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The Effect of *Berchemia berchemiaefolia* Extract in a Rat Model Acetic Acid-induced Irritable Bowel Syndrome

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Abstract

This study was conducted to reveal medicinal effect of Berchemia berchemiaefolia (BB) extract for induced IBS-rat model. IBS was induced by injecting acetic acid into target of Spraque-Dawley rats' colon. Control group and experimental group were categorized as follows; Group I: control group (n=5), Group II: induced IBS (n=5), Group III: induced IBS+BB LD_3 extract oral administration (n=5), Group Ⅳ: induced IBS+BB LD_{10} extract oral administration (n=5). The measurement of AWR was performed to determine the presence of disease leading and its alleviation. Also, we analyzed hematological factors, liver functional test, inflammatory factors, stress related factors, western blot and investigated significant differences in the variables among four groups.

This research result shows that feeding orally BB extract to induced IBS rat alleviates violent contraction of colon of those rats.

Keywords: *Irritable Bowel Syndrome, Berchemia berchemiaefolia, AWR, CRD, nrf2, iNOS, p38, cortisol, serotonin, IL-1β, IL-6, TNF-α*

1. Introduction

Irritable Bowel Syndrome (IBS) is the most common digestive illness which is due to violent contraction of colon and the functional colon trouble which lower the quality of life sharply. IBS symptom include: abdominal pain, bloating, discomfort, alteration of bowel habits [1]. Women are approximately two times more likely to have IBS than men [2]. Since the mid 80s, the westernization of Korea diet is driving the increase in the number of IBS patients.

Researchers still do not know what is causing IBS. While the cause of IBS is unknown, a disruption of the brain-gut axis (BGA) is thought to be important factor [1, 2]. Visceral hypersensitivity by disruption of BGA is believed to be a key underlying mechanism that causes pain [3]. According to the study [4], IBS is characterized by an overactivation of the hypothalamic-pituitary-adrenal axis and a proinflammatory cytokine increase. The risk of developing IBS increases six fold after acute gastrointestinal infection. As other risk factors, Genetic, environmental, and psychological factors seem to be important in the development of IBS [5].

IBS is a symptom-based diagnosis characterized by chronic abdominal pain, bloating, discomfort, and alteration of bowel habits [1]. But, for that reason a diversity of factors as colon hypersensitivity, activated immune system, and intestinal bacteria flora, medical

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treatment is difficult [6]. Available IBS therapies were diet, medication, psychotherapy, stress relief, and probiotics. Of these, commonly used medicines were antidiarrheal and serotonin agonist. Unfortunately serotonin agonists like 5HT3 led to a few side effects [6]. Therefore some research teams try to cure IBS by a new experiment using a medicine prepared from crude drugs. In our research IBS was induced by injecting acetic acid into target of Spraque-Dawley rats' colon. On the assumption that Persistent stimulation can confuse brain-gut axis, and we injected acetic acid into the colon of SD rat for four weeks [7].

Berchemia berchemiaefolia (BB) is Korea's natural monument. Traditionally *Berchemia berchemiaefolia* has been used for food additives for hundreds of years. In Korea' traditional remedy BB is believed to have anti-inflammatory effect. This plant was one of our novel natural drug candidates. Our ongoing study using *Berchemia berchemiaefolia* showed anti-inflammatory effect for rheumarthritis.

To find out effects of *Berchemia berchemiaefolia* we carried out behavior test, electric physiologic test, hematologic test, biochemical test, hormone test, inflammatory test, and western blot. Until now, there were few safe, effective, natural extract for IBS treatment. We believe that the result of the study will be helpful basic data to later research.

2. Materials and Methods

Animals

20 male Sprague-Dawley rats (aged at 4 weeks of 120 g averaged body weight) were purchased from central lab. Animal Inc (Seoul, Korea). Each animal was individually housed in a cage under standard laboratory conditions of 12/12 hours light/dark cycle at 25℃ and 60% humidity and was allowed to access food and water ad libitum for 2 weeks. All experiments were approved by the Ethics Committee of Catholic University of Pusan and were in accordance with the guidelines of the International Association for the Study of Pain (IASP).

Irritable Bowel Syndrome Leading

Method of disease leading was used modificated method of Fang-Yuan [8]. The animal model of IBS visceral hypersensitivity was induced by intracolonic infusion of 0.5% acetic acid (5 ㎕/g *rat weight*) in saline per day for 2 weeks.

Berchemia berchemiaefolia Extract

Roots of *Berchemia berchemiaefolia* were purchased from oriental medicine store in Pusan. We rinsed its roots clean, dried it in the shade for a week, chopped roots into small pieces by grinder, and chopped sources were lyophilized by freeze dryer. After filling 15 mL tube with 1 milligrams of lyophilized sample and 10 milligrams of 70% ethanol, tube whirled into rounded mixed machine for 18 hours. Only supernatant was collected, vaporized in 40℃ and also lyophilized. Yield of extracted material was 5.6%.

Study Design

Control group and experimental group were categorized as follows; Group Ⅰ: control group (n=5), Group Ⅱ: induced IBS and water oral administration (n=5), Group Ⅲ: induced IBS and BB (0.086 mg/g *rat weight*) extract oral administration (n=5), Group Ⅳ: induced IBS and BB (0.285 mg/g *rat weight*) extract oral administration (n=5). Oral administration dosage of *Berchemia berchemiaefolia*(BB) was used 0.086 mg/g *rat weight* and 0.285 mg/g *rat weight* based on lethal dose for 50 percent kill of *Berchemia floribunda*.[9] Time schedule as follows[Figure 1]: 1~2 weeks - executing colon irritation by acetic acid, 3~4 weeks – resting

time, then measured visual examination of abdominal withdrawal reflex, 5~8 weeks - feeding natural sample once a day, afterward measured visual examination of abdominal withdrawal reflex and electric physiologic test, finally sacrificed.

