ORIGINAL ARTICLE

Comparison of the Effects of Wet Cupping Therapy and Exercise on Brain-derived Neurotrophic Factor Expression in Stroke-animal Model

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ABSTRACT

Introduction: Cupping therapy has been widely utilized as a complementary therapy, including in the treatment of stroke. This study aims to compare the effects of wet cupping therapy and exercise on brain-derived neurotrophic factor (BDNF) expression in animal models of stroke. **Materials and methods:** This study employed a post-test-only control group design. Twenty-four 3-month-old male Wistar rats were divided into four groups: sham group (n=6), stroke group (n=6), stroke+cupping group (n=6), and stroke+exercise group (n=6). Stroke animal models were induced by unilateral common carotid artery occlusion (UCCAO). Wet cupping therapy was administered twice a week for three weeks to the stroke+cupping group. In contrast, the stroke+exercise group participated in swimming activities scheduled for 20 minutes per day, three times a week over the same three-week period. **Results:** The mean of nerve cells expressing BDNF in the sham group was 41.33±22.17; the stroke group was 17.66±11.03; the stroke+cupping group was 24.50±26.82; and the stroke+exercise group was 60.16±30.94. Analysis of multiple comparisons among groups proved that there were no significant differences in BDNF expression between the stroke and sham group (p=0.102) and between the stroke+cupping and stroke group (p=0.626). Meanwhile, the BDNF expression in the stroke+exercise group was significantly higher than in the stroke group (p=0.06). Apart from that, the BDNF expression in the stroke+exercise group was significantly higher than stroke+ cupping group (p=0.018). **Conclusion:** In the stroke animal model, exercise demonstrated greater efficacy in upregulating the expression of BDNF compared to wet cupping therapy.
Malaysian Journal of Medicine and Health Sciences (2024) 20(4): 124-129. doi:10.47836/mjmhs20.4.16

Keywords: Wet cupping therapy, Exercise, Stroke, BDNF, Animal model

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INTRODUCTION

Stroke turns out to be the second leading cause of death and disability worldwide. The prevalence of stroke globally reached 80.1 million, 87% of which ischemic stroke [1]. In Indonesia, the prevalence of stroke amounted to 10.9 per mile [2]. Moreover, post-stroke movement disorders have caused limitations in mobility, day-to-day activities, social participation, and return to work [3]. On the other hand, several factors attributed to the severity of disability include age, neurological disorder, cognitive impairment, depression, and social support [4].

Thus far, stroke infarction therapy has predominantly focused on targeting the pathological processes, often involving the administration of N-Methyl-D-aspartic acid receptor (NMDAR) antagonists. However, the outcomes of such approaches have frequently fallen short of expectations. A new therapeutic strategy has emerged, which emphasizes neuroprotection, repair, and regeneration of nerve cells. Central to this strategy is the neurotrophin known as brain-derived neurotrophic factor (BDNF), renowned for its neuroprotective effects. BDNF plays a pivotal role in various neuronal processes, including synapse maturation, synapse plasticity, neurite growth, and arborization. Its neuroprotective function primarily operates through two distinct signaling pathways, both activated by the tyrosine kinase receptor

Trk B. These pathways are the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase/extracellular-signal regulated kinase (MAPK/ERK) pathway. These pathways exert influence over critical cellular processes such as the cell cycle, division, and survival by regulating specific transcription factors, ultimately serving to prevent neuronal apoptosis and promote overall neuroprotection and repair in the context of stroke [5].

However, it's noteworthy that the concentration of BDNF tends to decrease in stroke patients [6]. This decline underscores the importance of interventions such as exercise, which have been demonstrated to boost BDNF levels and potentially counteract the detrimental effects associated with reduced BDNF concentrations in stroke patients. Hereby, exercise becomes the most effective method of minimizing motor impairment in stroke patients [3]. Notably, post-stroke exercise regimens have been found to specifically contribute to increased BDNF concentration in the cortex [7], further highlighting the therapeutic potential of exercise in enhancing neurological recovery and functional outcomes following stroke.

