원발성 월경통에 대한 桂枝茯苓丸 열수 추출물의 진통 효과

대구한의대학교 한의과학부 부인과학실
조수연, 김동철

ABSTRACT

Analgesic Effects of Gyejibokryeong-hwan Aqueous Extracts on the Rat Model of Primary Dysmenorrhea

Su-Yun Cho, Dong-Chul Kim
Dept. of Korean Obstetrics & Gynecology, College of Korean Medicine, Daegu Haany University

Objectives: The objective of this in vivo study is to observe the analgesic effects or improvements of Gyejibokryeong-hwan aqueous extracts (GJBRHe) on the primary dysmenorrhea (PD) in rats as compared to those of Indomethacin (IND).

Methods: The rats were administered with estradiol benzoate for 10 days and oxytocin 1 hour after the last 10th administration of estradiol benzoate to make the primary dysmenorrhea rat model. Gyejibokryeong-hwan aqueous extracts 500, 250 and 125 mg/kg were orally administered, for 10 days once a day. Then the changes on the body weights and gains during experimental periods, uterine weights and gross inspections, abdominal writhing response for analgesic activities, uterus lipid peroxidation (malondialdehyde (MDA) levels), antioxidant defense system - glutathione (GSH) contents, activities of superoxide dismutase (SOD) and catalase (CAT). Nuclear factor-κB (NF-κB) and Cyclooxygenase (COX)-2 mRNA expressions were monitored with uterus histopathology and immunohistochemistry for tumor necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS). The results of Gyejibokryeong-hwan aqueous extracts were compared to those of Indomethacin administered rats.

Results: As results of estradiol benzoate and oxytocin treatment, noticeable decreases of body weights and gains, uterus GSH contents, SOD and CAT activities, increases of abdominal writhing responses, uterus lipid peroxidation (MDA level), uterus weights, NF-κB and COX-2 mRNA expressions were observed with increases of TNF-α and iNOS immunolabeled cells, inflammatory cell infiltrations, congestion and enlargement of the uterus at gross and histopathological inspections. These means classic inflammatory and oxidative stress mediated primary dysmenorrhea are relatively well induced. However, these signs were favorably and dose-dependently inhibited by administration of three different dosages of Gyejibokryeong-hwan aqueous extracts, but lesser than those of Indomethacin.

Conclusions: The results obtained in this study suggest that Gyejibokryeong-hwan aqueous extracts has favorable analgesic and refinement activities dose-dependently on the estradiol benzoate and oxytocin treatment-induced primary dysmenorrhea signs.

Key Words: Gyejibokryeong-hwan (GJBRH), Primary Dysmenorrhea, Oxidative stress, Inflammation, Indomethacin

Corresponding author(Dong-Chul Kim) : Pohang Korean Hospital of Daegu Haany University, 907-8, Daegam-dong, Nam-gu, Pohang-si, Gyeongsangbuk-do, Korea
Tel : 054-271-8002 Fax : 054-281-7464 E-mail : kdc072@dhu.ac.kr
I. Introduction

There are two types of dysmenorrhea, primary and secondary\(^1,2\). Secondary dysmenorrhea is associated with pelvic pathology including adenomyosis, endometriosis, and less common than primary dysmenorrhea\(^1,2\). Primary dysmenorrhea (PD) is painful menstruation with no detectable organic disease in pelvis\(^1,2\). PD is one of the most common gynecological problems in young female, and more common in adolescents than adults\(^1,2\). Its prevalence varies between 45% and 95\%.\(^3\) Epidemiological studies show that 15% of female adolescents have severe PD\(^9\). The etiology of PD has not been clearly elucidated\(^1,2,6\). Some studies show that PD may be due to increased or abnormal uterine activity by uterine prostaglandins\(^1,2,7\).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are common drugs used to treat PD\(^6\). So we selected Indomethacin (IND) as reference drug in the present study. Their effects are rapid, but NSAIDs have many side effects on the kidneys, liver, and digestive tract\(^6-10\). Considering these disadvantages, herbal medicine can be a feasible alternative.

