

Neuroprotective Effects of Pine Bark and *Aloe vera* on the Locomotor Activity in Focal Cerebral Ischemia: Possible Antioxidant Mechanisms

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Abstract

Objective: Free radical induced neural damage is concerned in brain ischemia and antioxidants are reported to have neuroprotective activity. We investigated the protective effects of antioxidant plants that are Pine Bark and *Aloe vera* extracts against movement activity and oxidative stress in ischemia rats.

Materials and methods: Fifty rats were randomly separated into six groups (n=7); control group, middle cerebral artery occlusion (MCAO), MCAO+pine bark 1 (25 mg/kg), MCAO+pine bark 2 (05 mg/kg), MCAO+*Aloe vera* 1 (50 mg/kg), MCAO+*Aloe vera* 2 (100 mg/kg). All extracts were infused (IP) to animals for 14 days before MCAO. Then the middle cerebral artery of rats was occluded for 2 h and reperused for 22 h. The brain malondialdehyde (MDA), Thiol levels and locomotor activity on rotarod test were assessed.

Results: The MDA levels of brain were non-significantly increased in MCAO group vs control group. The thiol level of brain was significantly decreased in MCAO group compared to control group (p<0.005). The thiol level in groups pine bark 2 and *Aloe vera* 2 increased significantly vs MCAO group (p<0.01). The time stands rates on rotarod in MCAO group decreased significantly compared to control group. It was also increased significantly in pine bark 1 and pine bark 2 groups vs MCAO group (p<0.005). Time stands rates on rotarod in *Aloe vera* 1 and *Aloe vera* 2 groups were increased significantly compared to MCAO group (p<0.005).

Conclusion: Increasing endogenous antioxidant enzymatic activities indicate that *Aloe vera* and pine bark have neuroprotective role. Thus, herbal treatment of pine bark and *Aloe vera* may improve the function of ischemia-reperfusion brain injury related disorders.

Keywords: Pine bark; *Aloe vera*; Oxidative stress; Rat; Cerebral ischemia

Introduction

Stroke is one of the principal causes of death and disability worldwide. Cerebral ischemia is the result of insufficient cerebral blood flow for cerebral metabolic functions [1]. Oxidative stress and inflammation have an important role in cerebral infarction which mediated by ischemia and reperfusion. Reperfusion injury stimulates many pathological mechanisms such as leukocyte infiltration, oxidative stress, inflammation, destruction of blood-brain barrier, platelet activation, nitric oxide release, and apoptosis. Consequently, potent anti-inflammatory and antioxidant mediators may be beneficial in the treatment of cerebral ischemia and reperfusion injury. The lack of effective and widely applicable pharmacological treatments for ischemic stroke patients may explain a growing interest in the traditional medicines [2].

Pine bark: extract is rich in flavonoids and was found to be among the most powerful natural antioxidants [3,4]. It has been shown to recycle the ascorbic radical and to protect vitamin E against oxidation [5,6]. It is a potent antioxidant and much of its pharmacological activity comes from antioxidant activity, as it enhances synthesis of anti-oxidative enzymes and regenerate vitamins C and E, but also acts as a free-radical scavenger [7]. For this reason, the diverse beneficial effects of Pine Bark have been claimed to protect against various degenerative conditions caused by oxidative stress [8].

***Aloe vera*:** is a perennial plant belonging to the family of Liliaceae, which includes about 360 species. The plant has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. *Aloe vera* has been used for many centuries for its curative and therapeutic

properties [9,10], its antibacterial, antifungal and antiviral activities and of its ability to treat hyperlipidemia and psoriasis symptoms [11]. Topical and oral uses of *Aloe vera* gel have been shown to increase the collagen content in experimental dermal wounds in rats [3,12]. Both the *Aloe vera* gel and skin are purported to have anti diabetic and cytoprotective activities. *Aloe vera* has also been reported to have antioxidant activities [5-13]. Considering these beneficial effects of Pine bark and *Aloe vera*, the objective of this study was to investigate the effects of long-term administration of Pine bark and *Aloe vera* on locomotor activity using Rotarod test. Markers of oxidative stress, mainly, lipid peroxides levels (reported as malondialdehyde, MDA) and total thiol(SH) content, were also measured in brain.

Materials and Methods

Preparing the plant extract

Hydro-alcoholic extract of Pine bark and *Aloe vera* was developed (Figure 1). Pine bark and *Aloe vera* were prepared from Mashhad

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City, Khorasan Province, Iran, and identified by botanists in Ferdowsi University of Mashhad, Iran, and a voucher specimen was deposited. The plants were then dried at room temperature. To prepare the hydroalcoholic extract, 50 g of the chopped and dried aerial parts of the plant was soaked in ethanol (50%) for 48 h and filtered through paper filter. The extract was then dried with rotary.

