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Research Article

Exposure of Histone Deacetylase-2 Inhibitor Curcumin and Its Analogues in Self-Nano Emulsifying Drug Delivery System Change Memory and Cognitive Function, Anxiety, and Social Interaction Behavior in Mouse

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ABSTRACT

Class 1 and 2 histone deacetylase inhibitors (HDACI) have been reported as novel therapeutic approaches to treat neurodegenerative disorders, depression, anxiety, and cognitive deficits. HDACI ameliorated deficits in cognition and stress-related behaviors in a wide range of neurologic and psychiatric disorders. Preclinically, behavioral bioassay can be used to predict the influence of new compounds for treatment of these illnesses. Curcumin and its new analogues PGV-0 and PGV-1 have been reported to inhibit HDAC2. However, reports regarding the effect of curcumin and its analogues on memory and cognitive function, anxiety, and social interaction behavior are as yet to be examined. Mice were divided into control and treated groups. Brain disorder was induced by oral administration of 10% ethanol in sodium-CMC for 7 days. Curcumin, PGV-0, PGV-1, and sodium butyrate (as positive control) were then given orally once a day for 21 days. The behavior tests of social interaction, open field, radial 8-arm-maze, and passive avoidance were performed on day 29. To increase dissolution and bioavailability of the compounds, they were formulated in a self-nano emulsifying drug delivery system (SNEDDS). Brains were isolated and analyzed using PCR to investigate the expression of genes related to neurobehavioral disorders hdac2, trkB, and bdnf. In different doses, curcumin, PGV-0, and PGV-1 increased social interaction capability, declined anxiety level, and improved long-term memory and cognitive function. The mechanism proposed is: HDACI curcumin and its analogues (PGV-0 and PGV-1) that keep the histone protein in acetylation state increase bdnf expression. The increased trkB expression is increasing the activation of the bdnf gene because trkB is the primary receptor of bdnf that supports the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. Thus, those mechanisms could improve long-term memory and cognitive function, increase social interaction, and reduce anxiety in ethanol-induced mice with brain disorders.

INTRODUCTION

Ethanol-induced adult brain damage resulted in a decrease in cognitive functions such as learning and memory disorders. It was caused by oxidative stress that inducted the formation of free radicals, causing cell damage and necrosis (Heaton *et al.*, 2000). The acute application of ethanol in 7 days caused hippocampus CA1 damage in mice (Adiningsih, 2013). Sodium butyrate was well known as a histone deacetylation inhibiting compound in the hippocampus that related to recovery of memory and cognitive functions (Cohen *et al.*, 2014) and treating stress and anxiety by increasing the

balance of excitatory and inhibitory neural pathways in the brain (Fukuchi *et al*., 2009; Machado-Vieira *et al*., 2012). It was reported to be able to penetrate the blood-brain barrier (BBB) in an in vivo study (Simonini et. al., 2006).

Up to the present time, the results of the studies of curcumin (Figure 1(a)) showed that curcumin reduced oxidative stress (Frautschy *et al*., 2001), played a role in brain-derived neurotrophic factor (BDNF) and ERK/P38 signaling kinase, and increased histone acetylation that could induct neurogenesis (Deng *et al*., 2010; Istyastono *et al*., 2016; Liu *et al*., 2005; Mancuso *et al*., 2011). Blocking HDAC activity during cortical development using the HDAC inhibitor suberoylanilide hydroxamic acid increased acetylation of histone that leads to increased neurogenesis (Yuniarti *et al*., 2013) and reduced cortical interneuron and astrocyte in mouse brain (Yuniarti *et al*., 2018).

Pentagamavunon-0 (PGV-0) with IUPAC named 2,5-bis-(4'-hydroxy-3'-methoxy)-benzylidene cyclopentanone was one of the modified curcumin compound structures in the middle chain, representing the modification of β-diketone into cyclopentanone as illustrated in Figure 1(b) and also Pentagamavunon-1 (PGV-1) or 2,5-bis-(4'-hydroxy-3',5'-dimethylbenzylidine)-cyclopentanone (Figure 1(c)). They were reported to have several pharmacological activities such as antiinflammation (Sardjiman, 2000) by inhibiting Cox-1 and Cox-2 (Yuniarti *et al*., 2012), anticancer (Nurulita and Meiyanto, 2000), and antioxidants that were better than curcumin (Sardjiman *et al*., 1997).

Curcumin, PGV-0, and PGV-1 were very little to dissolve in water, so their dissolubility and their biological availability were low (Hakim *et al*., 2006). Therefore, it was necessary to prepare a formulation using a proper conduction system to overcome the low dissolubility and biological availability of curcumin and PGV-0 formulations with the self-nano emulsifying drug delivery system (SNEDDS) method. In vitro tests of cancer cells showed that curcumin nanoparticulation preparation was able to more effectively penetrate the walls of cancer cells and normal cells as antiinflammation (Tsai *et al*., 2011). A preliminary study showed that orally application of curcumin nanoemulsion gave a higher concentration in blood plasma than curcumin without any nanoparticle formulation (Mancuso *et al*., 2011). Here we investigate the effect of curcumin and its analogues (PGV-0 and PGV-1) in SNEDDS and non-SNEDDS formulations on the memory and cognitive function, anxiety, and social interaction behavior after ethanol-induced brain disorders in mice and propose their mechanism based on gene expression related to neurobehavioral disorders hdac2, trkB, and bdnf.

