However, the mice at dose of 20mg/kg BW tend to be more explorative as shown in movement tracking and heatmaps visualization. As depression disorder are related to several synapses neurotransmitter disfunction such as serotonin, dopamine, norepinephrine and GABA (50,51) we evaluate

the predictive mechanism of anthocyanin related to the recent results. We were then performed in silico analysis to interact the six anthocyanin and GABA agonist drug (baclofen) against $GABA_B$ receptor respectively. We found that six anthocyanin have more negative binding affinity than baclofen. Delphinidin has the most negative binding affinity followed by petunidin, pelargonidin, malvidin, cyanidin and peonidin. The more negative value of binding affinity is predicted better binding interaction to target molecule (52). Among six anthocyanin, only delphinidin and malvidin have the similar amino residues interaction with baclofen at $GABA_B$ receptor. Baclofen is a selective $GABA_B$ receptor agonist in human (53). $GABA_B$ receptor is a metabotropic receptor which are mediates the inhibitory neurotransmission in brain (10). $GABA_B$ receptor is involved in emotional regulation. $GABA_B$ deficient mice showed some anxiety- and panic-like behavior (54). The administration of $GABA_B$ agonist, i.e. baclofen effectively relieves the symptoms of post-traumatic stress disorder and the accompanying symptoms of depression and anxiety (55). Initially, baclofen is used as muscle relaxant, but recent report show the superiority of baclofen as $GABA_B$ agonist has been proven as sleep quality improvement and reduce the transient insomnia (56)(57). We assumed that delphinidin and malvidin probably have the similar agonist properties against $GABA_B$ receptor. Supporting this finding, earlier research was revealed the neuroprotective effect of anthocyanin on prenatally ethanol-induced stress oxidative via the $GABA_{B1}$ receptor modulation (58). Hypothetically, we are raising a further research question that anthocyanin might be act as muscle relaxant therefore impress the duration of immobilization on mice without reducing the movement performance.

CONCLUSION

The present study shows that the administration of anthocyanin extract from purple sweet potatoes at developmental age of prenatally stressed offspring, affect the behavioral parameters on mice. In the future, we would like to establish whether anthocyanin has advantages on miscellaneous behavioral analysis.