Blood Sampling and Processing

Under sedation with ether, approximately 5 mL blood was taken from abdominal aorta. Complete blood count test was measured using LC-600 (HORIBA, Japan) immediately. The rest sample was centrifuged, and then the serum was separated and stored at - 80℃ until assayed. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were measured using Cobas 6000 analyzer series (Roche diagnostics, Switzerland) on the principle of Chemiluminescence immunoassay (ECLIA). Inflammatory marker, high sensitivity C-reactive protein test and D-dimer test were measured using CHI7600-210 (Hitachi, Japan) on the principle of ECLIA [10]. Serum cortisol and IgE level was measured using Beckman Coulter AU5800 chemistry system on the principle of ECLIA. Serum IL-6, TNF-α, and IL-1β were measured using ELISA kit (R&D system, America). 1 mL blood was centrifuged 1500g for 10 minutes, then Platelet poor plasma (PPP) was collected. Serum serotonin (5-HT) level in PPP was measured using high performance liquid chromatography.

Measurement of Response to Graded Colorectal Distention

Visceral hypersensitivity was measured by grading the response of rats to colorectal distention (CRD) [11, 12, and 13]. First of all, rats were weakly sedated with ether. Then a flexible balloon made of a surgical glove finger attached to 3-way coked valve was inserted 8 cm into the descending colon and rectum via the anus. SD rats were placed in small plastic cage and allowed to adapt for 30 mins. CRD was performed by inflating the balloon to a constant pressure measured using a sphygmomanometer connected to a pressure transducer. The balloon was inflated to various pressures: 20, 40, and 60 mmHg, for a 20 sec stimulation period followed by a 5 mins rest. Behavioral responses to CRD were measured by visual observation of the abdominal withdrawal reflex (AWR) by a blinded observer and the assignments of an AWR score were as follows [11, 13]: 0 = Normal behavior without response; 1 = Brief head movement at the onset of the stimulus followed by immobility; 2 = Contraction of abdominal muscles; 3 = Lifting of the abdomen off the platform; 4 = Body arching and lifting of pelvic structures.

Electromyography (EMG)

The visceral hypersensitivity of the SD rats to CRD was quantified by measuring the electromyography (EMG) activities of oblique muscles [14]. Shortly before sacrificing animal, Visual AWR for CRD was measured. On its state carefully, rat was under anaesthetization, and then carefully was dissected. Electrodes(needle-electrode, tungsten microelectrode 125 ㎛, 12 MΩ) were inserted into the external oblique musculature. Ground pasted to mid-distal sternum. The EMG signal was recorded for five minutes. The EMG data was measured using Keypoint Portable (Medtronic Co. America).

Tissue Harvesting

After EMG test, rat were deeply anesthetized with ether and then blood was collected and segments of liver, kidney, medulla oblongata was dissected. The tissues were stored at -80℃ before using.

Western Blot

Segments of liver, kidney, and medulla were homogenized using homogenizer (Intron Biotechnology, Gyeonggi-do, Korea) with lysis buffer (PRO-PREP^{TM}, Protein Extraction Solution). Homogenized samples were centrifuged 13,000 rpm for ten minutes. Protein absorbance of supernatant was measured using X-ma spectrophotometer (Human Cor. Korea) [15] at 595 nm. Extracting about each tissues, equal amounts of protein (50 μg) were fractionated on 10% sodium dodecyl sulfate-polyacrylamide gels in running buffer (25 mmol/L Tris, 0.25mol/L glycine, 0.1% sodium dodecyl sulfate, pH 8.3) at 90 V and then electroblotted to nitrocellulose membranes[16]. p38, iNOS, and Nrf2 were detected by monoclonal antibodies (Santa Cruz Biotech, Inc. Santa Cruz, CA). Membranes were blocked at room temperature with 5% nonfat milk in Tris-buffered saline containing 0.05% Tween-20 and then incubated overnight at 4°c with the following primary antibodies: Histone monoclonal antibody(Cell Signaling Technology, INC. USA ; dilutions, each 1:2,000, 1:500, 1:20,000) Then the membranes were washed three times in Tween-20 and incubated with the corresponding secondary antibody (Santa Cruz Biochemicals; dilutions, each 1:8,000, 1:3,000, 1:10,000) conjugated to horseradish peroxidase at room temperature. Immunoreactive bands were visualized with the chemoluminescence kit (Santa Cruz Biochemicals) according to the manufacturer's instructions. Band intensities and molecular weight were quantified by using a Vision Works Image Software (UVP, Cambridge, UK) [17. 18].

Data Analysis

All results are reported as mean ± S.D. Statistical analysis was performed using SPSS 18, 1-way and 2-way analysis of variance (ANOVA) for multiple-group comparisons, Chi-square test for data count. The probability of null hypothesis < 0.05 ($P < 0.05$) was considered statistically significant.