Gyejibokryeong-hwan (GJBRH, Guizhifuling-wan in China and Keishibukuryo-gan in Japan) is a polyherbal prescription that has been commonly used to treat gynecological diseases including uterine disorders and blood stasis syndrome, listed in 《金匱要略》\(^11-3\). Studies have reported effects of GJBRH on several conditions, including endometriosis\(^4\), adenomyosis\(^5\), etc.

Therefore, in this study, we intended to observe the possibilities that GJBRH has favorable analgesic effects on the rat model of PD, as compared to those of IND (NSAIDs, a non-selective COX inhibitor).

II. Materials and methods

1. Animals and husbandry

Total seventy-three healthy female Sprague-Dawley (SD: Crl:CD1) rats (6 weeks old: OrientBio, Seungnam, Gyeonggi, Korea: Body weights ranged in 140-160 g upon receipt), were used after 7 days of acclimatization. Animals were allocated 4 or 5 per polycarbonate cage in a temperature and humidity controlled room (20–25°C, 50–55%). Light and dark cycle was 12 hours : 12 hours. Standard rodent feed (Cat. No. 38057: Purinafeed, Seungnam, Korea) was supplied freely, water was supplied freely as well. Prior to this animal experiment, all laboratory animals were processed in accordance with national regulations on the use and welfare of laboratory animals, and this experiment obtained the Institutional Animal Care and Use Committee in Daegu Haany University (Gyeongsan, Gyeongbuk, Korea) [Approval No. DHU2018-034, June 04, 2018]’s approval. All animals were overnight fasted (water was not restrict,
about 18 hours) before initial administration of estradiol benzoate and test substances, and at a termination (before last 10th estradiol benzoate and test substances), to decrease individual differences from food intakes, respectively. Six groups, 10 rats in each were used in this experiment: total 50 PD rats and 10 intact vehicle rats were selected base on the body weights (average 184.03±6.95 g, ranged in 172.0-198.0 g) after 7 days of acclimatization, before initial estradiol benzoate and test substances as follows (Table 1, Fig. 1). Thirteen of the 73 rats were eliminated from this experiment.

2. Achievement of primary dysmenorrhea (PD) rat model

Rat model of PD was induced using estradiol benzoate and oxytocin (E/O)\(^{18}\). The rats were treated with estradiol benzoate (Sigma-Aldrich, St. Louise, MO, USA) by subcutaneous injection for 10 days. The injection amount of estradiol benzoate is 2.5 mg/kg on the 1st and 10th day, and 1 mg/kg on 2nd-9th day. One hour after the last administration of estradiol benzoate, oxytocin (Sigma-Aldrich, St. Louise, MO, USA) 1 U/kg was administered by peritoneal injection. Appropriate amounts of estradiol benzoate and oxytocin was dissolved or suspended in physiological saline, and treated by parenteral routes in a volume of 5 ml/kg using 26 G needles attached to 5 ml syringes. Only equal volume of physiological saline was subcutaneously treated instead of estradiol benzoate and intraperitoneally treated instead of oxytocin in intact control rats, to provide same stresses from restrain and administration, in the current experiment (Table 1, Fig. 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inducer</th>
<th>Dose of treatment drug</th>
<th>Animal ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact vehicle</td>
<td>Saline</td>
<td>5 ml/kg, distilled water oral administration</td>
<td>R01-R10</td>
</tr>
<tr>
<td>PD</td>
<td>E/O</td>
<td>5 ml/kg, distilled water oral administration</td>
<td>R11-R20</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>E/O</td>
<td>5 mg/kg oral administration</td>
<td>R21-R30</td>
</tr>
<tr>
<td>GJBRHe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The highest</td>
<td>E/O</td>
<td>500 mg/kg oral administration</td>
<td>R31-R40</td>
</tr>
<tr>
<td>The middle</td>
<td>E/O</td>
<td>250 mg/kg oral administration</td>
<td>R41-R50</td>
</tr>
<tr>
<td>The lowest</td>
<td>E/O</td>
<td>125 mg/kg oral administration</td>
<td>R51-R60</td>
</tr>
</tbody>
</table>
3. Preparation and administration of test materials