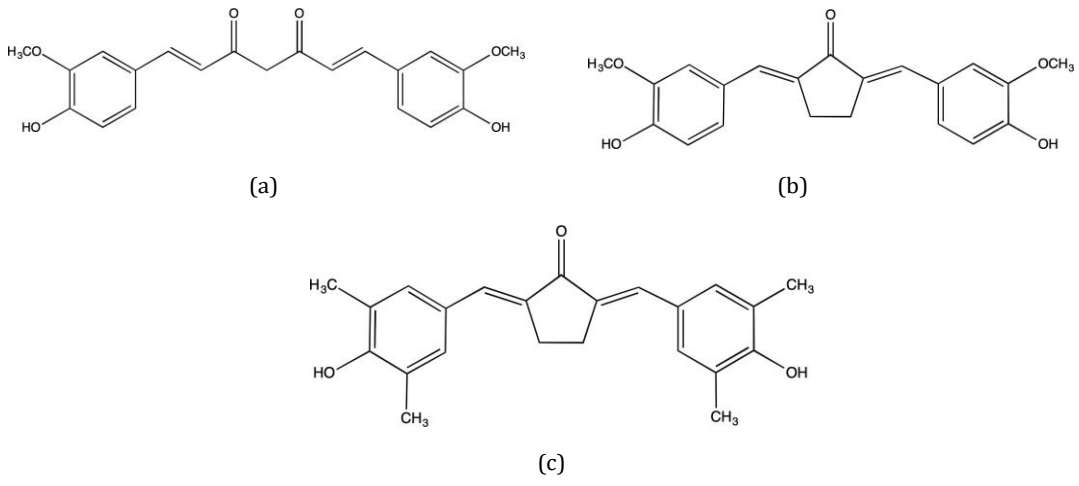

Figure 1. (a) Curcumin, (b) Pentagamavunon-0, (c) Pentagamavunon-1 structure

METHODS

Materials

Curcumin (Sigma Aldrich), PGV-0 and PGV-1 (synthesized by Prof. Sardjiman), ethanol 70% (CV. General Labora), sodium butyrate (Aldrich Chemistry), tween 20, tween 80, mygliol, CMC-sodium (Bratachem), mice fodder (Comfeed®), and aquadest (CV. General Labora). Adult and male Balb/c mice were bred and obtained from the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada (UGM).

Instruments

Glasses (IWAKI and Pyrex), injection syringe (Thermo®), injection syringe 1 mL (Terumo®), intraperitoneal and oral needle (Thermo®), mice cage (Lion Star®), handycam (Sony DCR SX65E), radial 8-arm-maze test instrument, social interaction instrument, passive avoidance instrument, and open field test instrument (Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, UGM).

Animal Treatments

All mice used in this study were handled according to the animal experimentation guidelines of Integrated Research and Testing Laboratory UGM numbers 00072/04/LPPT/VIII/2018 and 00054/04/LPPT/XII/2021. All efforts were made to minimize the number of mice used and their suffering. Mice were first adapted for a week and housed on a 12 h light/dark cycle with free access to food and water. Brain disorder was induction by exposing mice to 25 mL/kgBW ethanol 10% for 7 days. Mice were orally exposed to curcumin, PGV-0, PGV-1 5, 10, 20, and 40 mg/kgBW in SNEDDS formulation, and also sodium butyrate (as a positive control) of 1.2 g/kgBW intraperitoneally, and the SNEDDS vehicle (miglyol, tween 20, tween 80, and PEG) orally for 21 days. Subsequently, on the day 29 behavior tests were performed.

Behavior tests

Radial 8-arm-maze (Tarantino and Bucan, 2002)

The radial 8-arm-maze test was in the form of a labyrinth with 8 arms that have an end for each, and there were designs for opened-closed-door patterns in arranging fodder. No reward (N) meant that there was not any fodder (closed door), and reward (R) meant that there was fodder (opened door). Four patterns used in this test were pattern 1: NRNRNRNR; pattern 2: RRNNRRNN; pattern 3: NNRRNNRR; and pattern 4: RRNNNNRR. The mice were left in a fasting period of 24 hours before the test. Mice were put in the center of the labyrinth, given 6 minutes to explore the field and 5 minutes to scavenge for fodders at the ends of each arm. Parameters observed include frequency of failure entering filled arm and entering empty arm.

Passive avoidance test (IACUC Standard Procedure, 2018)

The instrument of the passive avoidance test consisted of two compartments, which were dark and well illuminated. Mice were put in a well-illuminated compartment, distantly facing the dark compartment, and then given 10 seconds to explore the compartment. Once mice came into the dark compartment, the door was closed and given foot-shock 0.5 mA for 2 seconds. Latency time started when the door was opened to the time when mice moved into the dark compartment with their four legs. The test was conducted twice with a 20-minute break in 4 days. The maximal latency time was 3 minutes. The parameters of the passive avoidance test included latency time 1 (long-term memory) and latency time 2 (short-term memory).

Social interaction test (Kaidanovich-Beilin *et al.*, 2011)

The instrument of the social interaction test consisted of 3 main compartments. The interaction that was scored in the test was contacting or touching a cage containing stranger mice. The session of each test was 10 minutes. There were two tests. Session I was referred to as the social affiliation test and aimed at measuring the preference of the mice to interact with the cage containing stranger mice. Session II was referred to as a social novelty test and aimed at measuring the preference of the mice to interact with the cage containing stranger 1 or stranger mice 2.

Open field test (Seo *et al.*, 2013)

The experimental instrument was square with a grid of a certain dimension at the bottom of it and illuminated by a 60-watt red lamp. Mice were first adapted to the experimental environment for a minute and then put in the center of the instrument. Subsequently, they were exploring the field for 5 minutes. The parameters observed included frequency of line crossing, center square entries, rearing, stretch attend posture, and duration of grooming, freezing, and center square duration as described in Seo *et al.* (2013).

Tissue preparation and PCR/q-RT PCR

Mice brains were dissected and collected in ice-cold phosphate buffered saline (PBS). They then inserted into the microtubes that contain

RNA stabilization solution (FavorgenTM, 1 mL solution for every 100 mg of samples). RNA isolations were performed in ± 30 mg of brain samples. Total RNA was isolated and quantified using FavorgenTM Favorprep Tissue Total RNA Purification Mini Kit 1 and Nanodrop 2000 Thermo Spectrophotometer ScientificTM (Thermofisher), respectively. cDNA was synthesized from 100 ng RNA using Reverse Transcription Kit II 100RXN (cDNA kit, SMOBIO®). All electrophoretic bands were visualized with the GelDoc Imager (BioRad), followed by densitometry analysis to see the intensity of gene expression bands with Image J software.