Evaluation of Visceral Sensitivity by Abdominal Withdrawal Reflex

Before test period (before oral administration), first visceral sensitivity to CRD was determined at 8 week of age. Group Ⅱ, Ⅲ, and Ⅳ treated with acetic acid exhibited higher mean AWR scores at all distension pressures tested than Group Ⅰ (Figure 2, upper figure) ($P < 0.05$). After test period (after oral administration), second visceral sensitivity to CRD was determined at 12 week of age. Group Ⅱ treated with acetic acid exhibited higher mean AWR scores at all distension pressures tested than Group Ⅰ, Ⅲ, and Ⅳ (Figure 2, below figure). Upper results showed that acetic acid treated SD rat Group(Group Ⅱ, Ⅲ, and Ⅳ) maintained IBS-induced state. Below results showed that *Berchemia berchemiaefolia* oral feeding(Group Ⅲ and Ⅳ)was effective to reduce visceral sensitivity for IBS-induced SD rat.

Evaluation of Visceral Sensitivity by EMG Activity in the External Oblique Muscle

As mentioned above, visceral sensitivity to CRD was determined at shortly before sacrifice. EMG results were described by Area under the curve (AUC) Figure 3) each data point was normalized to average baseline amplitude (defined as 100%). Area under the curve (AUC) was calculated for the 40-s period after CRD onset [19]. This result showed that Group Ⅱ (when 20 mmHg, 40 mmHg, and 60 mmHg, each response 175 AUC, 309 AUC, 351 AUC) still maintained a state of induced IBS. Also, Group Ⅱ EMG activity in the external oblique muscle significantly than Ⅲ and Ⅳ) ($P < 0.05$). This result was more quantitative evidence that *Berchemia berchemiaefolia* oral feeding(Group Ⅲ and Ⅳ)was effective to reduce visceral sensitivity for IBS-induced SD rat.

Hematological Marker and Serum IgE level

Complete blood count result showed that all groups were not significantly differences (Table 1). IgE levels in Group Ⅰ, Ⅲ, Ⅳ were significantly lower than those of Group Ⅱ (***P***<0.05)(figure 4). Only platelet function test of Group Ⅱ was significantly higher than those of other Groups.

Inflammatory Marker

Liver function test was fulfilled to investigate hepatotoxicity of for SD rat. There were no significant differences in AST, ALT, and ALP levels among four groups (***P***>0.05) (Table 3). Inflammatory markers were hsCRP, D-dimer, and cytokine (IL-1β, IL-6, TNF-α) of 12 week aged rat. Alos, hsCRP levels in Group Ⅰ, Ⅲ, Ⅳ were significantly lower than those of Group Ⅱ (***P***<0.05) (Table 2). But, D-dimer levels in Group Ⅰ, Ⅲ, Ⅳ were not significantly difference with those of Group Ⅱ (***P***<0.05). Otherwise all Cytokine levels in Group Ⅰ, Ⅲ, Ⅳ were significantly lower than those of Group Ⅱ (IL-1β:70.5 ng/mL, IL-6:109.1 ng/mL, TNF-α: 34.5 ng/mL) (***P***<0.05) (Figure 5).

Serum Hormone Level

Serum cortisol level(91.6μg/dL) of GroupⅡ was very higher than those of GroupⅠ(8.5μg/dL), GroupⅢ(35.5μg/dL) and GroupⅣ(21.1 μg/dL) (***P***<0.01). Similarly Serum 5-HT(serotonin) level(256.5μg/mL) of GroupⅡ was higher than those of GroupⅠ(115.1 μg/mL), GroupⅢ(181.5μg/mL) and GroupⅣ(155.8μg/mL) (***P***<0.05) (figure 6).

Western Blot

The iNOS, nrf2, and p38 of each organ (medulla oblongata, liver, and kidney) were analyzed by western blotting (Figure 7). When Compared with the controls(GroupⅠ) (100%), nrf2 expression(180%) of GroupⅡ in medulla oblongata was significant difference in comparison with GroupⅣ(90%) (***P***<0.05). But, It was not significant difference in comparison with GroupⅢ(170%)(***P***<0.05)(Figure 7a). nrf2 expression(177%) of GroupⅡ in liver was significant difference in comparison with and GroupⅢ(0.83) and GroupⅣ(110%). Similarly, nrf2 expression(119%) of GroupⅡ in kidney was significant difference in comparison with and GroupⅢ(0.71) and GroupⅣ(0.61%). iNOS expression(195%) of GroupⅡ in medulla oblongata was not significant difference in comparison with GroupⅢ(222%) and GroupⅣ(179%) (***P***>0.05). iNOS expression(285%) of GroupⅡ in liver was not significant difference in comparison with GroupⅢ(228%) and GroupⅣ(232%) (***P***>0.05). But unlike in medulla oblongata, iNOS expressions of GroupⅢ and GroupⅣ in liver and kidney showed a falling trend(figure 7b). Lastly p38 expression(254%) of GroupⅡin medulla oblongata was significant difference in comparison with GroupⅢ(140%) and GroupⅣ(164%) (***P***<0.01, ***P***<0.05). Also, Those(156%) of kidney was significant difference in comparison with GroupⅢ(95%) and GroupⅣ(100%) (***P***<0.05)(Figure 7c). On the contrary, Those(191%) of liver was significant difference in comparison with GroupⅢ(169%) and GroupⅣ(159%) (***P***<0.05)(Figure 7c).