GJBRHe, brown powders, were acquired via rotary vacuum evaporator (N-1110, Eyela, Tokyo, Japan) and programmable freeze dryer (FDB-5503, Operon, Kimpo, Korea) by routine methods. GJBRHe was acquired from 20 fold (Total 800 g) of traditional composition of GJBRH, 5 types of natural drugs listed in Table 2 – *Cinnamomi Ramulus, Hoelen, Moutan Cortex Radix, Persicae Semen and Paeoniae Radix Rubra*. They were purchased from local voucher (Je-cheon-Han-bang-Yak-cho, Jecheon, Chungbuk, Korea), after confirming the morphology under microscopy. Total 800 g of mixed herbs were boiled in 10 L distilled water (for 6 hours at 100°C), and evaporated in round automated flasked evaporator (Eyela N-1110, Tokyo, Japan), and completely lyophilized. Total 105.60 g (yield = 13.20%) of lyophilized GJBRHe were obtained, and they were stored at -20°C in a refrigerator to protect them from light and humidity until used. The voucher specimens which are documenting this purchase and some specimens of GJBRH have been deposited at herbarium of the Medical Research center for Globalization of Herbal Formulation of Daegu Haany University (Code GJBRHe2018KDC). Off-white powders of IND (Sigma-Aldrich, St. Louise, MO, USA) were used as standard references for PD. They were stored in a refrigerator at 4°C in order to protect them from humidity and light until used.

GJBRHe 500, 250 and 125 mg/kg, and 5 mg/kg of IND were orally treated for 10 days once a day, 30 minutes after estradiol benzoate treatment. GJBRHe was dissolved as 100, 50 and 25 mg/ml concentrations in distilled water as vehicle, and orally administered in a volume of 5 ml/kg as equivalence to 500, 250 and 125 mg/kg, using a 5 ml syringe attached zonde. Also, IND is dissolved in distilled water as 1 mg/ml concentration and orally treated in a volume of 5 ml/kg (equivalence to 5 mg/kg) respectively. In intact and PD control rats, same volume of distilled water was orally administrated instead of test substances, for 10 days once a day in the current experiments (Table 1, Fig. 1).
The highest dosages of GJBRRHe, 500 mg/kg were selected based on previous antidepressant-like activity study in a mouse model of reserpine-induced depression\(^1\), and 250 and 125 mg/kg were selected as middle and lowest dosage using common ratio 2. In addition, the dosage of IND 5 mg/kg and oral administration were also selected based on previous anti-inflammatory efficacy studies\(^2\).

Table 2. Composition of Gyejibokryeong-hwan Used in This Study

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Scientific name</th>
<th>Korean name</th>
<th>Produce region</th>
<th>Amounts (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamomi Ramulus</td>
<td>Cinnamomum cassia Presl</td>
<td>桂枝</td>
<td>Vietnam</td>
<td>8</td>
</tr>
<tr>
<td>Hoelen</td>
<td>Poria cocos Wolf</td>
<td>赤茯苓</td>
<td>Pyeongchang, Korea</td>
<td>8</td>
</tr>
<tr>
<td>Moutan Cortex Radicis</td>
<td>Paeonia suffruticosa Andrews</td>
<td>牧丹皮</td>
<td>Jecheon, Korea</td>
<td>8</td>
</tr>
<tr>
<td>Persicae Semen</td>
<td>Prunus persica Batsch</td>
<td>桃仁</td>
<td>South Africa</td>
<td>8</td>
</tr>
<tr>
<td>Paeoniae Radix Rubra</td>
<td>Paeonia lactiflora Pallas</td>
<td>赤芍</td>
<td>Uiseong, Korea</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

4. Changes in body weights

Individual body weights were measured every day from just immediately before initial estradiol benzoate and test substance treatment (Day 0) to the last 10th test material administration, the sacrifice day (Day 9) using an electronic automatic balance (XB320M, Precisa Instrument, Dietikon, Switzerland). To decrease the individual differences, the body weight gains after 10 days of treatments were calculated as follow Equation [1].

