INTISARI

Latar Belakang

Stres kronik merupakan faktor yang dapat menyebabkan penurunan fungsi memori, akibat terjadinya kerusakan pada hippocampus, suatu struktur di otak yang berperan penting dalam memori. *Centella asiatica* merupakan tanaman herbal yang berperan dalam peningkatan memori. *Brain-derined neurotrophic factor* (BDNF) mempunyai peran penting dalam proses pembentukan memori.

Tujuan Penelitian

Mengkaji efek neurotrofik dan neuroprotektif ekstrak etanol daun *Centella asiatica* terhadap memori spasial, kadar BDNF serum dan hippocampus serta kadar *Nitric Oxide* pada tikus pascastres listrik kronik.

Metode Penelitian

Dua puluh ekor tikus putih, jantan, umur 2 bulan, galur *Sprague Dawley*, dibagi menjadi 4 kelompok secara random, masing-masing 5 ekor tikus per kelompok, yaitu kelompok kontrol/aquades dan kelompok perlakuan yang mendapatkan *Centella asiatica* dengan tiga dosis (mg/kgBB) berbeda yaitu 150 (CeA150), 300 (CeA300) dan 600 (CeA600) per oral selama 28 hari berturut-turut. Tampilan memori tikus dinilai menggunakan *escape latency test* dan *probe test Morris Water Maze* sebelum dan setelah pemberian perlakuan sesuai kelompoknya dan stres listrik kronik selama 28 hari. Setiap tikus menjalani uji memori selama 7 hari sebelum dan setelah stres. Pemeriksaan kadar BDNF serum dari ekor dilakukan 4 kali yaitu 1). sebelum dan 2). setelah *Morris Water Maze* sebelum stres, 3). setelah stres dan 4). setelah *Morris Water Maze* pascastres. Pada hari terakhir uji memori, dilakukan pengambilan sampel darah dari vena retroorbital untuk pemeriksaan kadar BDNF serum dan *nitric oxide,* menggunakan metode ELISA. Tikus kemudian didekapitasi dan dilakukan isolasi hippocampus dari hemispherium cerebri dexter. Pemeriksaan kadar BDNF hippocampus dilakukan dengan metode ELISA. Data dianalisis dengan *General Linear Model Repeated Measurement, one way* ANOVA, *post hoc test* dan uji korelasi Pearson

Hasil Penelitian

Pengamatan tampilan memori spasial pascaperlakuan pada setiap kelompok tikus selama 6 hari berturut-turut pada uji *escape latency test Morris Water Maze* berdasarkan: 1). Rerata waktu (detik) hasilnya menunjukkan ada perbedaan bermakna rerata waktu *escape latency test* pascaperlakuan pada hari ke-2, hari ke-5 dan hari ke-6 (*p<0,05 GLM-RM vs kelompok kontrol stres); 2). Rerata jarak tempuh hasilnya menunjukkan ada perbedaan bermakna rerata jarak tempuh *escape latency test* pascaperlakuan pada hari ke-1, 2, 5 and 6 (*p<0,05 GLM-RM vs kelompok kontrol stres); 3). Rerata kecepatan pascaperlakuan hasilnya menunjukkan ada perbedaan bermakna rerata kecepatan *escape latency test* pascaperlakuan pada hari ke-2, 5 and 6 (*p<0,05 GLM-RM vs kelompok kontrol stres). Hasil pengamatan tampilan memori *probe test Morris Water Maze* pada kelompok kontrol stres, CeA150, CeA300 dan CeA600 berdasarkan: 1). rerata persentase waktu (%) yang

