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Anti-inflammatory Effect of Extract of Berchemia Berchemiaefolia in Rat Model with Irritable Bowel Syndrome

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Abstract. We feed orally *Berchemia berchemiaefolia* extract to Induced Irritable Bowel Syndrome (IBS) rat for four weeks. This research investigated animal behavior, animal electromyogram, inflammatory factors, cytokines, and biological factors of samples. This research result shows that feeding orally *Berchemia berchemiaefolia* extract to induced IBS rat alleviates violent contraction of colon of those rats.

Keywords: IBS, Irritable bowel syndrome, cytokine, western blot, AWR, animal model, anti-inflammation, medulla

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. Stress is one of the most forceful factors for cause of IBS. This physical and metal stress provide a stimulus to pituitary gland , and increase cortisol levels in the bloodstream. Prolonged stress status can lead to confusion of brain-gut axis, this confusion results in keen rectal senstion, finally abnormal contraction of rectal muscle [1]. Recently some research team try to cure IBS by a new experiment using a medicine prepared from crude drugs. This study was conducted to reveal medicinal effect of *Berchemia berchemiaefolia* (BB) extract for induced IBS-rat model.

2 Methods

1) Animal Groups
Animal: 5-weeks age female Spraque-Dawley rat
Extract oral administration: *Berchemia berchemiaefolia* 70% EtOH extract lethal dose 3%: 0.086 mg/g(rat weight), lethal dose 10%: 0.285 mg/g(rat weight) Control group and experimental group were categorized as follows; Groupl: control group (n=5), Group ll: induced IBS (n=5)

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GroupIII: induced IBS + extract oral administration(0.086 mg/g(rat weight)) (n=5); BB1
GroupIV: induced IBS + extract oral administration (0.285 mg/g(rat weight)) (n=5); BB2
2) Disease leading and physiological testing
a) Disease leading
-IBS was induced by injecting acetic acid into target of Spraque-Dawley rats's colon for two weeks [2].
b) Physiological testing
- The measurement of AWR was performed to determine the presence of disease leading and its alleviation.
- Evaluation of IBS levels: 1st (behavior test) -> quantitative analysis: electric physiologic test[3]
3. Hematologic examination and biochemical test
a) Hematologic factors – CBC, Hematocrit, oagulation factor, latelet aggregability test
b) Biochemical test
- anti-inflammatory factor, IgE, liver function test: using ECLIA
- IL-6, TNF-α, IL-1β: using ELISA kit
- iNOS, p38, Nrf2: using Western blot

3 Results

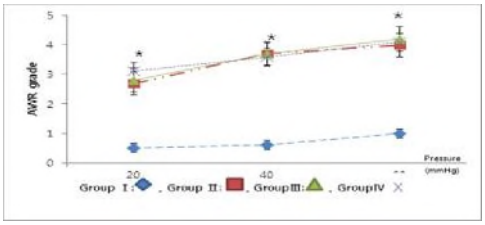

Fig. 2. First evaluation of visceral sensitivity in four groups.

Abdominal withdrawal reflex (AWR) scores were used as an index in response to distension pressure. AWR grades of GroupII, III and IV were significantly higher than those of GroupI. *****, ***P***<0.01 (compared with Group I). GroupI, Control (not IBS); Group II,(only IBS); Group III, IBS+BB1; Group IV, IBS+BB2.

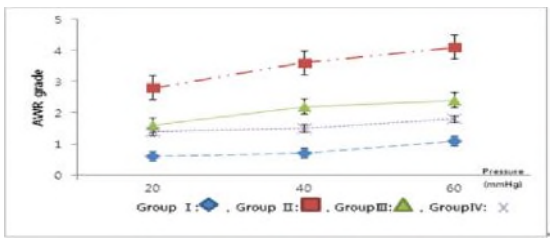

Fig. 3. Second evaluation of visceral sensitivity in four groups.

Abdominal withdrawal reflex (AWR) scores were used as an index in response to distension
pressure. AWR grades of GroupII were significantly higher than those of GroupI, III and IV. GroupI, Control (not IBS); Group II, (only IBS); Group III, IBS+BB1; Group IV, IBS+BB2.

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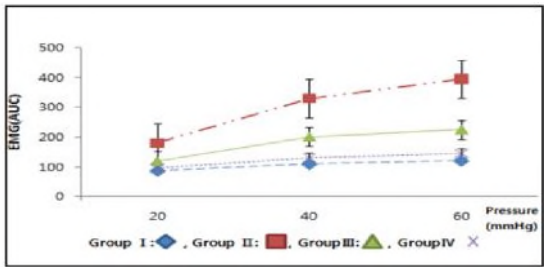

Fig. 4. Electromyographic (EMG) activity in the external oblique muscle in response to graded colorectal distension.

Area under the curve AUC) of EMG activity in the external oblique muscle in response to graded colorectal distension. GroupI, Control (not IBS); Group II, (only IBS); Group III, IBS+BB1; Group IV, IBS+BB2

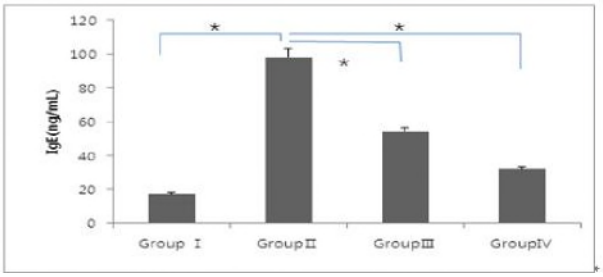

Fig. 8. Allergy-related marker (IgE) of four groups.