\[
\text{Body Weight Gains (g)} = \text{Body weights at Day 9 - Body weights at Day 0}
\]

5. Measurements of uterus weights and gross inspections

All animals were sacrificed at 1 hour after oxytocin treatment, and the weights of uterus were measured at g levels (absolute wet weights) after end of gross inspections under anesthesia with 2-3\% isoflurane (Hana Pharm. Co., Hwasung, Gyeonggi, Korea) in the mixture of 70\% N\(_2\)O and 28.5\% O\(_2\) via rodent inhalation anesthesia apparatus (Surgivet, Waukesha, WI, USA) and rodent ventilator (Model 687, Harvard Apparatus, Cambridge, Cambridgeshire, UK). To decrease the differences from individual body weights, the relative uterus weights (\% of body weights) were also calculated, using body weight at sacrifice and absolute weight as follow Equation [2].

\[
\text{Relative Uterus Weights (\% of Body Weight)} = \frac{\text{Uterus weight}}{\text{Body weight at sacrifice}} \times 100\%
\]
= [(Absolute uterus wet-weights/Body weight at sacrifice (the last 10th test material administration day)×100]

6. Abdominal writhing test
Analgesic effects were monitored through abdominal writhing response according to previously established methods. Individual rats were placed in separated boxes, general rat polycarbonate cages (280×420×180 mm; DJ-102, Daejong Instrument Ind. Co., Seoul, Korea), from 3 minutes after oxytocin intraperitoneal injection. The number of abdominal writhing was recorded for 30 min. For the precision and accuracy of the results, two well-trained scientists were performed the test in a double-blind manner.

7. Measurement of uterus lipid peroxidation
Uterus preferably left uterine horn in each rats were separated at 1 hour after oxytocin treatment after gross inspections and weighed, homogenized with a buffer consisting of 10 mM sucrose, 10 mM Tris–HCl, and 0.1 M MEDTA (pH 7.4) with bead beater (Model TacoTMPre, GeneResearch Biotechnology Corporation, Taichung, Taiwan) and ultrasonic cell disruptor (Model KS-750, Madell Technology Corporation, Ontario, CA, USA), and after that centrifuged at 12,000 rpm for 15 minutes as described by Zhan and Yang. Uterus tissue homogenates were stored at -150°C using ultradepfreezer (MDF-1156: Sanyo, Tokyo, Japan) until analysis. The concentrations of uterus lipid peroxidation were acquired by estimating MDA from the thiobarbituric acid test and UV/Vis spectrophotometer (OPTIZEN POP, Mecasys, Daejeon, Korea) at absorbance 525 nm, as μM of MDA/mg protein. Contents of total protein were measured by previous method that use bovine serum albumin (Invitrogen, Carlsbad, CA, USA) as internal standard.

8. Measurement of uterus antioxidant defense systems
To measure the GSH contents, prepared homogenates were mixed with 0.1 ml of 25% trichloroacetic acid (Merck, San Francisco, CA, USA), and then centrifuged at 4,200 rpm for 40 minutes at 4°C. GSH contents were measured by spectrophotometer at absorbance 412 nm using 2-nitrobenzoic acid (Sigma-Aldrich, St. Louise, MO, USA) as μM/mg protein. And then H2O2 was decomposed in the presence of CAT at 240 nm. Amount of enzyme required to decompose 1 nM of H2O2 per minute (at 25°C and pH 7.8) was used to express CAT activity. Results were expressed as μU/mg protein. Measurements of SOD activities were made in accordance with Sun et al. SOD was estimated based on the production of superoxide radicals (generated by xanthine and xanthine oxidase), that react with nitrotetrazolium blue to form formazan dye. SOD activity was measured at 560 nm spectrophotometrically by the degree of inhibition of this reaction. And SOD activity expressed as U/mg protein. One unit of SOD enzyme activity is equivalent
to the amount of enzyme which decreases the initial absorbance of nitroblue tetrazolium by 50% for 1 minute.