digunakan tikus untuk tetap berada pada kuadran target adalah: 53,33±2,43*; 55,77±4,16*; 63,33±4,07 dan 75,96±2,17 ($p<0,05$ *one way* ANOVA; *$p<0,05$ *post hoc* Bonferroni vs CeA600), 2). rerata persentase jarak tempuh (%) yang digunakan tikus untuk tetap berada pada kuadran target adalah: 47,48±3,21; 50,06±3,79; 50,92±4,16 dan 66,75±1,11* ($p<0,05$ *one way* ANOVA, *$p<0,05$ *post hoc* Tamhane, vs kelompok kontrol stres). Hasil pemeriksaan konsentrasi BDNF serum dari ekor secara serial menunjukkan tidak ada perbedaan bermakna antar kelompok pada sesi 1 dan sesi 2, yaitu sebelum pemberian stres dan perlakuan; namun ada perbedaan bermakna konsentrasi BDNF serum pada pengambilan darah sesi ke-3 dan ke-4. Rerata konsentrasi (pg/mL) BDNF serum pada sesi 3 dan sesi 4 pada kelompok kontrol stres, CeA150, CeA300 dan CeA600 berturut-turut adalah 1880$\pm$206 & 1924$\pm$239; 2284$\pm$126* & 2010$\pm$221; 2296$\pm$83* & 1864$\pm$105; dan 2713$\pm$70* & 2987$\pm$262* (*$p<0,05$ GLM-RM pada sesi 3 dan sesi 4 vs kelompok kontrol stres). Rerata konsentrasi (pg/mL) BDNF serum retroorbital pada kelompok kontrol stres, CeA150, CeA300 dan CeA600 berturut-turut adalah 1521$\pm$89,59*, 1490$\pm$202,90*, 1737$\pm$149,45, dan 2162$\pm$202 ($p<0,05$ *one way* ANOVA; *$p<0,05$ *post hoc* LSD vs CeA600). Rerata konsentrasi (pg/mL) BDNF hippocampus pada kelompok kontrol stres, CeA150, CeA300 dan CeA600 berturut-turut adalah 359,28$\pm$36,18, 432,34$\pm$24,65, 437,13$\pm$21,76, and 497,01$\pm$27,44* ($p<0,05$, *one way* ANOVA; *$p<0,05$ *post hoc* Bonferroni, vs kelompok kontrol stres). Rerata konsentrasi (µmol/L) *Nitric Oxide* serum pada kelompok kontrol stres, CeA150, CeA300 dan CeA600 berturut-turut adalah 5,938$\pm$0,519, 3,979$\pm$0,346*, 3,056$\pm$0,327*, dan 2,818$\pm$0,210* ($p<0,05$ *one way* ANOVA; *$p<0,05$ *post hoc* Bonferroni vs kelompok kontrol stres). Kelompok kontrol stres secara bermakna memiliki konsentrasi *nitric oxide* yang tertinggi dibandingkan kelompok perlakuan yang lain ($p<0,05$). Hasil analisis korelasi Pearson didapatkan ada korelasi positif antara 1). kadar BDNF serum sesi 3 dengan retensi memori ($r=0,466$, $p=0,038$), 2). kadar BDNF serum sesi 4 dengan retensi memori ($r=0,504$, $p=0,023$, 3). BDNF serum retroorbital dengan retensi memori ($r=0,556$, $p=0,011$), dan 4). kadar BDNF serum retroorbital dengan kadar BDNF hippocampus ($r=0,813$, $p=0,000$). Ada korelasi negatif antara 1). kadar NO serum dengan retensi memori berdasarkan parameter persentase waktu ($r=-0,699$; $p=0,001$) dan 2). kadar NO serum dengan kadar BDNF sesi 3 ($r=-0,478$; $p=0,033$).

Kesimpulan

Ekstrak ethanol daun *Centella asiatica* meningkatkan memori spasial tikus putih pascastes kronis melalui peningkatan kadar BDNF dan penurunan kadar *Nitric Oxide*

Kata kunci: *Centella asiatica,* memor*i*, *brain-derived neurotrophic factor, nitric oxide*

ABSTRACT

Background

Chronic stress causes memory impairment due to damage of hippocampus, a brain region plays important role in learning and memory. *Centella asiatica* is herbaceous plant that has medicinal value to improve learning and memory. Brain-derived neurotrophic factor (BDNF) has a significant role in memory formation process, while oxidative stress causes memory impairment.

Objective

This study aimed to investigate the effects of ethanol extracts of *Centella asiatica* leaf on memory spatial performance, serum and hippocampal BDNF, as well as serum Nitric Oxide (NO) concentration of rats following chronic electrical stress.