The expression of target genes was normalized to that of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (gapdh). The gene-specific primers (Macrogen) were as follows: hdac2: Hdac2-S, CGGTGTTTGATGGACTCTTTG; Hdac2-AS, CCTGATGCTTCTGACTTCTTG; trkB: trkB-S, GTCTGGAGGGTGCTATGCT; trkB-AS, CTACTGCTGGCGAAGTGACG; bdnf: Bdnf-S, TACCTGGATGCCGCAAACAT; Bdnf-AS, AGTTGGCCTTTGGATACCGG; gapdh: Gapdh-S, GTCGGTGTGAACGGATTTGG; Gapdh-AS, GACTCCACGACATACTCAGC.

Bdnf mRNA expression levels were determined quantitatively using real-time PCR based on the 2X Fast q-PCR Master Mix protocol (SYBR, no ROX) ExcelTaqTM (Smobio). Five cDNA templates were analyzed as recommended by the manufacturer. qRT-PCR was performed by a PCR T100TM Thermal Cycler (BioRad).

RESULTS AND DISCUSSION

Class 1 and 2 histone deacetylase inhibitors (HDACI) have been reported as novel therapeutic approaches to treat neurodegenerative disorders, depression, anxiety, and cognitive deficits. HDACI ameliorated deficit in cognition and stress-related behaviors in a wide range of neurologic and psychiatric disorders. Preclinically, behavioral bioassay can be used to predict the influence of new compounds for treatment of these illnesses. Curcumin and its new analogues PGV-0 and PGV-1 have been reported to inhibit HDAC2. Here we reported regarding their effect on memory and cognitive function, anxiety, and social interaction behavior by using neuro-behaviors radial 8-arm-maze, passive avoidance, open field, and social interaction tests.

A radial 8-arm-maze test was used to investigate the presence of a brain disorder in the form of a deficit in learning and memory or a decrease in cognitive function (Tarantino and Bucan, 2002). There were 4 patterns used to improve a cognitive map in the form of spatial relations. The results of the observation of patterns 1, 2, 3, and 4 could be seen in Figure 2 (Wulandari, 2016). Ethanol affected the cognitive and memory functions of mice, as indicated by the increase in frequency of failure as compared to the normal group in patterns 1, 2, and 4. It was clearly observed that the most effective testing compounds in affecting cognitive and memory function of ethanol-induced mice were curcumin 40, PGV-0 5, and PGV-1 10 mg/kgBW, as indicated by the decrease in the frequency of the failure in patterns 1, 2, and 4. The higher the frequency of the failure the mice made, the lower their cognitive memory function would be.

A passive avoidance test was commonly used to test various memory functions of experimental animals (usually rats and mice) and consisted of short-term and long-term working memory (Adiningsih, 2013). In the passive avoidance test, mice would be exposed to the stressor of repeated foot-shock so they would memorize and learn something from what they had experienced. The parameters observed in this test were latency time 1 and latency time 2. The latency time 1 represented long-term memory, while the latency time 2 represented short-term memory. It showed that the longer the latency, the better the memory and learning function of mice.

The results of the passive avoidance test could be seen in Figure 3. It was clearly observed that ethanol caused a decrease in memory function, as evidenced by the shorter latency time, opposite of sodium butyrate. It was proven that sodium butyrate, as a positive control of histone deacetylation inhibitor (HDACI), could improve learning capability and memory (Chuang *et al*., 2009; Fischer *et al*., 2007). The SNEDDS vehicle (consisting of miglyol, tween 20, tween 80, and PEG) did not have any significant effect from the control. Therefore, the most effective active compounds in improving memory and cognitive functions of mice were curcumin 20 and 40 and PGV-0 40 mg/kgBW. Curcumin was naturally neuroprotective because it had high antioxidant content, and PGV-0 also had high antioxidant content (Sardjiman, 1997; Sardjiman 2000), as evidenced by the capability to improve memory and cognitive functions in ethanol-induced mice's brains.

A social interaction test was conducted to find out characteristic neuropsychiatric disorders in social behavioral disorders and social adaptation processes, including

depression, autism spectrum disorders (ASD), bipolar disorders, obsessive-compulsive disorders, and schizophrenia (Kaidanovich-Beilin *et al*., 2011). The results were illustrated in Figure 4. In session 1, contact duration of mice with cage containing stranger mice 1 (Figure 4(a)) was indicative by significant difference between ethanol group and sodium butyrate, curcumin 5, 10, 20 mg/kgBW, PGV-0 5 mg/kgBW, and PGV-1 5, 10, 20 mg/kgBW based on post-hoc LSD test. It is suggested that the use of sodium butyrate for 21 days was able to alleviate social adaptation interaction disorder in ethanol-induced mice brains and prolonged social adaptation interaction duration in normal mice.

The second session results could be seen in Figure 4(b). There was a significant difference among the ethanol group and curcumin 20, PGV-1 20, 40, and PGV-1 10 mg/kgBW. Interaction with the cage containing stranger mice 2 lasted longer than that with the cage containing stranger mice 1, and it indicated the social interaction behavior tended to like the new environment more than the prior environment (in which there were stranger mice 1 that had been known before). Kaidanovich-Beilin *et al*. (2011) used control mice of wild type and GSK-α mutant on their study, and the results showed that the wild type was more likely to interact with cage-containing stranger mice 2 than with cage-containing stranger mice 1, and it was indicative of normal social behavior in social interaction with new subjects. Mice are more likely to interact with the cage containing stranger mice 1, and it indicated the presence of anxiety and fear in interacting with stranger mice 1 that has been known before.