3. Discussion

Previous studies have shown that Being infused rat pup with saline containing 0.5% acetic acid intracolonically provoked Irritable Bowel Syndrome of laboratory animals [20]. In our study, a new IBS model of visceral sensitivity dysfunction was established via a lot of stimulation using acetic acid [22]. Some difference between previous studies and our research are animal age and the number of stimulus. Although the younger animals are performed, the more possible they have IBS, our study used increasing the number of stimulus instead. Thankfully causing IBS was successful and real duration inducing IBS was above six weeks (Figure 2, 3). Similar researches in the way that animal used adult rats were different from our study in the way increasing the number of stimulus [21]. After BB extract oral administration, evaluation of visceral sensitivity by AWR was a little ambiguous because AWR of GroupⅢ was not clear-cut difference with GroupⅡ (Figure 2 bellow). Consequently our study used more accurate EMG method as alternative method [22, 23]. The result of electromyographic activity in the external oblique muscle to graded colorectal distension showed clear-cut difference between GroupⅢ and GroupⅡ. As a result, it is thought that *Berchemia berchemiaefolia* alleviates visceral sensitivity causing acetic acid. Against all expectations, hematological marker was not significant difference among any Groups except platelet function test (PFT) (table 1). Probably a rise in PFT of GroupⅡ was expected to be connected with 5-HT level (serotonin) and this question was later research task. IgE level, on the other hand, was significant difference between GroupⅡ and the others Groups (figure 4). Animal in pro-inflammatory state increases IgE, with reason, activated mast cells due to cytokine release histamine [24]. But this research was not distinction among distribution of hemogram of Groups. Previous study showed that the role of gastric mast cells, enterohromaffin-like cell, play a leading part in regulation of IgE [24, 25] Perhaps a rise of serum IgE level was expected to be caused by topical location of enterohromaffin-like cell or lack of systemic inflammation in our study. We identified unforeseen hepatic necrosis and dysfunction of BB extract by measuring live function test(ALP, ALT, AST) before confirming inflammatory state of animals[28](Table 3). These results showed that therapeutic dose of BB extract is proper. This study showed that level of inflammatory markers (hsCRP, IL-1, IL-6, and TNF-α) of groupⅡ were elevated than those of the other groups (Figure 2, 5). The result indicated that inflammatory status in GroupⅡ caused by acetic acid had being maintained continuously and on the other hand GroupⅢ and Ⅳ which was induced IBS four weeks ago was not inflammatory status caused by acetic acid. Previous research was mentioned that a dysfunction of brain-gut interaction was positive correlation with inflammatory state [13, 19, 22, 24, 26, and 27]. This research also was guessed that inflammatory state caused dysfunction of brain-gut interaction. Minutely describing, HsCRP level of GroupⅡ was significant higher than those of GroupⅢ,Ⅳ and this point was evidence that mast cell such as EC-like cell and fat cell received a stimulus from IL-6[29]. Proinflammatory cytokine such as IL-1β and TNF-alpha is a cytokine which promotes systemic inflammation [30]. Due to their proinflammatory action, these cytokine tend to make a disease worse by producing fever, inflammation, tissue destruction, and, in some cases, even shock and death [30, 31] Previous study mentioned that blocking IL-1 or TNF has been highly effective in patients with inflammatory bowel disease [31, 32]. We suggest that oral administration of BB extract prevent proinflammatory cytokine activating because these level of GroupⅢ andⅣ were remarkable lower than those of Group Ⅱ in our study. IL-6 is a cytokine that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine and when especially other tissue damage leading to inflammation, IL-6 also plays a role in

fighting infection [35]. Perhaps this result was evidence that increased level of IL-6 played a role as fighter against IBS (figure 5). These pro-inflammatory cytokines, in turn, increase indolamine 2, 3 dioxygenase (IDO) activity in liver, lack tryptophan that was in charge of a biochemical precursor of serotonin and less central availability leads to alterations in serotonin transmission [37]. As noted above, increased 5-HT(serotonin) in GroupⅡ level might be reasonable(figure 6) and these hormone test was measured in order to examine different way as looking at hypothalamic–pituitary–adrenal axis [33]. Physical stress such as intracolonic infusion of 0.5% acetic acid activate the HPA axis [38]. It was known that Cortisol was increased because of activated HPA axis and serotonin was increased to be active neurotransmitter concerned in mediating stress [34, 37]. Also previous studies also showed that cortisol and 5-HT (serotonin) level of IBS patients have higher those of non-IBS patients [33, 34]. Similarly two hormones level of GroupⅡ induced IBS was higher those of GroupⅢ, Ⅳ that expected to healed IBS by natural extract(Figure 6). The result of nrf2 western blot showed that nrf2 expression of GroupⅢ and Ⅳ was lower than nrf2 expression of GroupⅡ in each organs except GroupⅢ in medulla oblongata(figure 7a). We have grave doubts as to be the constant existence of reactive free radical produced by physical stress in GroupⅡ[39] and to be decreased state of reactive free radical in GroupⅢ, Ⅳ because feeding natural extract alleviated physical stress of IBS-induced rat. Considering result of medulla oblongata deeply involved with nrf2, *Berchemia berchemiaefolia* may be more effective in high dosage. Inducible NOS(iNOS) was activated by proinflammatory cytokines (IL-1, TNF alpha and IFN gamma) and produced a lot of quantities of NO upon stimulation[40]. However, in contrast with result of cytokines, iNOS expression was not significant difference among experimental Groups and showed a tendency that iNOS expression of GroupⅡ were a little higher than those of other Groups(figure 7b). The result of p38 western blot showed that p38 expression of GroupⅢ and Ⅳ was significant lower than p38 expression of GroupⅡ in medulla oblongata and kidney(figure 7c). Previous studies showed that IL-1βinduced p38 expression and activating condition was usually oxidative stress, DNA damage, UV condition[41, 42]. Considering result of IL-1βand p38, It was also evidence that extract of *Berchemia berchemiaefolia* alleviated stress of IBS-induced rat and improved continued inflammatory condition.