Currently, complementary therapy has been widely implemented, including for stroke, and wet cupping therapy is one of which. Wet cupping therapy is a method of therapy applying negative pressure and punctures to the skin [8]. Research revealed that wet cupping therapy could improve the motor skill of stroke patients [9], yet the mechanism remains unclear. Commonly, it exposes negative pressure on the skin, followed by punctures [8]. Wet cupping therapy can lead to complex biological responses, such as biochemical and circulatory effects. Consequently, the resulting inflammatory stimulus triggers an endogenous repair mechanism or stimulates neurotrophic [10].

Wet cupping therapy is a complementary treatment that is widely used by Indonesian people. Research conducted by Shao (2023) shows that wet cupping therapy combined with rehabilitation is effective in improving the function of stroke sufferers, but it is not yet known whether the increase in function is due to wet cupping therapy or the effects of rehabilitation. Exercise stands as a cornerstone in stroke rehabilitation therapy [11], with wet cupping therapy also demonstrating enhancements in upper extremity function [12]. While exercise is known to increase BDNF expression, the impact of wet cupping therapy on BDNF remains unclear. Acupuncture, a therapeutic method similar to wet cupping therapy, involves pricking the skin as a stressor, increasing BDNF expression [13, 14]. Building upon this background, the current study posits that wet cupping therapy is capable of increasing BDNF expression. However, it's essential to note that while exercise therapy effectively improves function post-rehabilitation, some stroke patients encounter challenges in engaging in exercise due to limitations stemming from post-stroke disabilities.

The hypothesis of this study is wet cupping therapy surpasses exercise in enhancing BDNF expression within the subventricular area. This anticipated elevation in BDNF expression is anticipated to stimulate neural plasticity, consequently leading to an augmentation in motor function among stroke patients.

MATERIALS AND METHODS

Study design

The present study employed a posttest-only control group design. In addition, the study protocol has been approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Ethics #2.KE.073.06.2021). We used Federer formula for the sample size calculation: (n-1) (t-1) > 15, (n= number of samples; t= number of groups). By the formula: (n-1) (4-1) > 15; n-1 > 5 = 6 rats per group. The inclusion criteria were male Wistar strain rats, aged 3 months, body weight 300±50 grams, healthy, and the exclusion criteria were sick or dead rats. Twenty-four rats were categorised into four groups randomly: sham group (n=6), stroke group (n=6), stroke+cupping (n=6), and stroke+exercise (n=6). Fig. 1 showed the time line of study design.

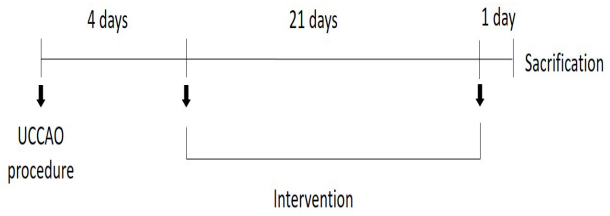

Fig. 1: The timeline of study design.

Experiment on Animal

The experimental procedure commenced with the housing of rats in quarantine within cages, accommodating four to five rats per cage, for a duration of seven days under a controlled environment featuring 12-hour light-dark cycles (with lights off at 7:30 pm) and maintained at a constant temperature of 24°C. Throughout this period, access to food (pellet HI-PRO-VITE 594, Charoen Pokphand Indonesia) and tap water was provided ad libitum to ensure optimal health and well-being of the animals.

Prior to treatment initiation, unilateral common carotid artery occlusion (UCCAO) was performed. This stroke model was selected based on its close resemblance to human ischemic stroke [15], characterized by the absence of craniotomy, ease of manipulation, precise control over the ischemic duration, and the presence of a significant ischemic penumbra [16]. Anesthesia induction was achieved using ketamine (Bernofarm Ltd, Indonesia)