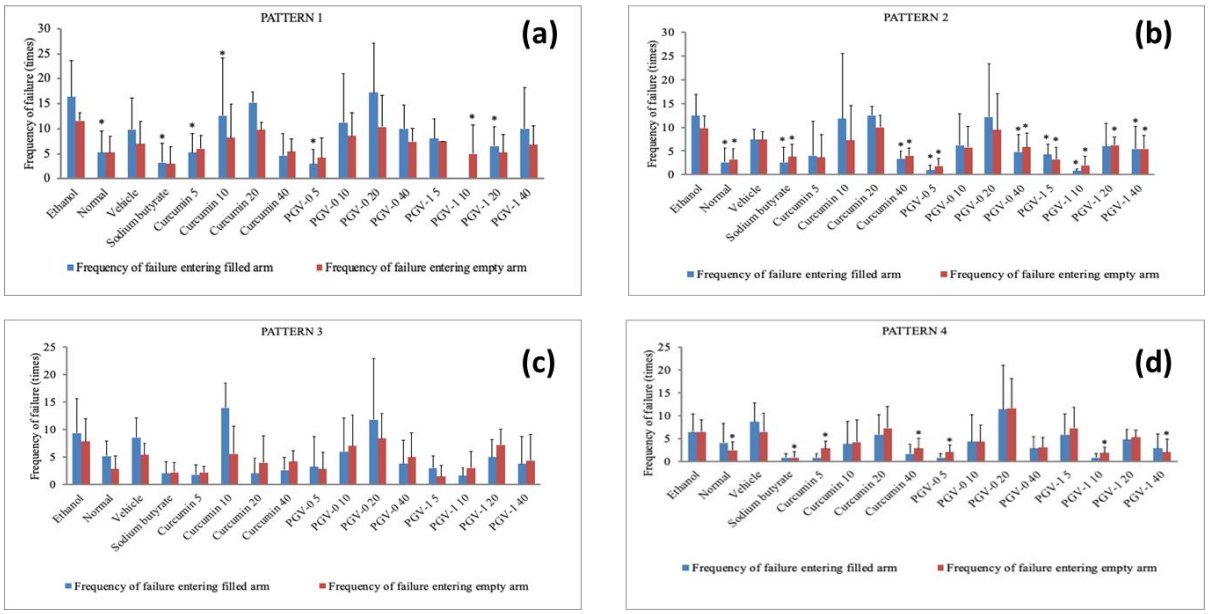

Figure 2. Histogram of frequency of failure made by mice on radial 8-arm-maze test in patterns 1 (a), 2 (b), 3 (c), and 4 (d) (X±SD) among treatment group, n=10. (*) sig. p<0.05, significant difference between ethanol group andtreatment group.

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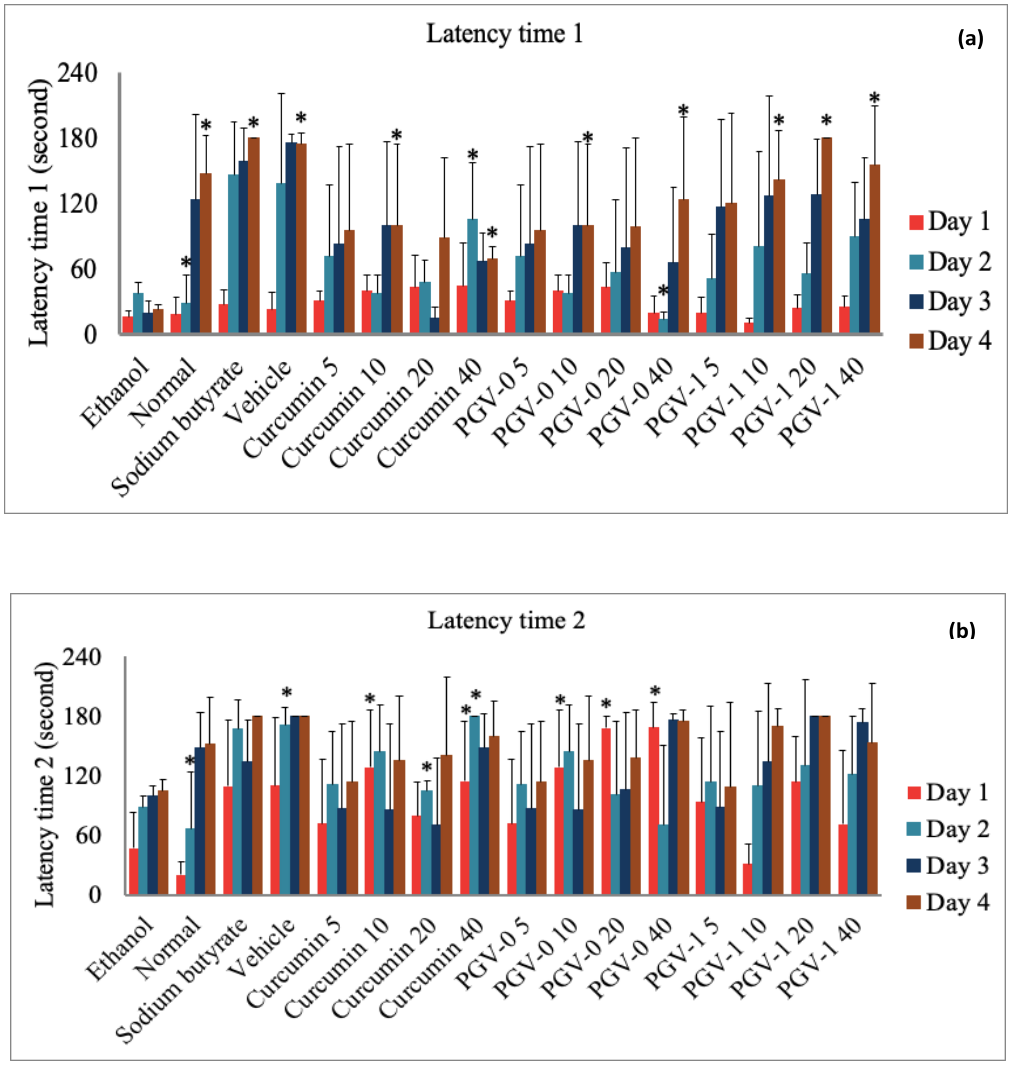

Figure 3. Histogram of latency time (X±SD) in short-term memory (latency time 1, a) and long-term memory (latency time 2, b) among the treatment groups, n=10. (*) sig. $p<0.05$, significant difference between ethanol groups and treatment group.

An open field test was used to know the phenotype brain disorders of anxiety (Bailey and Crawley, 2009; Tarantino and Bucan, 2002). The results could be seen in Figure 5. The anxiety of ethanol-induced mice was indicated by all parameters as compared to the normal group. Sodium butyrate had a significant impact on mice, as indicated by grooming in comparison between the sodium butyrate and ethanol groups. Curcumin 20 mg/kgBW-influenced anxiety, as indicated by an increase in frequency of line crossing, decreases in frequency of stretch-attend posture, and decreases in duration of grooming and freezing. PGV-0 10 mg/kgBW effected anxiety as indicated by increase in frequency of line crossing and decrease in grooming duration, while PGV-0 20 mg/kgBW influenced anxiety of mice as indicated by a decrease in frequency of stretch attend posture and grooming duration. PGV-1 40 mg/kgBW influenced anxiety by increasing the frequency of line crossing, grooming, and freezing durations.