4. Conclusion

In summary, *Berchemia berchemiaefolia* extract oral administration reduced inflammatory condition of IBS-induced experimental Group, decreased oxidative stress by inflammatory condition and balance proper level of stress related hormone. Consequently, *Berchemia berchemiaefolia* extract was effective in improving IBS.

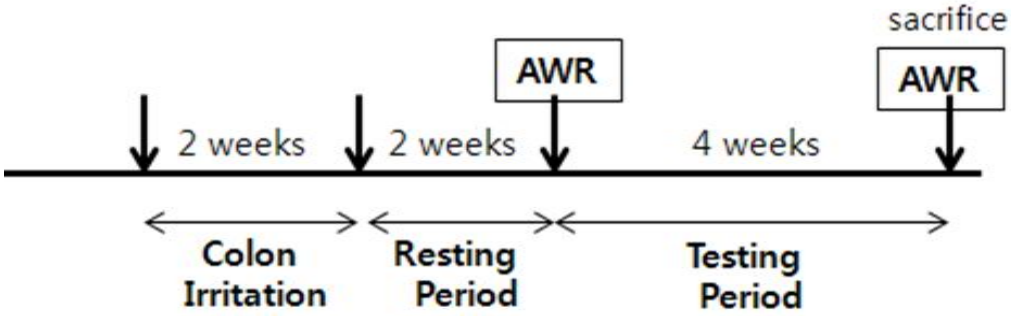

Figure 1. Timelines for Colon Irritation and Testing for Rats. Colon Irritation had been Continued in Aged 6 Weeks Rats for 2 Weeks It was Followed by a Resting Period of 2 Weeks. Before Sacrifice, visual AWR and EMG were measured. Abbreviation: AWR, abdominal withdrawal reflex

Table 1. Hematological Markers of the Study Population

Variable	Group			
	Group Ⅰ	Ⅱ	Ⅲ	Ⅳ
Erythrocyte (×10^4 /mm³)	811.5±28.4	841.6±29.1	825.1±11.4	841.3±22.8
Leukocyte (×10^2/mm³)				
Neutrophil	39.1±6.5	35.7±4.1	35.2±3.7	42.5±6.6
Lymphocyte	11.5±2.5	16.7±3.1	13.1±2.8	12.9±1.9
Monocyte	2.9±1.1	3.9±0.7	3.6±0.6	3.5±0.6
Eosinophil	1.8±0.3	1.8±0.9	2.1±0.5	1.8±0.7
Basophil	1.4±0.5	1.6±0.9	1.2±0.4	1.6±0.5
Platelet (×10^4/mm³)	91.4±7.5	90.5±11.1	89.5±5.1	86.4±7.2
PT (INR)	1.9	1.7	1.8	1.7
aPPT (INR)	2.0	1.8	2.1	1.8
PFT (ARU)	265.1	345.9*	259.9	263.2

Data art expressed ± SD. ***, P***<0.05 (compared with Group Ⅰ, Ⅲ and Ⅳ).
Abbreviation: ARU, aspirin reaction unit; INR, international normalized ratio; PT, prothrombin time; aPPT, activated partial thromboplastin time; PFT, platelet function test.

Table 2. Inflammatory Markers of the Study Population

Variable	Group			
	Ⅰ	Ⅱ	Ⅲ	Ⅵ
hsCRP (mg/dL)	0.20±0.38	6.14±0.76*	0.89±0.51	0.41±0.38
D-dimer (ng/mL)	323.4±16.33	351.1±51.28	331.9±11.12	333.6±13.17

Data art expressed SD. ***, *P*<0.05 (compared with Group Ⅰ, Ⅲ and Ⅳ).
Abbreviation: hsCRP, high sensitivity C-

Table 3. Liver Function Test of the Study Population

Variable	Group			
	Ⅰ	Ⅱ	Ⅲ	Ⅵ
ALP (IU/L)	504.12±8.80	492.64±7.76	478.71±8.14	493.5±7.31
ALT (IU/L)	81.43±1.15	78.14±2.26	76.51±0.95	80.89±1.01
AST (IU/L)	49.12±2.07	51.85±1.71	37.61±1.25	25.99±1.60

Data art expressed SD.
There were no significant differences in AST, ALT and ALP levels among four groups (***P*>**0.05).
Abbreviation: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

