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Original Article

Effect of Extract of Rhubarb Root on Secondary Brain Injury in Rats with Traumatic Brain Injury

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ABSTRACT

Background and Aim: To investigate the effects of Extract (Ext) of Rhubarb Root on secondary brain injury after Traumatic Brain Injury (TBI) in rats. **Methods:** Adult male Wistar rats were randomly divided into three groups: sham group, TBI group, and TBI+Ext group (*n*=15 per group). Rat TBI model was made by using the modified Feeney's method. In TBI+ Ext group, Ext was administered orally at a dosage of 500 mg/kg after TBI once a day for 5 days. On the 5th day after TBI, motor function and brain water content were evaluated in each group. As oxidative stress indices in brain tissue, MDA level, SOD and CAT activities were assayed. **Results:** Beam walking performance impairment and brain edema were significantly reduced in TBI+Ext group compared with TBI group; meanwhile, level of MDA significantly were decreased and activities of SOD and CAT significantly were increased in brain tissue. **Conclusion:** The extract of Rhubarb Root can reduce secondary brain injury and improve outcomes of the oxidative stress in injured brain following TBI.

Keywords: Rhubarb root, Traumatic brain injury, Experimental model, Secondary brain injury.

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INTRODUCTION

Traumatic Brain Injury (TBI) is one of the major causes of mortality and neurological disability. There are more than 10 million people suffering from it every year,[1] and nearly 80% of these patients have cognitive deficit.[2] TBI has been classified into primary injury and secondary injury, and the latter plays a crucial role in the clinical outcome of patients with TBI.[3] Posttraumatic inflammation can aggravate secondary brain injury and result in neurological deterioration.[4] Therefore, reduce of inflammation after TBI contributes to better prognosis of TBI patients. On the order hand, posttraumatic inflammation may interact with oxidative stress in pathophysiology of the secondary brain injury after traumatic brain injury. Oxidative stress is a significant contributor to the secondary sequelae of Traumatic Brain Injury (TBI), and may mediate subsequent neurobehavioral deficits and histopathology.[5] The pathological sequelae of Traumatic Brain Injury (TBI) include increased oxidative stress due to the production of Reactive Oxygen Species (ROS). Regulation of ROS levels following TBI is determined primarily by antioxidant enzyme activity that in turn can be influenced by Nerve Growth Factor (NGF). Rhubarb is a perennial plant that grows from thick short rhizomes, comprising the genus Rheum. The plant is indigenous to Asia. Varieties of rhubarb have a long history as medicinal plants in traditional medicine in DPR of Korea. Rhubarb is used as a strong laxative and for its astringent effect on the mucous membranes of the mouth and the nasal cavity. The roots and stems are rich in anthraquinones, such as emodin (1,3,8-trihydroxy-6-methyl-9, 10-anthraquinone) and rhein. These substances are cathartic and laxative, which explains the sporadic abuse of Rhubarb as a slimming agent. Rhubarb root has properties that make it a highly effective laxative. Its astringent qualities help to improve bowel tone after it has purged the intestines, making it an excellent agent for improving the tone and health of the digestive tract. Its laxative effects make it a valuable aid in the treatment of chronic constipation, hemorrhoids, and gastroenteritis. So far, we could not find the literatures and research works related to the effect of rhubarb root on the brain injury after TBI. Thus, here we investigate the neuroprotective effects of Rhubarb Root extract (Ext) against TBI in a rat model.

MATERIALS AND METHODS

Preparation of Rhubarb Root Extract

Rhubarb Root were obtained from Mannyon pharmaceutical company, DPR Korea and identified by comparison with the voucher specimen deposited at National drug certification institute of Pyongyang, DPR Korea. The roots were cleaned, dried, ground, weighed and homogenized in 92% ethanol at a ratio of

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1:10 of sample to ethanol and left to soak for 3 days at 25°C with occasional shaking and stirring. The mixture was then filtered and the resulting liquid was concentrated under reduced pressure at 45°C in rotary evaporator to yield a dark gummy-yellow extract (7%, w/w). The concentrated extract was then kept in the incubator at 45°C for 3 days to evaporate the ethanol residue yielding the crude root extract. Extracts were then dissolved in 10% Tween-20 before being orally administrated to animals in concentrations of 500 mg/kg body weight.