Hippocampus was a part of the brain situated in the temporal medial lobe directly connected to learning and memory processes, mood, and anxiety disorder (Gruart, 2006; Onksen, 2011; Saladin, 2006). Kushner *et al*. 2000 and Bouayed *et al*. 2009 suggested that

the acute consumption of ethanol could cause abnormality of GABA receptor function that played an important role in anxiety. Meanwhile, Sethi *et al*. (2009) reported that curcumin in nanoparticle form could pass BBB into brain tissue with passive diffusion. This allows for curcumin to have an effect on improving memory deficits related to aging and oxidative stress through BDNF, ERK/P38 signaling kinases, and increasing histone acetylation of the hippocampus that could induct neurogenesis. The increase in neurogenesis in the hippocampus could improve the function of the hippocampus, which plays an important role in cognitive memory function and anxiety (Deng *et al*., 2010; Liu *et al*., 2005; Mancuso *et al*., 2011). Anxiety is one of the symptoms of depression. Additionally, Kim *et al*. (2008) reported that curcumin had pharmacological activity as an antidepressant through BDNF activation. BDNF is a gene regulated by the epigenetic mechanism by which histones are modified to histone acetylation, which involves enzyme activity in the transfer of acetyl histone transferase and histone deacetylase (Ellenbroek and Youn, 2016).

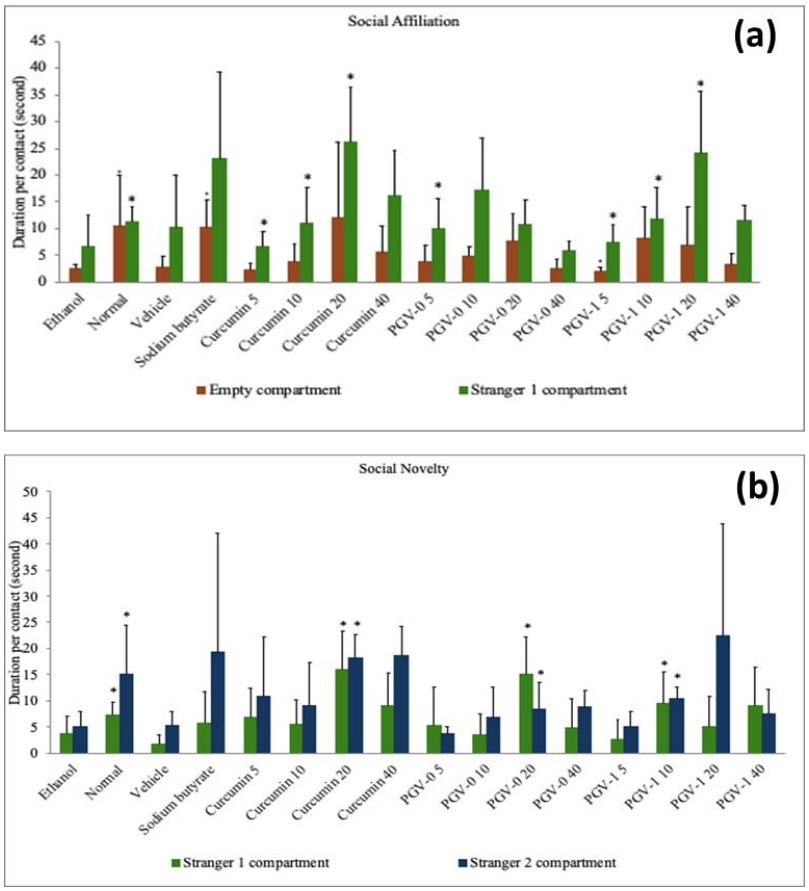

Figure 4. Histogram of session 1 (social affiliation, a) and session 2 (social novelty, b) in social interaction test (X±SD) among the treatment groups (n=10). (*) sig. p<0.05, significant difference between ethanol group and treatment group