Animals

Male Wister rats weighing 200-250 g were used in the present study purchased from Mashhad University of Medical sciences and kept under standard housing conditions at a temperature between 20°C and 25°C, with a 12 h light-dark cycle. All animals had free access to food and water. All animals were treated in accordance with the guidelines for care and use of laboratory animals prepared by the Animal Research Ethic Committee of North Kharasan University of Medical Sciences, Bojnurd, Iran and in conformity with EU Directive 2010/63/EU for animal experiments. Animals were randomly separated into six groups (n=7); control group, middle cerebral artery occlusion (MCAO), MCAO+pine bark 1 (50 mg/kg), MCAO+pine bark 2 (100 mg/kg), MCAO+Aloe vera 1 (50 mg/kg), MCAO+Aloe vera 2 (100 mg/kg). All extracts were infused (IP) to animals for 14 days before MCAO [10].

MCAO method: animals were 200-250 g anesthetized with ketamine/xylazine (150:10 mg/kg) mixture. The external carotid artery was isolated and coagulated, was closed with sutures occluding the middle cerebral artery for 2 hours. Body temperature was regulated at 37 C with a temperature control system. After 22 hours, animals were killed, brain and serum blood were extracted [5] (Figure 2).

Biochemistry

After the behavioural study, the animals were then sacrificed, and the brains were removed and dissected on an ice-cold surface and conserved for biochemical measurements.

MDA

For MDA measurement, the brain extracts were added to the detection solution of MDA in an Eppendorf tube and then boiled for 15 min. Beginning tissue weight and homogenized with KCl then centrifuged in 1500 rpm for 10 minutes. After centrifugation at 1000 × g for 10 min, the supernatant was collected. Relative MDA units were determined using an MD-M5 microplate reader at 532 nm [14].

Thiol

Total SH groups were measured using DTNB (2, 2-dithiodibenzoic acid) as reagent. This reagent reacts with the SH groups to produce a yellow coloured complex which has a peak absorbance at 412 nm. Briefly, 1 mL Tris-EDTA (ethylenediaminetetraacetic acid) buffer (pH=8.6) was added to 50 µL brain homogenate in 1 mL cuvettes and sample absorbance was read at 412 nm against Tri s-EDTA buffer al one (A). Then, 20 µL DTNB reagents (10 mM in methanol) were added to the mixture and after 15 min (at laboratory temperature) the sample absorbance was read again. The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation [15]:

$$\text{Total thiol concentration (mM)} = (A_2 - A_1 - B) \times 1.070.05 \times 13.6$$

Behavioural tests: rotarod test

Animals were tested on the rotarod at a speed of 8 rpm. Animals 3 days before ischemia and 24 hours after ischemia were tested with rotarod. The animal was placed on the rotating drum and the time spent to reach the criterion of remaining on the rotarod for 2 min without falling down was recorded.

Statistical analysis

Values are reported as mean ± SEM. Statistical analysis was performed with SPSS software version 13. Statistic calculations were carried out with one-way analysis of variance (ANOVA) for multiple pair wise comparisons of groups. A significant difference was defined as p<0.05.

Results

MDA

Figure illustrated that the MDA levels of brain were non-significantly increased in MCAO group compared to control group. MDA level in *Aloe vera* 2 group was significantly increased compared to control group (p<0.05) and in pine bark 1 and pine bark 2 groups were non-significantly decreased compared to MCAO group. In *Aloe vera* 1 group, MDA level was same compared to group MCAO.

Thiol

At the end of our study, the total thiol concentration of brain was significantly decreased in MCAO group compared to control group (p<0.005). Pretreatment of the animals by 100 mg/kg of the extracts decreased the total thiol concentration in the brain tissues significantly compared to MCAO group (p<0.01). The total thiol concentration in pine bark 1 and *Aloe vera* 1 groups was increased non-significantly compared to MCAO group.

Rotarod test

Figure was shown that time stands rates on rotarod in MCAO group decreased significantly compared to control group. The time stands on rotarod in pine bark 1 and pine bark 2 groups increased significantly compared to MCAO group (p<0.005). Time stands rates on rotarod in groups *Aloe vera* 1 and *Aloe vera* 2 were increased significantly compared to MCAO group (p<0.005).

Discussion

In this study, we provide evidence that Pine bark and *Aloe vera* are potent neuroprotectant in transient brain ischemia. We used the middle cerebral artery occlusion (MCAO) model with reperfusion

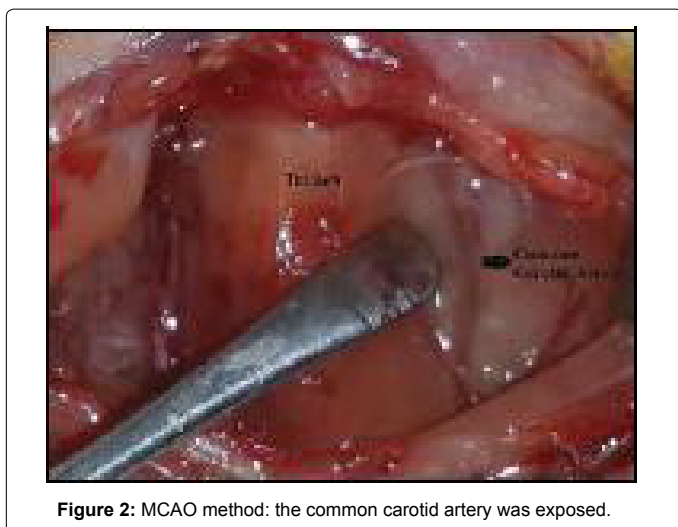


Figure 2: MCAO method: the common carotid artery was exposed.