9. Realtime RT-PCR analysis

The NF-κB and COX-2 mRNA expressions on the prepared left uterus horns were decided by realtime reverse transcriptase polymerase chain reaction (RT-PCR), separately based on the previous report\(^\text{25}\). RNA was extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA). The RNA concentrations and quality was measured by CFX96TM Real-Time System (Bio-Rad, Hercules, CA, USA). For removing contaminating DNA, samples were treated with recombinant DNase I (DNA-free; Ambion, Austin, TX, USA). RNA was reverse transcribed by the reagent High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions. Analysis was performed using ABI Step One Plus Sequence Detection System (Applied Biosystems, Foster City, CA, USA), and they were calculated as relative to vehicle control. The following thermal conditions were applied as 10 minutes at 94℃ and 39 cycles of 15 seconds at 94℃, 20 seconds at 57℃ and 30 seconds at 72℃. The data were normalized by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression, using comparative threshold cycle method\(^\text{26}\). The sequences of the PCR oligonucleotide primers were mentioned in Table 3.

Table 3. Oligonucleotides for Realtime RT-PCR Used in This Study

<table>
<thead>
<tr>
<th>Target</th>
<th>5′-3′</th>
<th>Sequence</th>
<th>NCBI* accession No. (production size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear factor-κB</td>
<td>Sense</td>
<td>GCGCATCCAGACCAACAATAA</td>
<td>NM_001276711 (424 bp)</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>GCAGGAAGCGATGGGACACT</td>
<td></td>
</tr>
<tr>
<td>Cyclooxygenase-2</td>
<td>Sense</td>
<td>CTGCACTGTGGCTGATGTCACT</td>
<td>S67722 (1061 bp)</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>AGGACCGCTCATCTCCAGGTTAATC</td>
<td></td>
</tr>
<tr>
<td>Glyceraldehydes 3-phosphate dehydrogenase</td>
<td>Sense</td>
<td>TGGTGAAGGTCGGGTGTAAC</td>
<td>NM_017008 (258 bp)</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>TTCCCCATTCTCAGCTCGTGCA</td>
<td></td>
</tr>
</tbody>
</table>

*NCBI*: national center for biotechnology information

10. Histopathology

Samples from uterus, the right uterine horn were crossly trimmed and fixed in 10% neutral buffered formalin. And the samples were embedded in paraffin, sectioned (3-4 μm), and stained with Hematoxylin and eosin (HE) for general histopathology\(^\text{27}\), and the histopathological profiles from each sample were monitored under optical microscope (Model 80i, Nikon, Tokyo, Japan). To more detail changes, total and mucosal thicknesses of right uterus horn (μm).
the mean numbers of inflammatory cells infiltrated in the mucosa (cells/mm²) were calculated using a automated computer-based image analyzer (iSolution FL ver. 9.1, IMT iSolution Inc. Vancouver, Quebec, Canada), in the previous method. The histopathologist was blinded to group distribution in this analysis.

11. Immunohistochemistry

The changes of proinflammatory cytokine - TNF-α and iNOS, responsible for the production of a significant amount of NO immunoreactive cells on the uterus mucosa were observed by Avidin-biotin-peroxidase complex (ABC)-based immunohistochemical methods (Table 4) using purified primary antibodies with ABC & peroxidase substrate kit (Vector Labs, Burlingame, CA, USA). In brief, endogenous peroxidase activities were blocked by being incubated in methanol and 0.3% H₂O₂ for 30 minutes, and non-specific bindings of immunoglobulin were blocked with normal equine serum blocking solution for 1 hour in humidity chamber after heating (95-100°C) based epitope retrievals in 10 mM citrate buffers (pH 6.0). Primary antisera was treated overnight in humidity chamber at temperature 4°C, and after that incubated with biotinylated universal secondary antibody and ABC reagents for 1 hour in humidity chamber at room temperature. After all, sections were reacted with peroxidase substrate kit at room temperature for 3 minutes. All sections were rinse in 0.01 M PBS for 3 times, between each step. The cells that were occupied by over 20% of immunoreactivities, the density, of each TNF-α and iNOS were regarded as positive in this study, and the numbers of TNF-α and iNOS-immunolabeled cells located in restrict view filed of uterus mucosa (cells/mm²) were measured using a computer-based automated image analyzer, in accordance with previous reports, respectively. The histopathologist was also blinded to group distribution in this analysis.