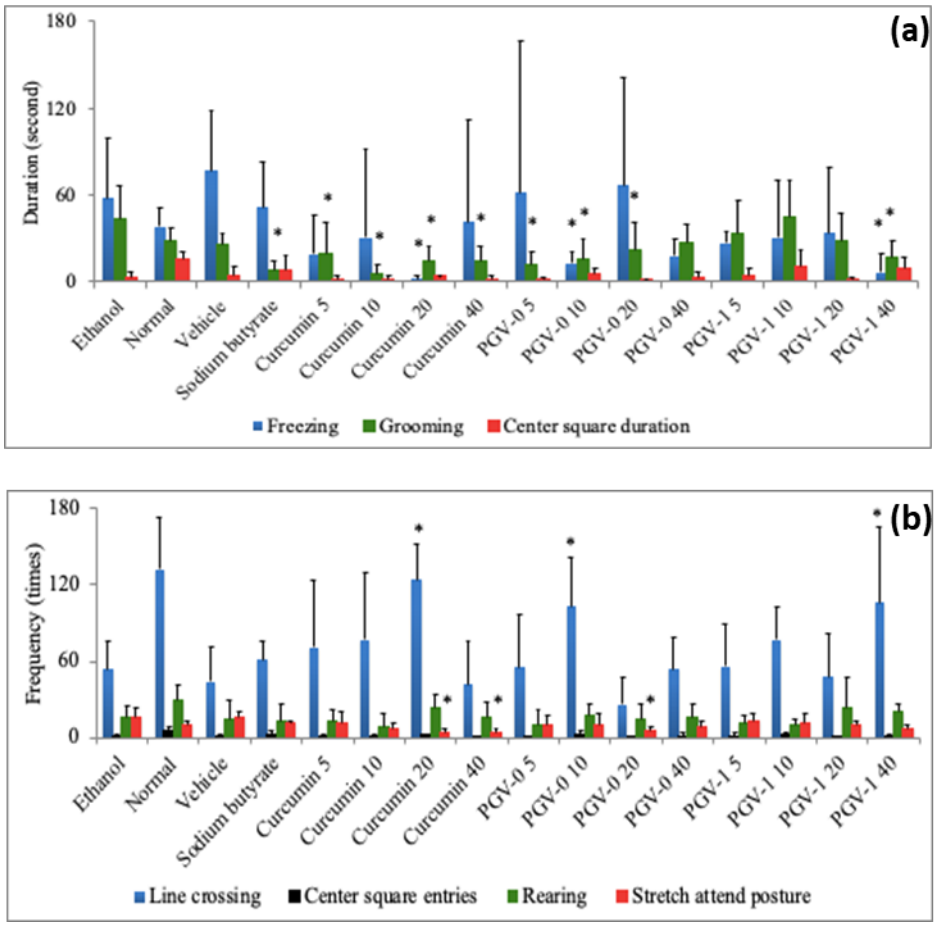

Figure 5. Histogram of parameters in open field test include freezing, grooming, center square duration (a) and line crossing, center square entries, rearing, stretch attend posture (b) (X±SD) among the treatment groups, n=10. (*) sig. $p<0.05$, significant difference between ethanol group and treatment group.

Histone deacetylase inhibitor compounds that keep the histone protein in acetylation state are reported to be able to increase BDNF expression through the activation of CREB-mediated transcription (Volmar and Wahlestedt, 2015). Curcumin increases BDNF levels and activates TrkB (Wang *et al*., 2008). This study is in line with the research from Wang *et al*. (2008) and Volmar and Wahlestedt (2015), which reported that curcumin and PGV-0 were potent ligands of HDAC2 in silico (Istyastono *et al*., 2016) and inhibit HDAC2 in vitro (Yuniarti *et al*., 2017). Moreover, this study shows that in different doses, curcumin, PGV-0, and PGV-1 increased social interaction, reduced depression in an open field test, and increased long-term memory and cognitive function in ethanol-induced mouse brains (Table 1). These phenotypes were followed by suppression of hdac2 (Figure 6, Table 2) and increased trkB (Figure 7, Table 2) gene expressions (Azizah, 2019) and bdnf upregulation in vivo (Figure 8). TrkB is a gene associated with learning and memory and anxiety. Increased TrkB expression is suspected through activation of the BDNF gene because it is known that TrkB is the primary receptor of BDNF (Kozisek *et al*., 2008). The results of our research on curcumin and its analogues PGV-0 were in line with that study, and also Kim *et al*. (2008) reported that the pharmacological activity of curcumin as an antidepressant increased BDNF expression, thus increasing the expression of TrkB. Overall,

here we propose a mechanism that HDACI curcumin and its analogues (PGV-0 and PGV-1) that keep the histone protein in acetylation state (Yuniarti *et al*., 2013) increase bdnf expression. The increased trkB expression is increasing the activation of the bdnf gene due to trkB being the primary receptor of bdnf that supports the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. Thus, those mechanisms could improve long-term memory and cognitive function, increase social interaction capability, and reduce anxiety (Figure 9; Kim *et al*., 2017 with modification).

PGV-0 and PGV-1 were curcumin analog compounds with higher lipophilicity (Sardjiman, 2000) that enabled them to pass BBB. To improve their solubility and bioavailability in biological fluids, here in this study we formulate curcumin and its analogues into nanoemulsions that have SNEDDS mechanisms. One of the pharmacological activities of curcumin, PGV-0, and PGV-1 was antioxidant (Sardjiman 1997, Sardjiman 2000). Generally, the antioxidant played a role in the neurogenesis process of the hippocampus through balancing regulation of mitosis activity, cell cycle arrest, differentiation, and cell apoptosis (Casadesus *et al*., 2004). It was possible that the pharmacological activity of curcumin, PGV-0, and PGV-1 as an antioxidant could influence learning and memory through the formation of new neurons in the hippocampus. Sodium butyrate was known as an HDAC inhibitor that could improve learning and memory (Govindarajan *et al*., 2011). Ethanol-induced non-permanent and reversible cortex abnormality that plays a role in histone acetylation through the HDAC enzyme and influences chromatin structure and gene-specificity that contributes to homeostasis. Peterson and Laniel (2004) also suggested that histone modification was dynamic and reversible in nature.

The behavioral test method (Tarantino and Bucan, 2002) used in this study had several weaknesses, such as the lack of a calculation of the duration of mice in freezing conditions in the center of the instrument. The time mice took in the center of the instrument could be used to predict their thinking capability.

Table 1. Summary of doses affecting neuro-behavior tests.

Compound	Memory and cognitive function		Social interaction	
	Radial 8-arm-maze	Passive avoidance	Social interaction	Open field
Curcumin	40 mg/kgBW	20, 40 mg/kgBW	5, 10, 20 mg/kgBW	20 mg/kgBW
PGV-0	5 mg/kgBW	40 mg/kgBW	5, 20, 40 mg/kgBW	10, 20 mg/kgBW
PGV-1	10 mg/kgBW	-	5, 10, 20 mg/kgBW	40 mg/kgBW

Different doses of curcumin, PGV-0, and PGV-1: (1) increased social interaction capability, (2) reduced depression in open field test, and (3) increased long term memory and cognitive function in passive avoidance and radial 8-arm-maze test.

Table 2. Quantification of *hdac2* and *trkB* expression level relative to normal group (n=5).

Group	*hdac2*		*trkB*	
	hdac2 expression level (Mean±SEM)	Decreased of *hdac2* expression level (%)	*trkB* expression level (Mean±SEM)	Increased of *trkB* expression level (%)
Normal	1.114±0.106	-	0.908±0.042	-
Ethanol	0.734±0.007*	-38±0.112	0.867±0.031	-4.1±0.047
Butyrate	0.327±0.018*	-78.7±0.018	1.393±0.044**	48.5±0.044
Curc20	0.619±0.026*	-49.5±0.026	1.002±0.034**	9.4±0.034
Curc40	0.673±0.012*	-44.1±0.012	1.042±0.021**	13.4±0.021
PGV-0 20	0.592±0.036*	-52.2±0.063	1.083±0.028**	17.5±0.059
PGV-0 40	0.542±0.028*	-57.2±0.075	1.231±0.043**	32.3±0.086

(*) sig. p<0.1, significant difference between normal group and treatment group
(**) sig. p<0.1, significant difference between ethanol group and treatment group
(-) and (+) values indicate decreased and increased gene expression, respectively.