at a dosage of 80 mg/kg body weight and xylazine (Interchemie werken "De Adelaar" BV, Netherland) at a dosage of 10 mg/kg body weight administered intraperitoneally, with subsequent increments of 40 mg/kgBW every 40 minutes to maintain anesthesia depth, evidenced by the absence of movement in response to a painful stimulus. Throughout the procedure, continuous monitoring of vital signs including breathing, heart rate, and urine output was conducted to ensure physiological stability. Rats were positioned supine and securely fixed to a rodent surgical table using adhesive tape after shaving the hair on their necks and making a small midline incision of approximately 1-2 cm. Subsequently, the unilateral common carotid artery was carefully separated from surrounding connective tissue and occluded using a bulldog clamp (Dieffenbach, German) for a duration of three hours. A sham group underwent identical surgical procedures without carotid artery occlusion. Strict aseptic techniques were adhered to during all surgical interventions, involving disinfection with alcohol swabs (OneMed, Indonesia) and povidone-iodine (Mundipharma Healthcare, Indonesia). Saline (Widatra Bhakti Ltd, Indonesia) was administered for irrigation during induction, and post-surgery, the wound was dressed with sterile covering followed by a single intravenous injection of Ampicillin at a dosage of 100 mg/kg body weight. Consciousness was monitored postoperatively, and stroke occurrence was assessed using a ladder rung test as a behavioral indicator, wherein a decrease in scores reflected diminished motor performance attributed to stroke [17]. Additionally, body weight and wound status were meticulously monitored on a daily basis to evaluate recovery progress.

Interventions

In the intervention groups, wet cupping therapy and exercise were performed four days after UCCAO. Wet cupping therapy was executed by applying ten punctures using lancets, followed by skin suction with negative pressure of -200 mmHg for five minutes on the right and left back using cups of 2 cm diameter [18]. Wet cupping therapy was performed twice a week for three weeks.

On the other hand, the exercise included a swimming activity scheduled for 20 minutes per day [19]. Swimming protocol was made 20 minutes taking into account the capabilities of the mice. If unable to do so, termination will be carried out. Rats swam individually in deep water tanks with the same dimensions with a 1.2 m diameter and 1.1 m height. Further, the water depth was set at 0.7 m and water temperature was maintained at 28-30oC [20]. This swimming exercise was performed three times a week for three weeks. The training dose was given for three weeks based on research conducted by Kim (2013), where experimental animals who exercised for 21 days could improve motor function and increase BDNF expression [21]. In the intervention groups, wet cupping therapy and exercise were performed four days after UCCAO.

Immunohistochemistry

Time point of animal termination and brain sample collection was done within the same day. The brain sample was examined directly after it was collected. Brain tissues were collected and preserved in neutral buffer formalin at 4oC overnight. Afterwards, they were put in paraffin and cut into slices (4 microns) using a microtome (BQ-318D). After incubation at 75oC for one-half hours, the slices were immersed in xylene for ten minutes and in clean xylene for another ten minutes. Subsequently, these slices were then incubated in 100%, 95%, or 80% ethanol and purified water for three minutes each. Following that, the slices were incubated in a citrate buffer solution (antigen retrieval buffer). Then, the buffer solution was removed, and the slices were eluted with phosphate buffer solution (PBS) and incubated in 3% hydrogen peroxide in a wet box at room temperature for ten minutes to remove the endogenous peroxidase-blocking buffer. Next, normal goat serum was added at room temperature for 30 minutes, and excess liquid was removed. A dissolved BDNF antibody (1:1000; Elabscience, USA) was then injected into each slice to be incubated afterwards at 4oC overnight, and the slices were rinsed again using PBS. Henceforth, the secondary antibody buffer (1:2000) was injected into each slice. The slices were then incubated at room temperature for an hour. Afterwards, the slices were rinsed, stained, and hydrated to be placed on and examined using a microscope. Further, the quantitative analysis of this study adopted the Image-pro Plus 6.0 software.

Statistical analysis

The data collected from study was tabulated, and subsequent analysis was conducted utilizing the Statistical Package for the Social Sciences (SPSS) version 26 software.

RESULTS

Brain-derived neurotrophic factor (BDNF) expression

The immunohistochemical technique applied in this study suggested that BDNF was expressed in the cytoplasm of nerve cells in the subventricular area. Such an expression was indicated by a brown color reaction to the anti-BDNF antibody (Fig. 2). The mean of nerve cells expressing BDNF in the sham group was 41.33±22.17; the stroke group was 17.66±11.03; the stroke+cupping group was 24.50±26.82; and the stroke+exercise group was 60.16±30.94 (Fig. 3).