Subjects and Groups

Adult male Wistar rats (3 weeks old, 250~300 g) were provided by Laboratory Animal Centre of the Hamhung University of Medical Sciences and adapted in a lab environment before experiments for a week. 45 rats are randomly chosen and divided into three groups (Sham, TBI and TBI+Ext). During the experiment, feed and water were available to rats at any time. The temperature was maintained at 20±2°C and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Pyongyang University of Medical Sciences. Rats were randomly divided into three groups: sham group, TBI group and TBI+Ext group (n=15 for each). Rat model of TBI was established according to the modified Feeney's free weight-drop method.[1] Sham rats received right parietal craniotomy alone without head trauma. Rats in the other two groups suffered TBI; in TBI+ Ext group the extract of Rhubarb Root was orally administered at the dose of 500mg/kg once a day for 5 days, while sham and TBI groups received equal volume of 0.9%saline solution. On the 5th day after operative procedure, rats in each group received beam walking test.[2] Then all the rats were sacrificed and brain tissue assays including water content and the biochemical indices were performed.

Beam Walking Test for Evaluation of Motor Function

Under the distraction of bright light and loud white noise, rats escaped along a narrow wooden beam (2.5 cm wide and 120 cm long) to enter a darkened goal box. A score of 7 was given when animals traversed the beam with 2 or less foot slips; 6 was given with less than 50% foot slips; 5 was given for more than 50% but less than 100% foot slips; 4 was given for 100% foot slips; 3 was given for traversal with the affected limb extended and not reaching the surface of the beam; 2 was given when the animal was able to balance on the beam but not traverse it; and 1 was given when the animal could not balance on the beam.[2]

Measurement of Water Content in Brain Tissue

After the rats were killed, brains were rapidly harvested and cut through midline. Both cerebral hemispheres (injured and uninjured) were immediately weighed to gain the wet weight, and then placed in an oven at 100°C for 72 hr to obtain dry weight. The brain water content was calculated as: (wet weight-dry weight)/wet weight×100%.[3]

Preparation of Brain Tissue Sample for Biochemical Assay

Based on literature survey,[4] the biochemical assay was undertaken on 5th day after TBI and immediately after killing the animals by decapitation, brains were put into ice, and cerebellums were removed. Brain tissue was frozen in liquid nitrogen and stored at -70°C for determination of the oxidative stress indices.

Malondialdehyde

Lipid Peroxides of hippocampus was expressed as Malondialdehyde (MDA) level. MDA assay was determined spectrophotometrically as Thiobarbituric Acid-Reactive Substances (TBARS) according to the method of Ohkawa *et al.*[5] Tissue lipid peroxide levels were expressed as nanomoles of TBARS formed per g tissue weight. The results are expressed as nmol/g wet weight.

Catalase

Catalase (CAT) activity was measured by the method of Aebi,[6] by tracking the decomposition of hydrogen peroxide by measuring decrease in extinction of H_2O_2 at 240 nm. The activity of CAT is expressed as rate constant of first order reaction K per gram tissue weight.

Superoxide Dismutase

Superoxide Dismutase (SOD) activity was estimated by the method of Misra and Fridovich.[7] Activity is expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% which is equal to U per gram tissue weight.

Statistical Analysis of Data

Results were expressed as the mean and SEM. Data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS 16.0 and the differences between the means assessed using Dunnet's multiple range test. A p value of <0.01 was taken as the level of statistical significance.

RESULTS

Beam Walking Test Score after TBI

Before operation all the rats underwent training on the wooden beam for 3 days and all of them achieved a normal score of 7. The rats in sham group still kept the score of 7.00 on 5th day after sham operation. But the rats in TBI group had a score of 4.83±0.71 ($p<0.01$ compared with sham group), and those in TBI+Ext group had a score of 6.67±0.84 ($p<0.01$ compared with TBI group) (Figure 1).

Brain Water Content after TBI

On 5th day after injury, brain water content of injured side was 77.64%±0.57% in sham group, 81.53%±0.62% in TBI group ($p<0.01$ compared with sham group), and 78.82%±0.47% in TBI+Ext group ($p<0.01$ compared with TBI group). Brain water

Table 1: **The effects of extract on oxidative stress in hippocampus.**

	MDA (nmol/g wet tissue)	SOD ($\times10^{-1}$) (U/g wet tissue wt)	CAT (k/g wet tissue wt)
Sham	5.72±0.43	58.51±4.44	15.13±0.51
TBI	15.13±0.92**	23.25±2.42**	7.65±0.79**
TBI+Ext	10.27±0.76△△	48.92±3.15△△	13.85±0.63△△

Each value represents the mean±SEM of 15 rats per group. **p<0.01 as compared with sham group. △△p<0.01 as compared with TBI group. MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; TBI: Traumatic brain injury; Ext: Extract.

