The Effect of Berchemia Berchemiifolia to Moderate Gastric Mucosal Bleeding Caused by the Exposure to Acute Intense Stress

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Abstract: To evaluate the effect to moderate gastric ulcer caused by acute intense stress, this study used an experimental animal model of gastric ulcer triggered by waterlogging stress. Experimental animal groups are divided into control group; vehicle group; 200 mg/kg rat weight berchemia berchemiifolia extract oral administration group; 50 mg/kg rat weight berchemia berchemiifolia extract oral administration group; and 3 mg/kg rat weight ranitidine oral administration group (positive control). The substances were administered before imposing waterlogging stress. After 6 hours of waterlogging, the animals were sacrificed, and samples were collected. The level of gastric mucosal bleeding, inflammatory gastric cells, and inflammatory protein (iNOS, Nrf2, NF-kB) signal substances were analyzed. The results of analysis showed that the expression of inflammatory cells caused by gastric bleeding was significantly lowered in the berchemia berchemiifolia extract administration groups, and the expression of inflammatory proteins showed a similar tendency. Therefore, berchemia berchemiifolia extract is expected to become a candidate substance for new drugs and health foods for the treatment of gastritis and gastric ulcer.

Keywords – Acute intense stress, Berchemia berchemiifolia extract, iNOS, Nrf2, NF-kB

I. Introduction

The introduction of the paper should explain the nature of the problem, previous work, purpose, and the contribution of the paper. The contents of each section may be provided to understand easily about the paper. Acute, intense stress affects the immune system in various ways. According to recent studies, one of the most frequently found symptoms these days is gastric ulcer caused by gastric mucosal bleeding. Gastric ulcer is a hole in the lining of the stomach partially corroded by inflammation, and it occurs when the immune defense system of gastric mucosa is lowered. There are several causes of gastric ulcer, such as smoking, drugs, stress, helicobacter pylori infection, etc[1]. Under intense stress, gastric movement is increased and this triggers abnormal blood flow, and for instance, gastric mucosa can be damaged due to the lack of blood flow[2]. According to the Statistics Korea, the incidence rate of gastric cancer that gastric ulcer developed into was male 17.4 % (highest in the world), and female 7.8 % in 2015, which has meaningful implications on the seriousness of gastric ulcer. This mechanism is still being studied, but it involves the interaction between cytokine (related to cytokine) and signalling proteins.

Lipid peroxidation by oxygen free radical degrades the polyunsaturated fatty acids of cell membrane, causing the collapse of the membrane structure of gastric mucosal cells[3]. These changes in gastric tissues caused by external stimulation trigger the expression of nuclear factor (NF)-kB in cells[4], and when the normal NF-xB condition is broken down, tissues are damaged by acute inflammation[5]. In addition, nitric oxide (NO) is a substance to moderate various biological functions, and involved in the occurrence of inflammatory diseases[6]. NO synthase (NOS) enzyme is associated with the production of NO[7], and there are two types of NOS — constitutive NOS (cNOS) and inducible NOS (iNOS). When the protein expression of iNOS decreases, inflammation can be inhibited[8]. In this study, the protein expression of the two substances were quantitatively analyzed to reveal its gastritis inhibiting effect.

Berchemia berchemiifolia is a deciduous tree distributed in Korea and Japan. According to recent studies, it is found to have anti-inflammatory and anti-cancer efficacy through animal experiments[9]. As a natural edible substance, it is expected to have less side effects than existing chemosynthetic drugs.