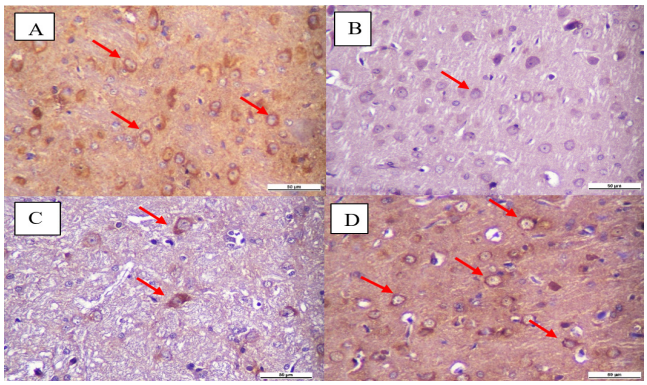

Fig. 2: Immunohistochemistry of BDNF expression in the subventricular area of the brain. The BDNF expression was examined using light microscope with magnification 400. A. Sham group, B. Stroke group, C. Stroke+cupping group, D. Stroke+exercise group. Red arrow: BDNF expression.

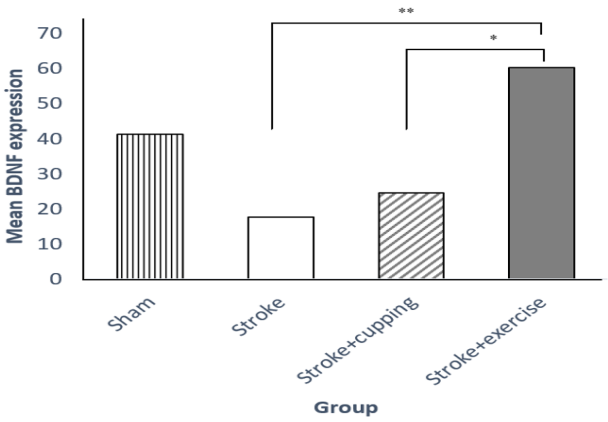

Fig. 3: Number of nerve cells expressing BDNF. The number of nerve cells expressing BDNF in stroke+exercise group is higher compared to the stroke+cupping group and stroke group. *p<0.05, **p<0.01

Analysis of multiple comparisons among groups employing the ANOVA test proved that BDNF expression in the stroke group was insignificantly lower compared to the sham group (p=0.102). In addition, the BDNF expression in the stroke+cupping group was insignificantly greater than the stroke group (p=0.626). Meanwhile, the BDNF expression in the stroke+exercise group was significantly higher than the stroke group (p=0.06). Apart from that, the BDNF expression in the stroke+exercise group was significantly higher than the stroke+cupping group (p=0.018).

DISCUSSION

Our society has been acquainted with wet cupping therapy since thousands of years ago. A wide range of studies revealed that wet cupping therapy is effective in reducing low back pain, neck pain, knee osteoarthritis, hyperlipidemia, headache, and hypertension. However, research on wet cupping associated with stroke remains limited number [9].

This study indicated that the BDNF expression in infarct or ischemic stroke in experimental animals was lower than the negative control. Infarct stroke correlates with the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), where in infarction stroke there is an increase in ROS production [22]. ROS and RNS trigger protein and DNA oxidation, resulting in toxic substances. One of the oxidative reaction products is malondialdehyde (MDA), a marker of stress in stroke [23]. Syafrita discovered a decrease in BDNF concentration and an increase in MDA in infarct stroke patients experiencing depression [6]. Further, the decrease in BDNF in infarct stroke patients was higher than in hemorrhagic stroke. It is important to note that the BDNF concentration in severe infarct strokes was lower than in mild infarct strokes. Also, the BDNF concentration does not correlate with the stroke duration [7].