that mimics many features of the stroke in humans, since the middle cerebral artery (MCA), which is the specific occlusion site in this model, is the most commonly affected vessel in both embolic and thrombotic strokes in humans [16]. It is well documented that MCAO results in behavioural and neurochemical abnormality in rat brain, probably by generating free radicals due to their high reactivity, they provoke damage to lipids, DNA, and proteins, leading to neuronal death and is consistent with previous studies [17]. Our study exhibited that pretreatment (prevention) with pine bark and *Aloe vera* retarded brain injury subsequent to MCAO in rats. Free radicals have been the focus of interest as a possible candidate for the elucidation of ischemia responses and potential therapeutic agents. The reactive oxygen species (ROS) threaten neuronal survival by their ability to propagate the initial attack on lipid rich membranes of the brain to cause lipid peroxidation, which then stimulates glycation of proteins, inactivation of enzymes and alterations in the structure and function of membrane and DNA damage [17]. After brain damage by either ischemic or hemorrhagic stroke, the production of free radicals' increases, and at the same time, a physiological system that removes excessive free radicals is impaired. Production of free radicals in the ischemic brain depends on the intensity, stage, and site of ischemia and occurrence of reperfusion. Increased levels of free radicals can damage all cellular components, including DNA, lipids, and proteins, which then leads to injury of neurons, glial cells, blood vessels, and nerve fibers. In order to prevent ischemia-reperfusion damage and abate the pre-existing damage, many approaches including free radical scavengers, anti-apoptotic and anti-inflammatory agents were tried [18,19]. Since MDA is end product of lipid peroxidation, the results indicate the cytotoxic effect of free radical by peroxidation on brain tissue, because it contains large amount of phospholipids that leading to neuronal death [21]. *Aloe vera* has been reported to have antioxidant activities [20]. In this study, we founded *Aloe vera* consumption decreased MDA level, thus it may partly prevent lipid peroxidation in brain then can be useful to reduce the effects of cerebral ischemia. Also *Aloe vera* purported to have anti diabetic and cytoprotective activities, which may ameliorate complications from diabetes and cardiovascular diseases. Pine bark treatment non-significantly reduced the tissue MDA levels. Despite complete inhibition of MDA, protection to neuronal damage was partial. This may be because of involvement of multiple pathways in global cerebral ischemia reperfusion injury. The beneficial effects of these flavonoids are attributed to their antioxidant and anti-inflammatory properties.

The significant alteration in the antioxidant enzyme activities during cerebral ischemia and reperfusion may be responsible for more neuro degeneration than ischemia [22]. Our findings suggested, thiol levels were significantly increased in *Aloe vera* 2 and Pine bark 2 groups compared to MCAO group. Thus, they may be promising candidates for the improvement of prophylactic treatment of brain ischemia. Thiol group containing agents such as bioflavonoids are reported to have free radicals scavenging effects. Investigators suggested that the scavenging mechanisms are based on an increased amount of reduced glutathione. We confirmed through this study, a flavonoid-derived compound had preventive value in focal ischemia because it reduced motor dysfunction. The rotarod test is a well-established procedure for testing balance and coordination aspects of motor performance in rats. Recent improvements in the model include implementation of accelerating and bidirectional rotarod, both of which enhance the sensitivity of the test. Recent studies have indicated that the accelerating rotarod task is a sensitive index for the assessment of motor impairment induced by traumatic brain injury, dopaminergic lesion, or ischemia in the rat. Importantly, this is one of the few behavioral tests that yield objective quantitative data. Thus, this test of motor performance is suitable for incorporation into neuroprotection studies to provide an important profile of motor function that will supplement standard histopathological analysis [7,22]. We next examined whether extracts of Pine bark and *Aloe vera* reduces the oxidative lesions in brain and subsequently results in a reduction of oxidative damage of lipids and proteins. The present results demonstrated that extracts of Pine bark 2 and *Aloe vera* 2 given to rats were very effective in reducing oxidative burden in brain. The treatment of plant extracts significantly ($p < 0.05$) improved the locomotor responses in a "rotarod test". This is in line with the apparent reduction of lipid peroxidation and protein carbonyl observed in brain of rats supplemented with these plant extracts. Several studies conducted elsewhere in recent years demonstrated an attenuation of neuronal cell death induced by oxidative stress by supplementation of phenolic anti-oxidants [23-26]. While a direct protective effect cannot be dismissed, the beneficial effects promoted by and Pine bark 2 and *Aloe vera* 2 extracts could also be attributed to lowering the free radical production and improving antioxidant activity within brain regions, which potentially could result in a reduction in lipid peroxidation and protein carbonyl content in brain and to improving motor activity.

Conclusion

In our studies directed toward demonstrating functional improvement of neurological function, in addition to reduction of MDA and increase of Thiol may improve the predictive value of animal models for clinical efficacy with novel neuroprotective agents.

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This study was done in Mashhad University of Medical Sciences, Iran.

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