To evaluate the impact of the anti-inflammatory efficacy of berchemia berchemiifolia on gastric ulcer, this study used an experimental animal model that imposed waterlogging stress. Certain concentration levels of berchemia berchemiifolia extract were orally administered to experimental animals, and they were locked in a cage and waterlogged up to the neck. Biological changes were analyzed to reveal the effect of berchemia berchemiifolia extract to moderate gastric ulcer.
II. Materials and Methods

2.1 Extraction and administration of test substances

Roots of *berchemia berchemifolia* were thoroughly washed and dried in the shade for over a week. The dried roots were ground in the blender and freeze-dehydrated. The dried sample (2g) and 10ml of 70% v/v ethanol were poured into a 15 ml tube, and were melted in a mixer for 18 hours. The melted sample was separated by centrifugation at 3,000 rpm, and the upper liquid phase was taken into a separate container, and concentrated at 40°C in an evaporator. The concentrated sample was again freeze-dehydrated. The sample used in this experiment was extracted from roots of *Berchemia berchemifolia* and the percent yield was 5.6%. The *berchemia berchemifolia* extract was orally administered (200 mg/kg rat weight, 50 mg/kg rat weight) to rats, and ranitidine 3 mg/kg was orally administered to the positive control. All substances were orally administered at 10 am.

2.2 Test animal groups and waterlogging stress

Thirty Sprague-Dawley (SD) rats (5 weeks old, 120-130g/body weight) were purchased from Joongang Animal Center, and housed in a cage with a filter. Living conditions were maintained at 22-24°C, 55-60% humidity, and a 12-hour light-dark cycle. Foods and clean water were provided regularly in a dedicated animal breeding room. According to the purpose of the experiment, they were divided into 5 different groups of 6 rats each as follows: 1) control, 2) vehicle, 3) 200 mg/kg rat weight *berchemia berchemifolia* extract oral administration (BBE 4x), 4) 50 mg/kg rat weight *berchemia berchemifolia* extract oral administration (BBE 1x), and 5) positive control (3 mg/kg rat weight ranitidine oral administration). *Berchemia berchemifolia* extract and ranitidine were orally administered before imposing waterlogging stress. After 6 hours of waterlogging, rats were sacrificed.

The waterlogging stress test was conducted in a water tank (60x100cm) and rats were locked in a square cage inside the tank. Except the control group, waterlogging stress was imposed on those in the rest groups for 6 hours.

2.3 Animal sacrifice and sampling

The rats were fasted for 24 hours before sacrificing. The weight of the rats were measured and recorded before anesthesia, and they were placed in supine position on a rat operating table (Dong Sew Science, Seoul, Korea). The lower abdomen was incised and 7-8 ml blood was collected from the abdominal aorta. A proper amount of blood was taken into an EDTA tube and serum separating tube. The stomach was incised between cardia and pylorus, and inflammatory areas were taken pictures of. Gastric tissues were collected for biopsy and protein analysis, and put in formaldehyde solution and E-tube.

2.4 Tissue staining and western blot

Parts of the stomach body and the stomach body close to pylorus were extracted and fixed in 10% neutral formalin. The collected parts were formatted in paraffin after following the procedure for tissue sample preparation. The samples were cut with a microtome, and stained with hematoxylin and eosin (HE). The sliced samples were observed with a optical microscope. The extracted stomach parts were cut fine with a scalpel, added with lysis buffer, and homogenized at 4°C. The samples were separated by centrifugation at 13,000 rpm, 4°C for 10 minutes. The Bio-Rad protein assay kit was added to the upper liquid phase, and their protein absorbance was measured at 595nm. Using the measured absorbance, the upper liquid was mixed with a proper amount of distilled water (D.W.) and sample buffer to ensure the samples have the same protein concentration. The prepared samples (18.5) were loaded into 10%-12% gel and electrophorized. In a stirrer, they were blocked at one hour interval using a blocking buffer (1X PBST, skim milk, sodium azide), and the blocking buffer was replaced with a new one twice. NFkB p65, iNOS, Nrf-2, and β-actin Anti body (Ab) were left at 4°C overnight. Ab was purchased from Santacruze. They were rinsed for 7 minutes 5 times using 1X PBST (10X PBST, D.W, 0.1% Tween 20), and the secondary Ab was reacted at the room temperature on the stirrer for 2 hours. Using ECL, they were expressed and analyzed[10,11], and their expression levels were measured with Image J software.

3.1 Gastric tissue bleeding

The right picture in Figure 1 shows the inside of the stomach of the rats that were under waterlogging stress for 6 hours. The dark spot on the lining of the stomach is caused by gastric mucosal bleeding. The table on the right shows the results of measuring the gastric mucosal bleeding spot using Image J.