In a systematic review, Karantali (2021) identified a decrease in BDNF concentration in infarct stroke patients. Moreover, research by Chaturvedi figured out a decrease in post-stroke BDNF concentration compared to healthy people [7]. The brain-derived neurotrophic factor (BDNF) is the neurotrophin expressed by the central nervous and peripheral nervous systems in adult mammals [6]. BDNF supports neural life through differentiation and maturation and is neuroprotective against neurotoxicity, cerebral ischemia, and hypoglycemia [7]. It also has a vital role in regulating synaptic plasticity. BDNF can penetrate the blood-brain barrier, and its serum and brain levels are the same [6]. ROS and RNS initiate oxidation in biomolecules, such as proteins and DNA, causing toxic substances to form. Oxidative DNA damage exposes an inevitable consequence of the dysfunction of the blood-brain barrier and degeneration and apoptosis [5].

In terms of immune function and anti-inflammatory processes, two theories underlie wet cupping therapy, including the “activation of the Immune system” theory and the “release of Nitric Oxide” theory [8]. Subadi (2014) revealed that wet cupping therapy triggers the expression of the transcription factor of nuclear factor-kappa B (NFkB) [24]. The transcription factor of NFkB consists of p50 and p65 heterodimers. NFkB dimers are retained in the cytoplasm because they bond with IkB. Oxidants activated IkB kinases, resulting in IkB phosphorylation. Phosphorylated IkB is then degraded by the proteasome, generating NFkB dis-inhibition, and translocated to the cell nucleus to bind the promoter and control gene expression. The gene products range from tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and matrix metalloproteinase 9 (MMP9). Meanwhile, NFkB also regulates the expression of an anti-apoptotic protein, referred to as Bcl-2, contributing to the survival of nerve cells [25]. Other research suggested that BDNF is the potential biomarker for neuron survival [26]. This study denoted that wet cupping therapy in the UCCAO experimental animals showed higher BDNF expression

than the positive control animals, although it was insignificant (Fig. 2). Moreover, an infarct stroke causes a decrease in blood flow to the brain, triggering the death of brain cells and an inflammatory reaction [27], and a decrease in BDNF concentration [28]. Besides, tumour necrosis factor (TNF), interleukin 1 (IL-1), and interleukin-6 (IL-6) in ischemic stroke patients will also significantly increase [29]. Qudah identified that exposure to TNF-α and IL-6 on colonic smooth muscle contributed to an increased BDNF concentration [30].

In this study, the BDNF expression in the stroke+exercise group (60.16±30.94) was significantly higher than in the stroke group (17.66±11.03) and the stroke+cupping group (24.50±26.82). The increased BDNF expression correlated with improved oxygenation and is considered a key mechanism for improving brain function. In a randomised control trial, Hsu (2021) discovered an increase in BDNF serum in stroke patients after high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), where BDNF levels after having HIIT were higher than having MICT [31]. Another study employing randomised control trial design in stroke patients trained with constraint-induced movement therapy (CIMT) with virtual reality on their upper limbs signified an increase in BDNF concentration compared to conventional training [32]. The increased energy needs due to exercise have resulted in mitochondrial biogenesis and increased oxygen consumption. Exercise is associated with an increase in the ketone of D-b-hydroxybutyrate (DBHB), an energy metabolism. This study was limited in its research method, where it applied a posttest-only control group design; therefore, it cannot describe the direct effects similar to pre and post-experimental designs.

CONCLUSION

In this experimental study utilizing the UCCAO animal model, exercise exhibited significantly greater efficacy in upregulating BDNF expression compared to wet cupping therapy, indicating a pronounced and preferential effect of exercise in enhancing neurotrophic factors within the context of cerebral ischemia.

ACKNOWLEDGEMENT

We thank Universitas Airlangga for support and funding this research.