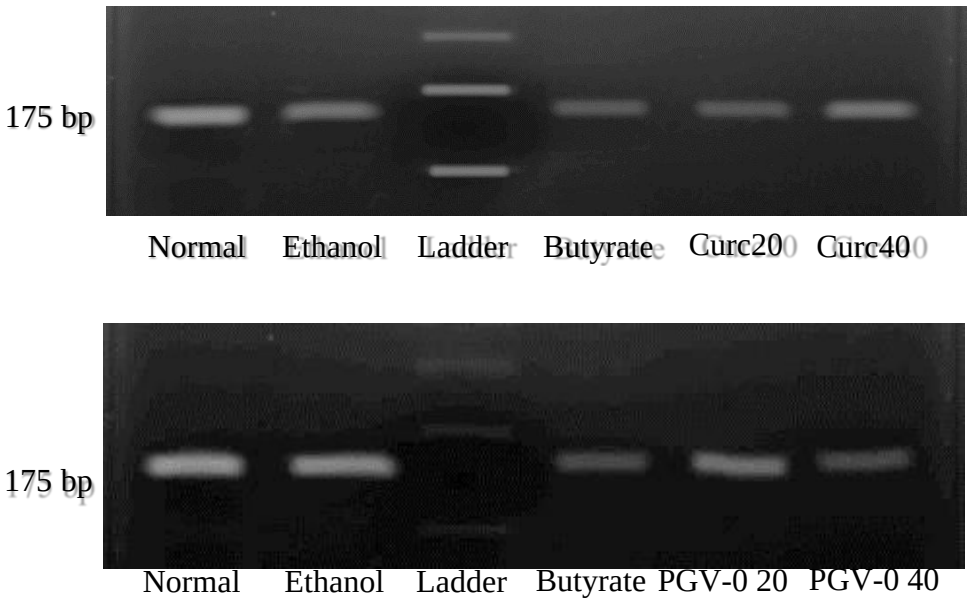

Figure 6. Curcumin and its analog PGV-0 suppressed *hdac2* gene expressions. The *hdac2* electrophoretic band appeared at 175 bp (n=5).

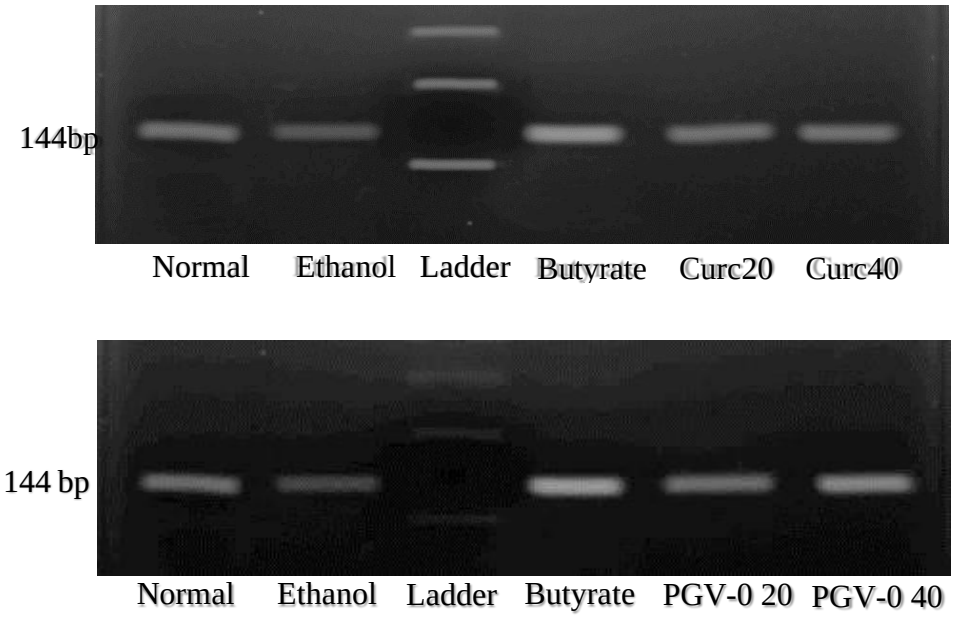

Figure 7. Curcumin and its analog PGV-0 increased *trkB* gene expressions. The *trkB* gene electrophoretic band appeared at 144 bp (n=5).

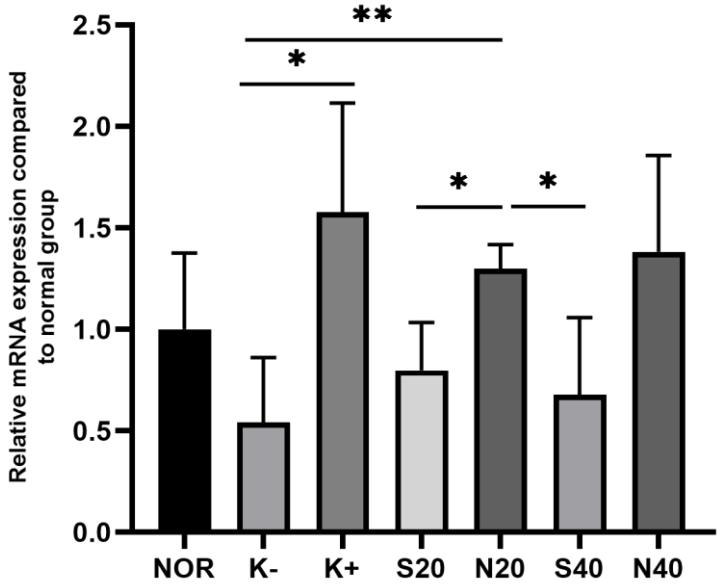

Figure 8. qRT-PCR of *bdnf* gene expressions. Results were expressed as mean ± SEM (n=5). The results were analyzed by ANOVA/Kruskall-Wallis and Independent sample t-test/Mann-Whitney. Significant results were marked with the notation *p<0.05; **p<0.01. (NOR = normal group; K- = negative control (10% ethanol v/v p.o); K+ = positive control (sodium butyrate 1.2 g/kgBW i.p); S20 = PGV-0 suspension 20 mg/kgBW p.o; N20 = SNEDDS PGV-0 20 mg/kgBW p.o; S40 = PGV-0 suspension 40 mg/kgBW p.o; N40 = SNEDDS PGV-0 40 mg/kgBW p.o).