With the naked eyes, you can see that the conditions of the gastric mucosal bleeding spots of the substance administered groups are better than those of the vehicle group. The quantitative results of the bleeding spots also show that the number of bleeding spots of the vehicle was 614.6 ± 6.5, while that of the 200 mg/kg
**berchemia berchemiifolia** extract administration group (4Xberchemia berchemiifolia), 226.3 ± 8.6, and the 50 mg/kg **berchemia berchemiifolia** extract administration group (1Xberchemia berchemiifolia), 137.2 ± 8.8. The results of statistical analysis also indicate that the 200 mg/kg and 50 mg/kg **berchemia berchemiifolia** extract administration groups show significantly lower bleeding than the vehicle group (p<0.01).

### 3.2 HE-stained gastric tissues

Figure 2 shows the HE-stained gastric tissues (epithelial tissues on the top, and connective tissues on the bottom). The gastric mucosa of the normal group kept the epidermal cells of the epithelial tissues in good conditions, while the considerable amount of the epidermal cells of the vehicle group were damaged and broken away. In the case of the 50 mg/kg **berchemia berchemiifolia** extract administration group, the epidermal cells in the gastric mucosa tissues were broken away more significantly than those of the normal group, but compared with those of the vehicle group, their conditions were relatively good. In the case of the 200 mg/kg **berchemia berchemiifolia** extract administration group (4Xberchemia berchemiifolia), however, their conditions were significantly improved, as good as those of the normal group. Compared to the vehicle group, their cells were markedly less damaged.

### 3.3 Protein expression in gastric tissues

Figure 3 shows the analysis results of protein expression in gastric tissues using the western blot technique. The protein expression in the normal group was set as the base level (=1), and compared with other groups. Except the low-concentration **berchemia berchemiifolia** extract administration group, all the experimental groups showed lower iNOS expression than the vehicle group.

In the case of NF-κB protein expression in gastric mucosa tissues, the 200 mg/kg and 50 mg/kg administration groups were approximately 38%(0.62) and 50%(0.4) lower than the vehicle group (1.00) respectively (p<0.05). The iNOS expression in the 50 mg/kg administration group was approximately 30%(0.70) lower than the vehicle group (1.00), and the Nrf2 expression in the 200 mg/kg and 50 mg/kg administration groups were approximately 22%(0.77) and 25%(0.74) lower than the vehicle group respectively (p<0.05).

### IV. Figures and Tables

**Figure 1.** Measurement of gastric injury lesion

The gastric injury lesion size was quantified by measuring each lesion along its greatest diameter. Each groups of total pixel density expressed and averaged as the lesion index. Positive, Ranitidine 3 mg/kg; *, p<0.05; **, p<0.01, Analyzed by ANOVA and Duncan’s multiple range test.
Gastric ulcer caused by gastric mucosal damage, despite the use of medication and enhancers, results in complications such as bleeding, perforation and stricture, and often recurs after treatment. Thus, in addressing these issues, substances that function as a defense agent and reduce inflammatory responses to gastric mucosal damage based on the existing medication for regulating gastric juice secretion can be candidate substances of experimental significance.

V. Discussion

Gastric ulcer caused by gastric mucosal damage, despite the use of medication and enhancers, results in complications such as bleeding, perforation and stricture, and often recurs after treatment. Thus, in addressing these issues, substances that function as a defense agent and reduce inflammatory responses to gastric mucosal damage based on the existing medication for regulating gastric juice secretion can be candidate substances of experimental significance.
The Effect of Berchemia Berchemiifolia to Moderate Gastric Mucosal Bleeding Caused by the Exposure to Acute ...

This study was conducted to test the efficacy of orally-administered berchemia berchemiifolia extract to restore damaged gastric mucosa, and to test protein expression, and significant results were found after observing inflammatory responses.