CONFLICTS OF INTEREST

This study has no potential of conflicts of interest

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REFERENCE

1. American Psychiatric Association. Diagnostic and Statistical manual of Mental Disorders. 5th ed. Diagnostic and Statistical Manual of Mental Disorders. Arlington: American Psychiatric Association; 2013. 271–280 p.
2. World Health Organization. Depression and Other Common Mental Disorders:Global Health Estimates. Geneva: World Health Organization Licence:CC BY-NC-SA 3.0 IGO; 2017.
3. Maxwell SD, Fineberg AM, Murphy SK, Drabick DA, Ellman LM. Maternal Prenatal Stress and Other Developmental Risk Factors for Adolescent Depression: Spotlight on Sex Differences. J Abnorm Child Psychol. 2017;46(2):381–97.
4. Nast I, Bolten M, Meinlschmidt G, Hellhammer DH. How to measure prenatal stress? A systematic review of psychometric instruments to assess psychosocial stress during pregnancy. Paediatr Perinat Epidemiol. 2013;27(4):313–22.
5. Osborne S, Biaggi A, Chua TE, Du Preez A, Hazelgrove K, Nikkheslat N, et al. Antenatal depression programs cortisol stress reactivity in offspring through increased maternal inflammation and cortisol in pregnancy: The Psychiatry Research and Motherhood – Depression (PRAM-D) Study. Psychoneuroendocrinology [Internet]. 2018;98(December 2017):211–21. Available from: https://doi.org/10.1016/j.psyneuen.2018.06.017
6. Park S, Kim BN, Kim JW, Shin MS, Yoo HJ, Lee J, et al. Associations between maternal stress during pregnancy and offspring internalizing and externalizing problems in childhood. Int J Ment Health Syst. 2014;8(1):1–8.
7. King S, Laplante DP, Veru F, Dancause K, Turcotte-Tremblay A-M. Using Natural Disasters to Study the Effects of Prenatal Maternal Stress on Child Health and Development. Birth Defects Res Part C Embryo Today Rev. 2013;96(4):273–88.
8. Van Den Bergh BRH, Mulder EJH, Mennes M, Glover V. Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: Links and possible mechanisms. A review. Neurosci Biobehav Rev. 2005;29(2):237–58.
9. Pastor V, Antonelli MC, Pallarés ME. Unravelling the Link Between Prenatal Stress, Dopamine and Substance Use Disorder. Neurotox Res. 2017;31(1):169–86.
10. Jembrek M, Vlainic J. GABA Receptors: Pharmacological Potential and Pitfalls. Curr Pharm Des. 2015;21(34):4943–59.
11. Luscher B, Fuchs T. GABAergic control of depression-related brain states. Adv Pharmacol. 2015;73:97–144.
12. Ghose S, Winter MK, McCarson KE, Tamminga CA, Enna SJ. The GABA B receptor as a target for antidepressant drug action. Br J Pharmacol. 2011;162(1):1–17.
13. Jacobson LH, Vlachou S, Slattery DA, Li X, Cryan JF. The Gamma-Aminobutyric Acid B Receptor in Depression and Reward. Biol Psychiatry [Internet]. 2018;83(11):963–76. Available from: https://doi.org/10.1016/j.biopsych.2018.02.006
14. Duman RS, Sanacora G, Krystal JH. Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. Neuron [Internet]. 2019;102(1):75–90. Available from: https://doi.org/10.1016/j.neuron.2019.03.013
15. Shi Y, Dong J-W, Zhao J-H, Tang L-N, Zhang J-J. Herbal Insomnia Medications that Target GABAergic Systems: A Review of the Psychopharmacological Evidence. Curr Neuropharmacol. 2014;12(3):289–302.
16. Hritcu L, Ionita R, Postu PA, Gupta GK, Turkez H, Lima TC, et al. Antidepressant Flavonoids and Their Relationship with Oxidative Stress. Oxid Med Cell Longev. 2017;2017(Article ID 5762172):1–18.
17. Rahman MM, Ichiyanagi T, Komiyama T, Sato S, Konishi T. Effects of anthocyanins on psychological stress-induced oxidative stress and neurotransmitter