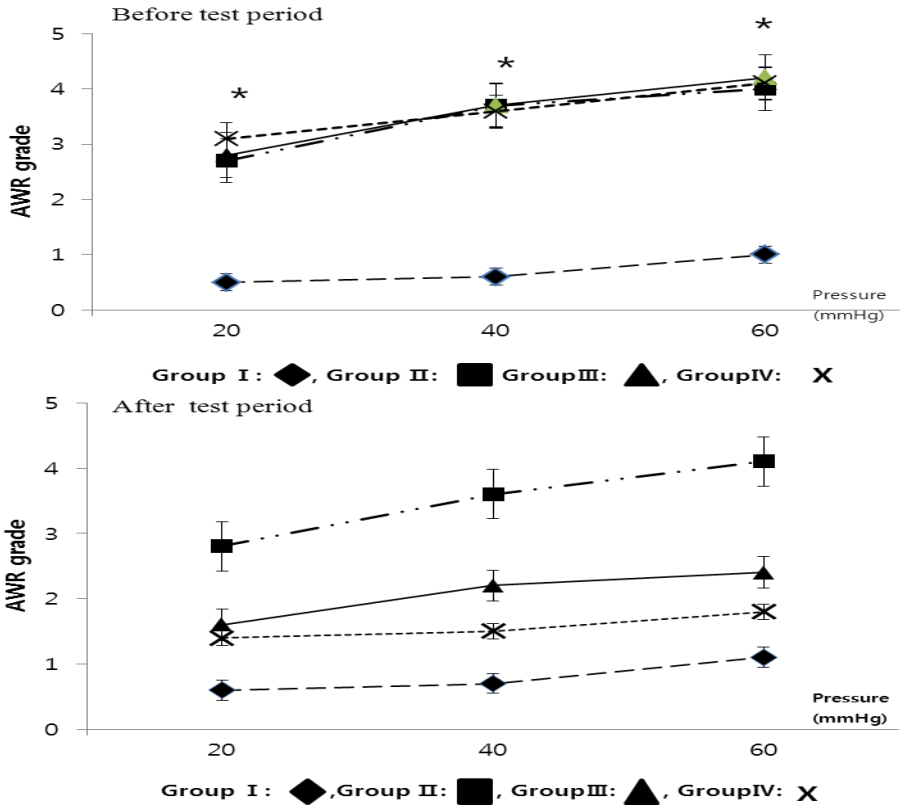

Figure 2. Evaluation of Visceral Sensitivity by Abdominal Withdrawal Reflex (AWR)

Abdominal withdrawal reflex (AWR) scores were used as an index in response to distension pressure. Before test period: AWR grades of Group Ⅱ, Ⅲ and Ⅳ were significantly higher than those of Group Ⅰ. *****, ***P***<0.05 (compared with Group Ⅰ). After test period: .AWR grades of Group Ⅱ were significantly higher than those of Group Ⅰ, Ⅲ and Ⅳ.
Group Ⅰ: Control (not IBS) Group Ⅱ: induced IBS and water oral administration (n=5), Group Ⅲ: induced IBS and BB (0.086 mg/g *rat weight*) extract oral administration (n=5), Group Ⅳ: induced IBS and BB (0.285 mg/g *rat weight*) extract oral administration (n=5).

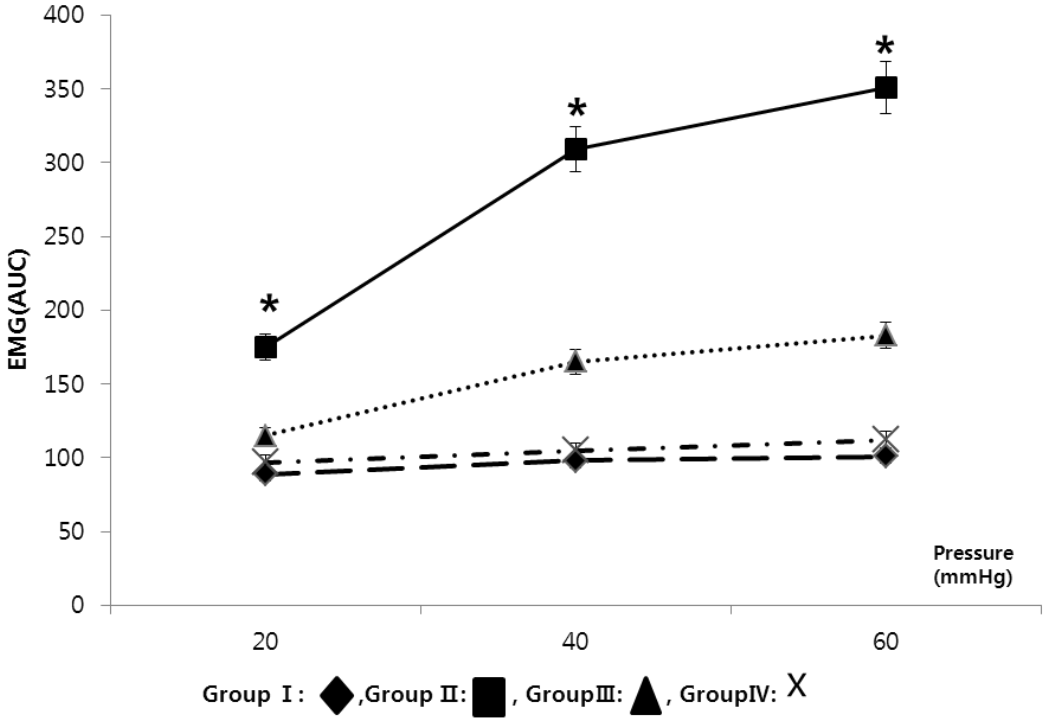

Figure 3. Electromyographic (EMG) Activity in the External Oblique Muscle in Response to Graded Colorectal Distension

Area under the curve AUC) of EMG activity in the external oblique muscle in response to graded colorectal distension.
Group Ⅰ: Control (not IBS) Group Ⅱ: induced IBS and water oral administration (n=5), Group Ⅲ: induced IBS and BB (0.086 mg/g *rat weight*) extract oral administration (n=5), Group Ⅳ: induced IBS and BB (0.285 mg/g *rat weight*) extract oral administration (n=5).