REFERENCES

1. Saini V, Guada L, Yavagal DR (2021) Global Epidemiology of Stroke and Access to Acute Ischemic Stroke Interventions. Neurology. https://doi.org/10.1212/WNL.0000000000012781
2. Ministry of Health Republic Indonesia (2018) Laporan Riset Kesehatan Dasar (Riskesdas) 2018. Jakarta: Badan Litbangkes
3. Hatem SM, Saussez G, della Faille M, Prist V, Zhang X, Dispa D, Bleyenheuft Y (2016) Rehabilitation of motor function after stroke: A multiple systematic review focused on techniques to stimulate upper extremity recovery. Front Hum Neurosci. https://doi.org/10.3389/fnhum.2016.00442
4. Lv Y, Sun Q, Li J, Zhang W, He Y, Zhou Y (2021) Disability status and its influencing factors among stroke patients in northeast china: A 3-year follow-up study. Neuropsychiatr Dis Treat. https://doi.org/10.2147/NDT.S320785
5. Liu W, Wang X, O'Connor M, Wang G, Han F (2020) Brain-Derived Neurotrophic Factor and Its Potential Therapeutic Role in Stroke Comorbidities. Neural Plast. https://doi.org/10.1155/2020/1969482
6. Syafrita Y, Amir D, Susanti R, Fadhilah I (2020) Relationship of brain-derived neurotrophic factor, malondialdehyde, and 8-hydroxy 2-deoxyguanosine with post-ischemic stroke depression. Dement e Neuropsychol. https://doi.org/10.1590/1980-57642020dn14-010007
7. Chaturvedi P, Singh A, Tiwari V, Thacker A (2020) Brain-derived neurotrophic factor levels in acute stroke and its clinical implications. Brain Circ. https://doi.org/10.4103/bc.bc_23_20
8. Al-Bedah AMN, Elsubai IS, Qureshi NA, Aboushanab TS, Ali GIM, El-Olemy AT, Khalil AAH, Khalil MKM, Alqaed MS (2019) The medical perspective of cupping therapy: Effects and mechanisms of action. J Tradit Complement Med. https://doi.org/10.1016/j.jtcme.2018.03.003
9. Chirali IZ (2014) Traditional Chinese Medicine Cupping Therapy: Third Edition, Third edit. Tradit Chinese Med Cupping Ther Third Ed. https://doi.org/10.1016/C2009-0-63931-3
10. Boese AC, Eckert A, Hamblin MH, Lee JP (2020) Human neural stem cells improve early stage stroke outcome in delayed tissue plasminogen activator-treated aged stroke brains. Exp Neurol. https://doi.org/10.1016/j.expneurol.2020.113275
11. Shao TY, Wang JX, Shou ST, Fidimanantsoa OL (2023) Wet cupping with rehabilitation training for upper-limb poststroke spasticity: A systematic review and meta-analysis of randomized controlled trials. Heliyon. https://doi.org/10.1016/j.heliyon.2023.e20623
12. Kim M, Han C (2023) The effectiveness and safety of cupping therapy for stroke survivors: A systematic review and meta-analysis of randomized controlled trials. J Korean Med. https://doi.org/10.13048/jkm.23025.e1
13. Ashcroft SK, Ironside DD, Johnson L, Kuys SS, Thompson-Butel AG (2022) Effect of Exercise on Brain-Derived Neurotrophic Factor in Stroke Survivors: A Systematic Review and Meta-Analysis. Stroke. https://doi.org/10.1161/STROKEAHA.122.039919
14. Qin S, Zhang Z, Zhao Y, et al (2022) The impact of acupuncture on neuroplasticity after ischemic