status. J Agric Food Chem. 2008;56(16):7545–50.
18. Steed LE, Truong VD. Anthocyanin content, antioxidant activity, and selected physical properties of flowable purple-fleshed sweetpotato purees. J Food Sci. 2008;73(5):215–21.
19. Adnyana IMO, Sudewi AR, Samatra DP, Suprapta D. Neuroprotective Effects of Purple Sweet Potato Balinese Cultivar in Wistar Rats With Ischemic Stroke. Open Access Maced J Med Sci. 2018;6(11):1959–64.
20. Prabawati RK, Ratnawati R, Rahayu M, Prakosa AG. Effect Anthocyanin of Purple Potato Gunung Kawi on Mda Levels, Expression of Caspase-3, and Spatial Memory Function on Diabetic Wistar Rats. MNJ (Malang Neurol Journal). 2019;5(1):34–41.
21. Darwatik, Ratnawati R, Rianawati S, Sarwono I. Pengaruh Antosianin Ubi Ungu terhadap TNF-α , Apoptosis dan Memori Spasial Hipokampus Tikus Model Diabetes Melitus Effect of Purple Sweet Potato Anthocyanin towards TNF-α , Apoptosis , and Spatial Memory in Hippocampus of Rat Model of Diabetes Mellitus. J Kedokt Brawijaya. 2018;30(1):12–8.
22. Ye D, Zhang L, Fan W, Zhang X, Dong E. Genipin normalizes depression-like behavior induced by prenatal stress through inhibiting DNMT1. Epigenetics. 2018;13(3):310–7.
23. Vanmierlo T, De Vry J, Nelissen E, Sierksma A, Roumans N, Steinbusch HWM, et al. Gestational stress in mouse dams negatively affects gestation and postpartum hippocampal BDNF and P11 protein levels. Mol Cell Neurosci [Internet]. 2018;88(February):292–9. Available from: https://doi.org/10.1016/j.mcn.2018.02.009
24. Fatchiyah F, Ratih D, Sari T, Safitri A, Cairns JRK. Phytochemical Compound and Nutritional Value in Black Rice from Java Island , Indonesia. Sys Rev Pharm. 2020;11(7):414–21.
25. Chen CC, Lin C, Chen MH, Chiang PY. Stability and quality of anthocyanin in purple sweet potato extracts. Foods. 2019;8(9):1–13.
26. Bind RH, Minney SM, Rosenfeld S, Hallock RM. The role of pheromone responses. J Am Assoc fro Lab Anim Sci. 2013;52(2):124–9.
27. Bertolin K, Murphy BD. Reproductive Tract Changes During the Mouse Estrous Cycle [Internet]. The Guide to Investigation of Mouse Pregnancy. Elsevier; 2014. 85–94 p. Available from: http://dx.doi.org/10.1016/B978-0-12-394445-0.00007-2
28. Frye C, Paris J, Rhodes M. Exploratory, anti-anxiety, social, and sexual behaviors of rats in behavioral estrus is attenuated with inhibition of 3α,5α-THP formation in the midbrain ventral tegmental area. Behav Brain Res. 2008;193(2):269–76.
29. Heyne GW, Plisch EH, Melberg CG, Sandgren EP, Peter JA, Lipinski RJ. A Simple and Reliable Method for Early Pregnancy Detection in Inbred Mice. J Am Assoc Lab Anim Sci [Internet]. 2015;54(4):368–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26224435%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4521569
30. Palacios-garcía I, Lara-vásquez A, Montiel JF, Díaz-véliz GF. Prenatal Stress Down-Regulates Reelin Expression by Methylation of Its Promoter and Induces Adult Behavioral Impairments in Rats Prenatal Stress Down-Regulates Reelin Expression by Methylation of Its Promoter and Induces Adult Behavioral Impairments in Ra. PlosONE. 2015;(February).
31. Notarangelo FM, Schwarcz R. Restraint Stress during Pregnancy Rapidly Raises Kynurenic Acid Levels in Mouse Placenta and Fetal Brain. Dev Neurosci. 2017;38(6):458–68.
32. Laviola G, Macrì S, Morley-Fletcher S, Adriani W. Risk-taking behavior in adolescent mice: Psychobiological determinants and early epigenetic influence. Neurosci Biobehav Rev. 2003;27(1–2):19–31.
33. Notarangelo FM, Schwarcz R. Restraint Stress during Pregnancy Rapidly Raises Kynurenic Acid Levels in Mouse Placenta and Fetal Brain. Dev Neurosci. 2017;38(6):458–68.
34. Salari A, Fatehi L, Motayagheni N, Homberg JR. Fluoxetine normalizes the effects of prenatal maternal stress on depression- and anxiety-like behaviors in mouse dams and male offspring. Behav Brain Res [Internet]. 2016; Available from: http://dx.doi.org/10.1016/j.bbr.2016.05.062
35. Iturra-Mena AM, Arriagada-Solimano M, Luttecke-Anders A, Dagnino-Subiabre A. Effects of prenatal stress on anxiety- and depressive-like behaviours are sex-specific in prepubertal rats. Vol. 30, Journal of Neuroendocrinology. 2018. 0–2 p.
36. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Vol. 85, Psychopharmacology. 1985. p. 367–70.
37. Yoshihara T, Nishimura T, Jing Z, Akther S, Fakhrul, A., A., K. M, Liang M, et al. Anxiety- and Depression-like Behavior in Mice Lacking The CD157/BST1 gene, a risk factor for Parkinson's Disease. Front Behav Neurosci. 2017;11(April):1–18.
38. Dallakyan S. Small-Molecule Library Screening by Docking with PyRx. In: John MW, editor. Methods in Molecular Biology. Switzerland; 2015. p. 243–50.
39. Alamri MAAMA. Pharmacophore and docking-based sequential virtual screening for the identification of novel Sigma 1 receptor ligands. Bioinformation. 2019;15(8):579–85.
40. Zafar T, Naik AQ, Shrivastava VK. Effect of cold stress on infanticide by female Swiss albino mice Mus musculus: A pilot study. J Anim Sci Technol. 2018;60(1):1–5.
41. Kristal MB. The biopsychology of maternal behavior in nonhuman mammals. ILAR J. 2009;50(1):51–63.
42. Maguire J, Mody I. Behavioral deficits in juveniles mediated by maternal stress hormones in mice. Neural Plast. 2016;2016.
43. Joewono HT, Sulistyono A, Haslinda Y, Aditiawarman. Maternal and perinatal outcome of women with obesity in pregnancy. Syst Rev Pharm. 2020;11(2):690–4.
44. Rakers F, Rupprecht S, Dreiling M, Bergmeier C, Witte OW, Schwab M. Transfer of maternal psychosocial stress to the fetus. Neurosci Biobehav Rev [Internet]. 2017; Available from: http://dx.doi.org/10.1016/j.neubiorev.2017.02.019
45. McGowan PO, Matthews SG. Prenatal stress, glucocorticoids, and developmental programming of the stress response. Endocrinology. 2018;159(1):69–82.
46. Austin MP, Leader LR, Reilly N. Prenatal stress, the hypothalamic-pituitary-adrenal axis, and fetal and infant neurobehaviour. Early Hum Dev. 2005;81(11):917–26.