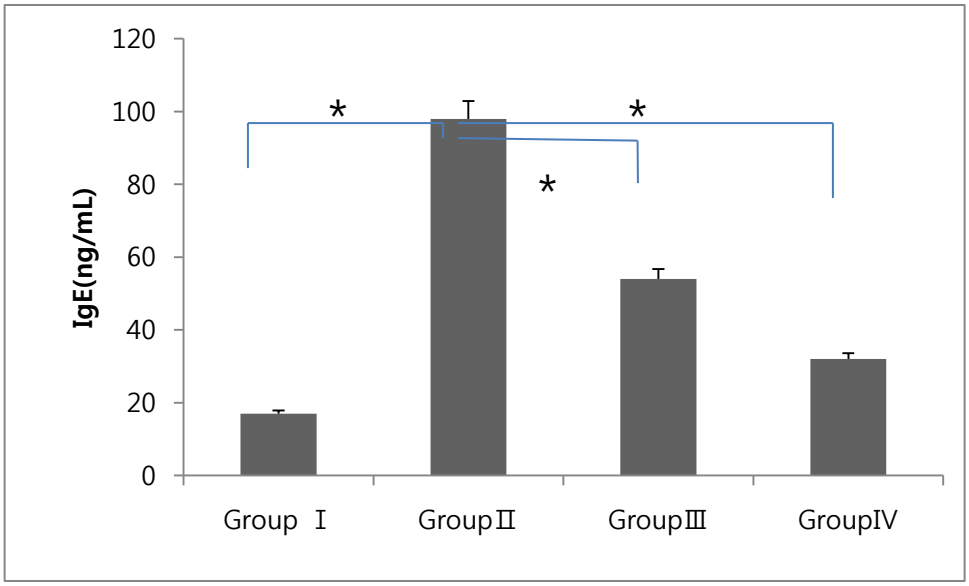

Figure 4. IgE level of Four Groups

IgE levels in Group Ⅰ, Ⅲ, Ⅳ were significantly lower than those of Group Ⅱ (***, *P***<0.05, compared with GroupⅡ).

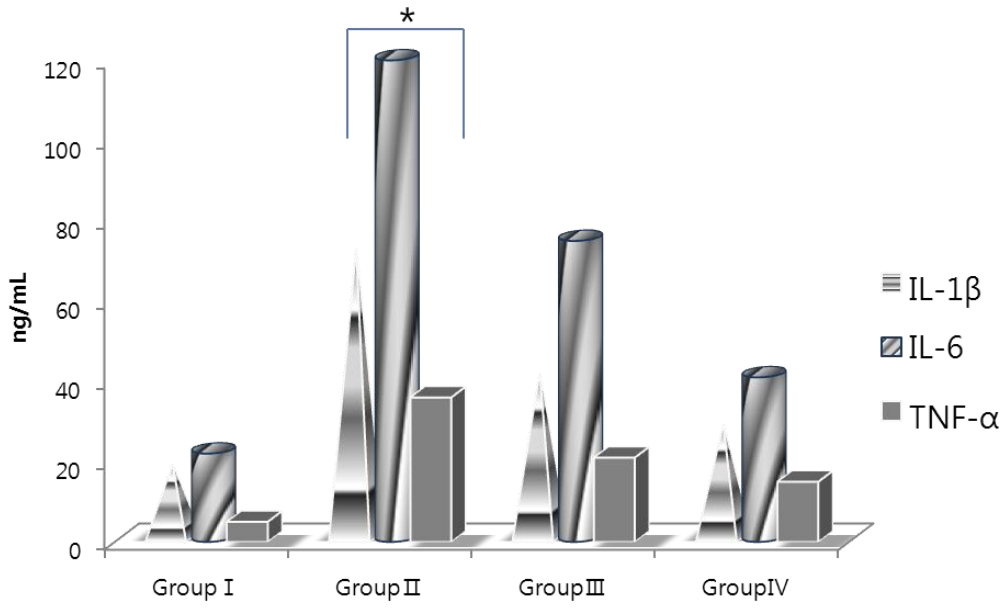

Figure 5. Cytokines Concentrations of Four Groups

Cytokines(IL-1β, IL-6, TNF-α) levels in Group Ⅲ, Ⅳ were significantly lower than those of Group Ⅱ (***, *P***<0.05, compared with GroupⅡ).