IgE levels in Group I,III,IV were significantly lower than those of Group II (***, *P***<0.05, compared with GroupII). GroupI, Control (not IBS); Group II, (only IBS); Group III, IBS+BB1; Group IV, IBS+BB2

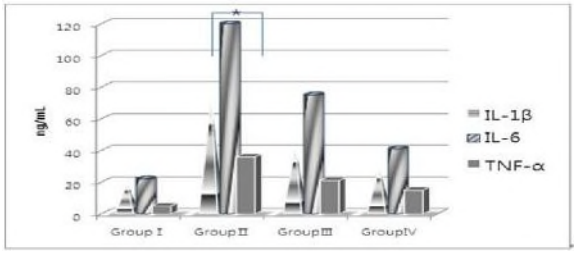

Fig. 12. Cytokines concentrations of four groups.

Cytokines(IL-113, IL-6, TNF-α) levels in Group III,IV were significantly lower than those of Group II (*, P<0.05, compared with GroupII).GroupI, Control (not IBS); Group II, (only IBS); Group III, IBS+BB1; Group IV, IBS+BB2

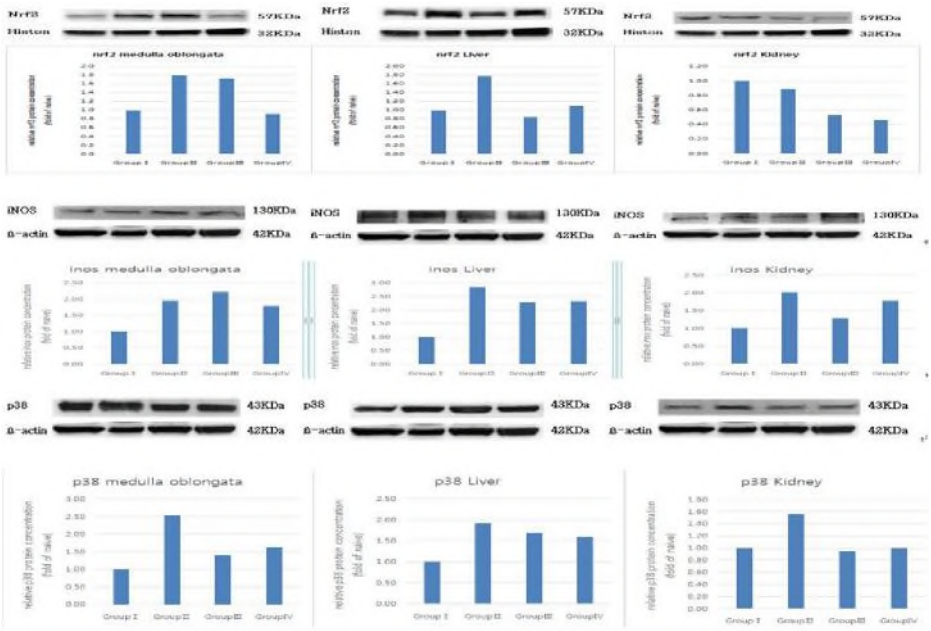

Fig. 9-11. Protein Expression in the medulla, liver and kidney

Nrf2 and p38 of GroupII were significantly higher than those of GroupI and III in three organs. There were no significant differences in iNOS levels among three groups with the exception of GroupI. GroupI, Control (not IBS); Group II, (only IBS); Group III, IBS+BB1; Group IV, IBS+BB2

4 Conclusions

Before feeding orally BB extract to rat, abdominal withdrawal reflex (AWR) test had been done. the result was that AWR of GroupI was lower than AWRs of GroupII, GroupIII, and GroupIV(fig 2) (P<0.05). After feeding orally BB extract, the result was that all Groups except GroupII showed a sharp decline of AWR(fig 3) (P<0.05). In electric physiologic test, the result also was that all Groups except GroupII showed a sharp decline of AWR(fig. 4)(P<0.05). After considering three factors, we reached this conclusion that feeding orally *Berchemia berchemiaefolia* extract to induced IBS rat alleviates violent contraction of colon of those rat and treatment can be more effective when its extract level is high(0.285 mg/g(rat weight)). In

Hematological markers of the study, the result of platelet function test was that result of GroupⅡexclusively was slightly difference (P>0.05) the rest didn't exist significance difference. As before AST, ALT, ALP and inflammatory factors between group comparison didn't be significance difference. Although immunoglobulin E(IgE), Cytokines(IL-1β, IL-6, TNF-α) levels of GroupⅡ was higher than level of BB extract dosage group and was significance difference(fig. 8, 12)(P<0.05). According to this results, It is expected that a rise in cytokines is causative of elevated colon sensibility. To find out characteristics of related inflammation factors on three organs(liver, kidney, medulla oblongata)[4], we carry out western blot of iNOS, p38, Nrf2[6]. As a result, levels of GroupⅡ(Nrf2, p38) was higher than the other groups. Generally Nrf2 ,the master regulator of the total antioxidant system that is available in all human cells[7], levels of GroupⅡ was in high concentration except Nrf2 levels of groupⅠin kidney. P38 ,mitogen-activated protein kinases that are responsive to stress stimuli such as cytokines, levels of GroupⅡ also was in high concentration(fig. 9.,11)[4,5]. But iNOS, enzymes catalyzing the production of nitric oxide (NO) from L-arginine, levels of GroupⅡ was irregular(fig. 10). Probably GroupⅡ animals had the highest stress exposure and its condition is directly attributable to secrete cytokines. Hematological results showed no change in distribution of macrophagpcyte, the defensive nitric oxide produced by macrophages reacted accordingly[1,5].
In short, *Berchemia berchemiaefolia* extract of high dose(0.285 mg/g(rat weight)) control effectively Nrf2 and p38 passway, maintain optimal levels of cytokines in body , We can draw a conclusion that its extract have Anti-inflammatory effect in rat model with irritable bowel syndrome.

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