47. Kim DR, Bale TL, Epperson CN. Prenatal Programming of Mental Illness: Current Understanding of Relationship and Mechanisms. Curr Psychiatry Rep. 2015;17(2):1–16.
48. Di Lorenzo A, Sobolev AP, Nabavi SF, Sureda A, Moghaddam AH, Khanjani S, et al. Antidepressive effects of a chemically characterized maqui berry extract (Aristotelia chilensis (molina) stuntz) in a mouse model of Post-stroke depression. Food Chem Toxicol [Internet]. 2019;129(April):434–43. Available from: https://doi.org/10.1016/j.fct.2019.04.023
49. Belovicova K, Bogi E, Csatlosova K, Dubovicky M. Animal tests for anxiety-like and depression-like behavior in rats. Interdiscip Toxicol. 2017;10(1):40–3.
50. Hasler G. Pathophysiology of depression: Do we have any solid evidence of interest to clinicians? World Psychiatry. 2010;9(3):155–61.
51. Fogaça M V, Duman RS. Cortical GABAergic Dysfunction in Stress and Depression : New Insights for Therapeutic Interventions. 2020;13(March 2019):1–20.
52. Dallakyan S, J.Olson A. Small Molecule Library Screening by Docking with PyRx. Methods Mol Biol. 2015;1263.
53. Reis GML, Duarte IDG. Baclofen, an agonist at peripheral GABA B receptors, induces antinociception via activation of TEA-sensitive potassium channels. Br J Pharmacol. 2006;149(6):733–9.
54. Möhler H. Neuropharmacology The GABA system in anxiety and depression and its therapeutic potential. Neuropharmacology [Internet]. 2012;62(1):42–53. Available from: http://dx.doi.org/10.1016/j.neuropharm.2011.08.040
55. Froestl W. Chemistry and pharmacology of GABA B receptor ligands. Vol. 58, Advances in Pharmacology. 2010. 19–62 p.
56. Veena NS, Sivaji K, Benerji G V, Babu MF, Kumari DR. Skeletal muscle relaxant property of baclofen by using rotarod on albino mice. Indian J Basic Appl Med Res. 2015;4(4):722–30.
57. Raad S, Wilkerson M, Jones KR, Orr WC. The effect of baclofen on objective and subjective sleep measures in a model of transient insomnia. Sleep Med [Internet]. 2020;72:130–4. Available from: https://doi.org/10.1016/j.sleep.2020.03.028
58. Shah SA, Yoon GH, Kim MO. Protection of the Developing Brain with Anthocyanins Against Ethanol-Induced Oxidative Stress and Neurodegeneration. Mol Neurobiol. 2015;51(3):1278–91.