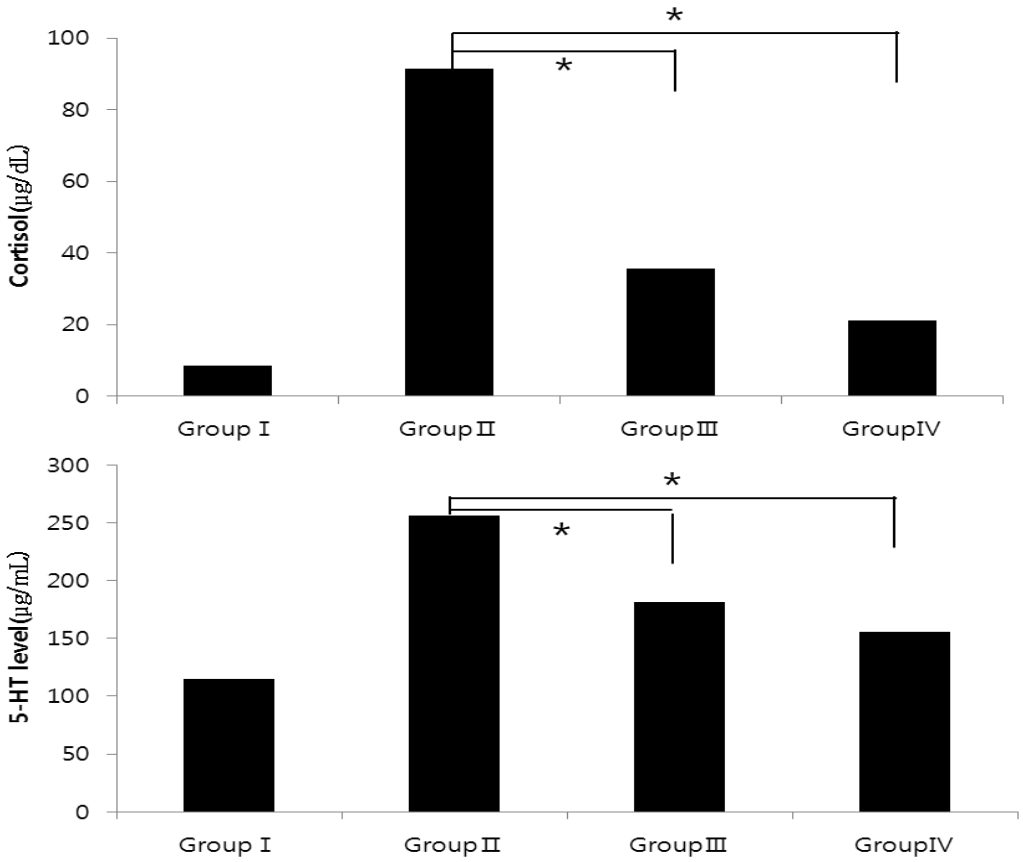

Figure 6. Level of Serum Hormone

The cortisol levels in Group Ⅲ and Ⅳ were lower than those of Group Ⅱ (***, P***<0.01, compared with Group Ⅱ). Serotonin levels(5-HT) in Group Ⅲ and Ⅳ were lower than those of Group Ⅱ (***, P***<0.05, compared with Group Ⅰ and Ⅲ).

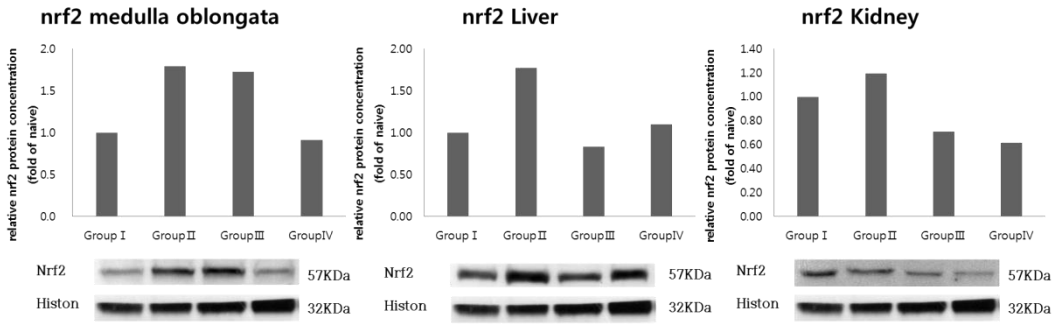

Figure 7 a

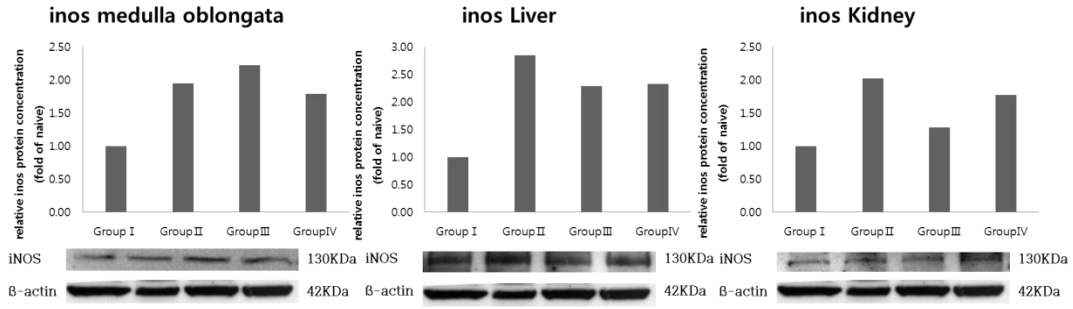

Figure 7 b

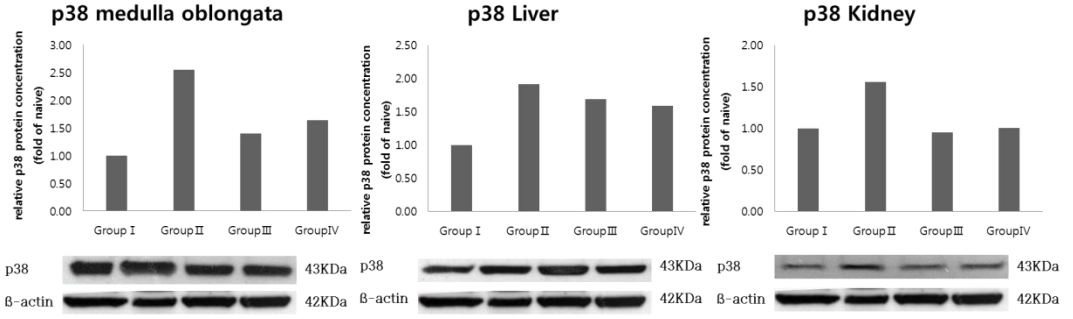

Figure 7 c

Figure 7. Protein Expression of Each Organ

a. nrf2 expression of medulla oblongata, liver, and kidney. nrf2 expression of GroupⅢ and Ⅳ was lower than nrf2 expression of GroupⅡ in each organs except GroupⅢ in medulla oblongata. b. iNOS expression of medulla oblongata, liver, and kidney. c. nrf2 expression of medulla oblongata, liver, and kidney. p38 expression of GroupⅢ and Ⅳ was significant lower than p38 expression of GroupⅡ in medulla oblongata and kidney.

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