stroke: a literature review and perspectives. Front Cell Neurosci. https://doi.org/10.3389/fncel.2022.817732
15. Li Y, Zhang J (2021) Animal models of stroke. Anim Model Exp Med. https://doi.org/10.1002/ame2.12179
16. Ma R, Xie Q, Li Y, Chen Z, Ren M, Chen H, Li H, Li J, Wang J (2020) Animal models of cerebral ischemia: A review. Biomed Pharmacother. https://doi.org/10.1016/j.biopha.2020.110686
17. Machin A, Divamillenia D, Fatimah N, et al (2022) The Effect of Green Tea with EGCG Active Compound in Enhancing the Expression of M2 Microglia Marker (CD206). Neurol India. https://doi.org/10.4103/0028-3886.344631
18. Subadi I, Kusumawardani MK, Qorib MF, Susilo I, Hidayati HB (2019) Wet cupping therapy improves mu opioid receptor expression and pain threshold in animal models of inflammation. Anaesthesia, Pain Intensive Care. https://doi.org/10.35975/apic.v23i4.1166
19. Wang R, Tian H, Guo D, Tian Q, Yao T, Kong X (2020) Impacts of exercise intervention on various diseases in rats. J Sport Heal Sci. https://doi.org/10.1016/j.jshs.2019.09.008
20. Veskoukis AS, Kyparos A, Paschalis V, Nikolaidis MG (2018) A novel swimming performance test in rats. Chin J Physiol. https://doi.org/10.4077/CJP.2018.BAG548
21. Kim G, Kim E (2013) The effects of antecedent exercise on motor function recovery and brain-derived neurotrophic factor expression after focal cerebral ischemia in rats. J Phys Ther Sci. https://doi.org/10.1589/jpts.25.5_553
22. Peters O, Back T, Lindauer U, Busch C, Megow D, Dreier J, Dirnagl U (1998) Increased formation of reactive oxygen species after permanent and reversible middle cerebral artery occlusion in the rat. J Cereb Blood Flow Metab. https://doi.org/10.1097/00004647-199802000-00011
23. Elsayed WM, Abdel-Gawad EHA, Mesallam DIA, El-Serafy TS (2020) The relationship between oxidative stress and acute ischemic stroke severity and functional outcome. Egypt J Neurol Psychiatry Neurosurg. https://doi.org/10.1186/s41983-020-00206-y
24. Subadi I, Laswati H (2014) Ekspresi β-Endorfin Pada Penurunan Nyeri Inflamasi Pasca Terapi Bekam Kering. J. VOK@ SINDO 2:1–7.
25. Chen H, Yoshioka H, Kim GS, Jung JE, Okami N, Sakata H, Maier CM, Narasimhan P, Goeders CE, Chan PH (2011) Oxidative stress in ischemic brain damage: Mechanisms of cell death and potential molecular targets for neuroprotection. Antioxidants Redox Signal. https://doi.org/10.1089/ars.2010.3576
26. Mojtabavi H, Shaka Z, Momtazmanesh S, Ajdari A, Rezaei N (2022) Circulating brain-derived neurotrophic factor as a potential biomarker in stroke: a systematic review and meta-analysis. J Transl Med. https://doi.org/10.1186/s12967-022-03312-y
27. Lambertsen KL, Biber K, Finsen B (2012) Inflammatory cytokines in experimental and human stroke. J Cereb Blood Flow Metab. https://doi.org/10.1038/jcbfm.2012.88
28. Karantali E, Kazis D, Papavasileiou V, et al (2021) Serum bdnf levels in acute stroke: A systematic review and meta-analysis. Med. https://doi.org/10.3390/medicina57030297
29. Zhu H, Hu S, Li Y, Sun Y, Xiong X, Hu X, Chen J, Qiu S (2022) Interleukins and Ischemic Stroke. Front Immunol. https://doi.org/10.3389/fimmu.2022.828447
30. Qudah M Al, Alfaqih M, Al-Shboul O, Saadeh R, Al-Dwairi A (2020) Effect of cytokine treatment on the expression and secretion of brain derived neurotrophic factor in the smooth muscle of the rat colon. Biomed Reports. https://doi.org/10.3892/br.2020.1302
31. Hsu CC, Fu TC, Huang SC, Chen CPC, Wang JS (2021) Increased serum brain-derived neurotrophic factor with high-intensity interval training in stroke patients: A randomized controlled trial. Ann Phys Rehabil Med. https://doi.org/10.1016/j.rehab.2020.03.010
32. Anjum AF, Khan HF, Sadiq N, Samad A, Jawwad G, Ayaz H (2019) Effects of Virtual Rehabilitation and Constraint Induced Movement Therapy on Brain Derived Neurotrophic Factor Mediated Motor Improvement in Stroke Patients. J Bahria Univ Med Dent Coll. https://doi.org/10.51985/jbumdc2019086