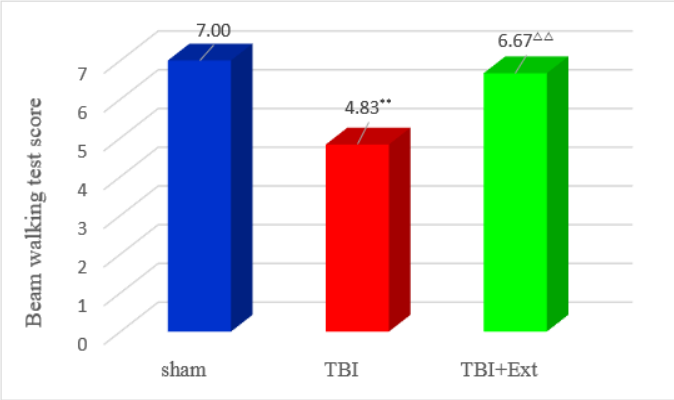

Figure 1: The effects of extract on beam walking test.

Each value represents the mean±SEM of 15 rats per group. **p<0.01 as compared with sham group. △△ p<0.01 as compared with TBI group. TBI: Traumatic brain injury; Ext: Extract.

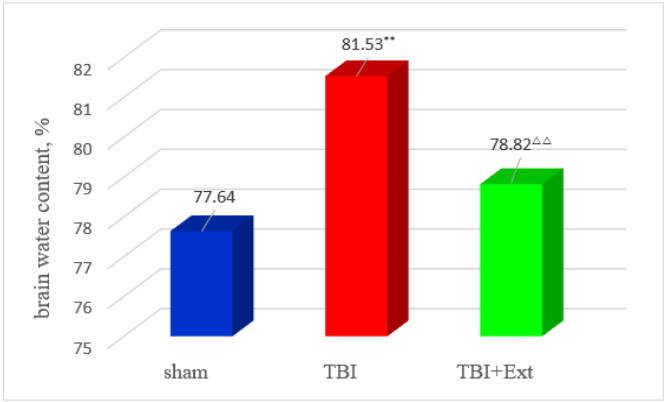

Figure 2: The effects of Extract on brain water content.

Each value represents the mean±SEM of 15 rats per group. **p<0.01 as compared with sham group. △△ p<0.01 as compared with TBI group.

contents of uninjured sides showed no statistically significant differences for each group (Figure 2).

Level of MDA, Activities of SOD and CAT in Brain Tissue

Table 1 showed that in TBI+Ext group, the level of MDA was significantly decreased and the activities of SOD and CAT were significantly increased compared with TBI group (p<0.01).

DISCUSSION

There is mass of evidence to suggest that inflammation plays a vital role in the pathophysiology of TBI. TBI initiates the inflammatory responses by blood brain barrier disruption, brain edema and inflammatory cell infiltration.[8] At the present time, there is no treatment with significant effect clinically. Therefore, exploration of new pharmacologic therapies that target the inflammatory process is necessary for TBI treatment. Recent studies demonstrated that rhubarb root can delay or stop the progression of chronic renal failure. One of the tannin components of rhubarb root, lindleyin, has been shown to act as an anti- inflammatory agent with fever-reducing properties similar to those of aspirin. Emodin, another component of rhubarb root, has been found to inhibit the growth of cancer cells. Herbalists in DPR of Korea as well as China also use rhubarb root for diseases and disorders in the upper body, including sinus and lung infections, nose bleeding, and eye infections. In the present experiment, we demonstrate that rhubarb root exerts neuroprotective effects in a rat weight-dropping model of TBI. All of the effect including beam working test, brain water content and oxidative stress in this present study supports the viewpoint that rhubarb root can reduce secondary inflammation to some extent and improve motor function recovery after TBI. And to our knowledge, it is the first time that rhubarb root is demonstrated to provide neuroprotective effects following TBI.

CONCLUSION

To our knowledge, it is the first time that rhubarb root is demonstrated to provide neuroprotective effects following TBI. Rhubarb root could retrain excessive inflammatory responses and possess strong neuroprotective effects. Honestly, the underlying molecular mechanism and interaction still needs further exploration. Rhubarb root can be expected to become a new drug for TBI treatment through affecting oxidative stress.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TBI: Traumatic Brain Injury; **ROS:** Reactive Oxygen Species; **NGF:** Nerve Growth Factor; **MDA:** Malondialdehyde; **CAT:** Catalase; **SOD:** Superoxide Dismutase.

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