To reveal the treatment effect on gastric mucosal damage, acute stress caused by waterlogging was imposed on animals to trigger gastric mucosal damage artificially. After the experiment, gastric tissues of each group were taken pictures of, and HE-stained to check changes in mucosal tissues. To reveal the defensive efficacy of the extract against inflammatory responses, protein expressions (NF-κB, iNOS, and Nrf-2) were tested.

In terms of histological changes, as shown in Figure 2, hemorrhagic erosion and damaged microvilli were found in the epithelial tissues of the vehicle group, and this mucosal barrier injury is attributable to the blocking of prostaglandin secretion caused by inflammatory responses. This is the result of blocking of the cell signaling proteins (COX, NF-κB etc.) in the process of the transition of arachidonic acid to prostaglandin[12]. On the contrary, in the experimental groups, similar results to the normal group were observed, and in particular, in the high-concentration administration group, gastric mucosal damage seemed to be very low.

Lipid peroxidation by oxygen free radical causes the collapse of the membrane structure of gastric mucosal cells. I-κB (inhibitory protein) is attached to NF-κB (transcription factor) in cytoplasm, and when the cytoplasm is stimulated, I-κB is separated from NF-κB and resolved, and the remaining p50-p65 heterodimer moves to a nucleus. This movement triggers the synthesis of various inflammatory expression factors in a nucleus, and the expression of the protein is generally increased on areas damaged by acute inflammation[13]. Figure 3.A shows that NFκ-B expression was noticeably lowered in the berchemia berchemiifolia extract administration groups compared to the control group, and from this fact, it can be inferred that berchemia berchemiifolia extract functions as an inhibitory agent against the expression of gastric irritation.

Nitric oxide (NO) is a free radical involved in inflammatory diseases, and NO synthase (NOS) enzyme is associated with the production of NO[14]. This study aimed to reveal the inflammation inhibiting effect of the reduced expression of inducible Nos (iNOS). Figure 3.B shows that there was no noticeable effect of inhibiting expression in the low-concentration berchemia berchemiifolia extract administration group, but effect in the high-concentration group was 22% compared to the control group, which indicates that berchemia berchemiifolia extract is effective against the expression of inflammation in gastric mucosa.

Nrf2 stands for “Nuclear factor (erythroid-derived 2)-like 2;” and it is an antioxidant protein, a family of basic leucine zipper (bZIP) proteins. Nrf2 is expressed under oxidative stress caused by inflammation[15]. The excessive expression of this protein is a proof of the accelerated oxidative stress. In this study, Nrf2 was quantitatively analyzed to reveal that the oral administration of berchemia berchemiifolia extract can control the oxidative stress caused by stimulation. Figure 3.C shows that compared to the control group, a statistically significant reduction of Nrf2 expression was found in the berchemia berchemiifolia extract administration groups. From this fact, it can be inferred that berchemia berchemiifolia extract is effective to inhibit oxidative stress.

VI. Conclusion

Using an animal model of acute waterlogging stress, this study reviewed the impact of berchemia berchemiifolia ethanol extract on bleeding caused by gastric ulcer, inflammatory expression in tissues, and the expression of inflammatory proteins (iNOS, Nrf2, NF-κB). In the 200 mg/kg and 50 mg/kg berchemia berchemiifolia extract oral administration groups, the level of bleeding and inflammatory expression were noticeably lower than in the control group, which indicates the high efficacy of berchemia berchemiifolia extract to improve gastric ulcer symptoms. In the test of the expression of the three proteins that indicate the level of anti-oxidation and inflammation, the berchemia berchemiifolia extract administration groups show lower expression levels. From this fact, the oral administration of berchemia berchemiifolia extract increases antioxidative activity, and inhibits inflammatory signalling, and thus it can be incurred that the extract increase the anti-ulcer effect. Therefore, berchemia berchemiifolia is expected to be a candidate substance for new drugs and health foods for the treatment of gastritis and gastric ulcer.

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References


DOI: 10.9790/3008-1106015964 www.iosrjournals.org 63 | Page
The Effect of Berchemia Berchemiifolia to Moderate Gastric Mucosal Bleeding Caused by the Exposure to Acute …