Materials and Methods

Twenty male (2 months) rats (*Sprague Dawley*) were divided randomly into four groups, each 5 per group, i.e.: control/aquadest group and groups treated with three different doses (mg/kg) of *Centella asiatica*: 150 (CeA150), 300 (CeA300) and 600 (CeA600), per oral, for 28 consecutive days. Memory performance was tested using escape latency test and probe test in Morris Water Maze before and after oral administration of ethanol extracts of *Centella asiatica* leaf followed by electrical stress for 28 days. Each rat underwent memory exercise for seven days before and after electrical stress and oral administration of ethanol extracts of *Centella asiatica* for twenty-eight days. Blood sampling was taken serially from rats' tail for four times: 1). before memory exercise, 2). after memory exercise (before stress), 3). after chronic stress, and 4). after memory exercise (following chronic stress). At the last day of memory exercise, blood sample was taken from retroorbital veins of the rat. Concentration of serum BDNF retroorbital and nitric oxide was assessed using ELISA method. The rats were subsequently sacrificed and hippocampus was isolated from right cerebral hemisphere. BDNF concentration from hippocampal tissue lysate was measured using ELISA method. Data were analyzed using General linear model repeated measurement, one way ANOVA, post hoc test and Pearson correlation

Results

The results of memory performance of escape latency test in Morris Water Maze after stress, from first day until sixth day based on: 1). mean time spent (seconds) showed significant difference vs control group at day-2, 5 and 6 (*p<0,05 GLM-RM vs control group); 2). mean path length (meter) of escape latency test showed significant difference vs control group at day-1, 2, 5 and 6 (*p<0,05 GLM-RM vs control group); 3). Mean velocity (meter/second) of escape latency test in water maze performance after stress showed significant difference vs control group at day-2, 5 and 6 (*p<0,05 GLM-RM vs control group). The memory performance of probe test in Morris Water Maze for control group, CeA150, CeA300, and CeA600 respectively based on: 1). mean time percentage (%) were 53,33±2,43*;

55,77±4,16*; 63,33±4,07 and 75,96±2,17 ($p<0,05$ one way ANOVA; *$p<0,05$ post hoc Bonferroni vs CeA600); 2). mean path length percentage (%) were 47,48±3,21; 50,06±3,79; 50,92±4,16 and 66,75±1,11* ($p<0,05$ one way ANOVA, *$p<0,05$ post hoc Tamhane, vs control group). Data BDNF serial from rat's tail showed that there was no significant difference in serum BDNF concentration between groups in first and second serum sampling, which was prior to chronic stress and administration of different treatments. However, there was significant difference in third and fourth serum sampling between groups. Mean concentration of serum BDNF (pg/mL) in third and fourth sampling for control group, CA150, CA300, and CA600, respectively were 1880±206 & 1924±239; 2284±126* & 2010±221; 2296±83* & 1864±105; 2713±70* & 2987±262* (*$p<0,05$, GLM-RM vs control group). Mean concentration of retroorbital serum BDNF (pg/mL) for control group, CeA150, CeA300, and CeA600 were 1521±89,59*, 1490±202,90*, 1737±149,45, and 2162±202, respectively ($p<0,05$ one way ANOVA; *$p<0,05$ post hoc LSD vs CeA600). Mean concentration of hippocampal BDNF (pg/mg) for control, CeA150, CeA300, and CeA600 groups were 359,28±36,18, 432,34±24,65, 437,13±21,76, and 497,01±27,44*, respectively ($p<0,05$, one way ANOVA; *$p<0,05$ post hoc Bonferroni vs control group). Mean concentration of serum NO (µmol/L) for control group, CeA150, CeA300, and CeA600 were 5,938±0,519, 3,979±0,346*, 3,056±0,327*, dan 2,818±0,210* ($p<0,05$ one way ANOVA; *$p<0,05$ post hoc Bonferroni vs control group). Control group had higher serum NO concentration compared to all treatments groups. Pearson correlation test revealed that there were significant positive correlation between: 1). concentration of serum BDNF third session and memory ($r=0,466$, $p=0,038$), 2). concentration of serum BDNF fourth session and memory ($r=0,504$, $p=0,023$), 3). Concentration of serum BDNF retroorbital and memory ($r=556$, $p=0,011$), and 4). concentration of serum BDNF retroorbital and hippocampal BDNF ($r=0,813$, $p=0,000$).; and there were significant negative correlation between concentration of nitric oxide and memory ($r=-0,669$, $p=0,001$) and between concentration of nitric oxide and serum BDNF third session ($r=-0,478$, $p=0,033$).

Conclusion:

Ethanol extracts of *Centella asiatica* leaf increases memory performance of rat after chronic stress due to elevation of BDNF concentration and reduction of Nitric Oxide production.

Keywords: *Centella asiatica,* learning and memory, BDNF, nitric oxide