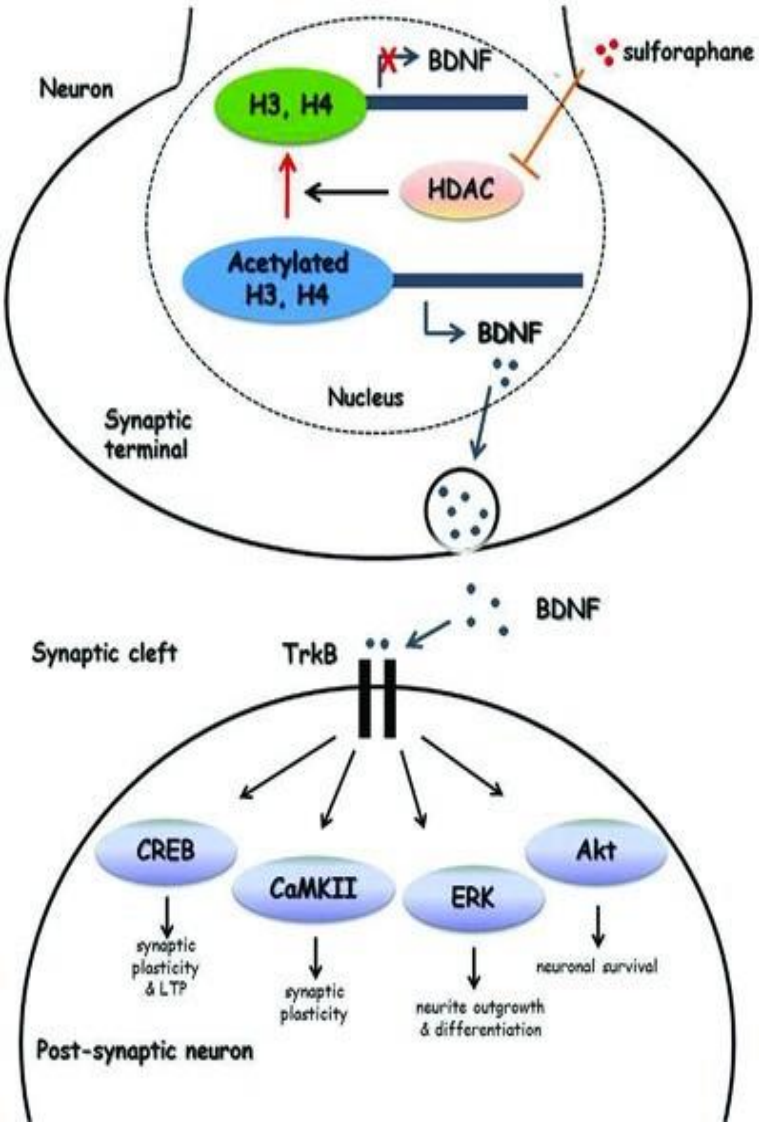

Figure 9. HDACI curcumin and its analogues (PGV-0 and PGV-1) that keep the histone protein in acetylation state increase *bdnf* expression. The increased *trkB* expression is increased the activation of the *bdnf* gene because trkB is primary receptor of bdnf that supports the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. Thus, those mechanisms could increase social interaction capability, decline anxiety level, and improve long-term memory and cognitive function in ethanol-induced mice brain disorder.

It could be correlated to the learning process and cognitive aspects. Also, in the radial 8-arm-maze test, Kaidanovich-Beilin *et al*. (2011) had several weaknesses, such as the lack of the calculation of the duration of the mice in the center of the instrument. The time mice took in the center of the instrument could be used to predict their anxiety and doubt in interaction.

Additionally, the Additionally, the results of the behavioral test could be influenced by controlled and uncontrolled variables, such as noise and light and the metabolism condition of each animal, respectively. It was necessary to conduct histological tests to observe improvement of the hippocampus after the exposure to curcumin, PGV-0, and PGV-1.

Examination of post-mortem samples of Alzheimer's disease patients shows that the abundance of HDAC2 increases even in the

early stages of Alzheimer's disease. The abundance of HDAC2 is a cause of decreased cognitive function in Alzheimer's disease. Curcumin and its analogues can inhibit HDAC2 in silico and in vitro selectively. In vivo, they alleviated social adaptation interaction disorders, declined the anxiety levels in the open field test, and improved memory and cognitive function in ethanol-induced mice brains after tested on radial 8-arm-maze and passive avoidance. These phenotypes were followed by suppression of hdac2 and increased trkB gene expressions by upregulating bdnf, a neurotrophin that supports the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. Taken together, these findings are providing insights into drug discovery to the field of neuropharmacology for potential treatments in cognitive and memory disorders on the target of HDAC2, such as Parkinson's disease, stroke, anxiety, schizophrenia, and alcohol-drug abuse, including Alzheimer's disease. Fifteen heterocyclic curcumin analogues that are modified on the keto-enol group, especially with the pyrazole group, enhance their permeability into the brain, thus exhibiting promise efficacy in preclinical studies and are suitable for anti-Alzheimer's candidates (Liu *et al*., 2008; Anas *et al*., 2022; Annisa *et al*., 2023).

CONCLUSIONS

In different doses, curcumin, PGV-0, and PGV-1 increased social interaction capability, declined anxiety level, and improved long-term memory and cognitive function. These phenotypes were followed by suppression of hdac2 and up-regulation of bdnf and trkB. The mechanism proposed is: HDACI curcumin and its analogues (PGV-0 and PGV-1) that keep the histone protein in acetylation state increase bdnf expression. The increased trkB expression is increasing the activation of the bdnf gene because trkB is the primary receptor of bdnf that supports the survival of existing neurons and encourages the growth and differentiation of new neurons and synapses. Thus, those mechanisms could improve long-term memory and cognitive function, increase social interaction, and reduce anxiety in ethanol-induced mice with brain disorders.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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