Table 4. Primary Antisera and Detection Kits Used in Immunohistochemistry

<table>
<thead>
<tr>
<th>Antiseras or detection kits</th>
<th>Code</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary antisera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-iNOS* polyclonal antibody</td>
<td>sc-651</td>
<td>Santa Cruz Biotechnology. Santa Cruz, CA, USA</td>
<td>1:100</td>
</tr>
<tr>
<td>Anti-tumor necrosis factor-α antibody</td>
<td>sc-52746</td>
<td>Santa Cruz Biotechnology. Santa Cruz, CA, USA</td>
<td>1:200</td>
</tr>
<tr>
<td>Detection kits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vectastain Elite ABC* Kit</td>
<td>PK-6200</td>
<td>Vector Lab., Burlingame, CA, USA</td>
<td>1:50</td>
</tr>
<tr>
<td>Peroxidase substrate kit</td>
<td>SK-4100</td>
<td>Vector Lab., Burlingame, CA, USA</td>
<td>1:50</td>
</tr>
</tbody>
</table>

All antisera were diluted using 0.01 M phosphate buffered saline (pH 7.2).

*iNOS : inducible nitric oxide synthase, *ABC : Avidin-biotin-peroxidase complex
12. Statistical analysis

All data were shown as mean±standard deviation (S.D.) of 10 rats. Multiple comparison tests for different dose groups were performed. Variance homogeneity was examined by the Levene test. If the Levene test didn't indicate significant deviations from variance homogeneity, the acquired data were analyzed by one way-analysis of variance (ANOVA) test, and then by least-significant differences multi-comparison (LSD) test to find out which pairs of group comparison were significantly different. If significant deviations from variance homogeneity was monitored at Levene test, Kruskal-Wallis H test that is a non-parametric comparison test was carried out. If a significant difference is monitored in the Kruskal-Wallis H test, the Mann-Whitney U (MW) test was carried out to determine the specific pairs of group comparison, that are significantly different. SPSS for Windows (Release 14.0K, IBM SPSS Inc., Armonk, NY, USA) is used for statistical analysis. In addition, the percent changes between PD control and intact vehicle were calculated for observing the severities of PD signs which were induced by estradiol benzoate and oxytocin administration in this experiment, and the percent changes as compared with PD control and test substances treated rats were also calculated for helping the understanding of the efficacy of test materials according to previous study\(^{29}\), as follow Equation [3] and [4], respectively.

\[
\text{EQUATION [3]. Percent Changes as Compared with Intact Vehicle Control (\%)} \\
= \left[\frac{(\text{Data of PD control rats}-\text{Data of intact vehicle control rat})}{\text{Data of intact vehicle control rats}}\right] \times 100
\]

\[
\text{EQUATION [4]. Percent Changes as Compared with PD Control (\%)} \\
= \left[\frac{(\text{Data of test material treated rats}-\text{Data of PD control rats})}{\text{Data of PD control rats}}\right] \times 100
\]

III. Results

1. Changes on the body weight and gains

Body weights were significantly (\(p<0.01\)) decreased in PD control from 2 days after initial estradiol benzoate treatment as compared with intact vehicle control. Accordingly, significant (\(p<0.01\)) decrease of the body weight gains for 10 days of administration period were also detected. However, significant (\(p<0.01\) or \(p<0.05\)) increases of body weights were detected from 7 days after initial estradiol benzoate administration in IND 5 mg/kg and GJBRHe 500 mg/kg administrated rats as compared to those of PD control rats, and they got significant (\(p<0.01\)) increases of body weight gains for 10 days of administration periods as compared to those of PD control rats, respectively. In addition, although no significant changes on the body weights were demonstrated in GJBRHe 250 and 125 mg/kg administrated rats, they showed significant (\(p<0.01\) or
p(0.05) increases of body weight gains for 10 days of administration periods as compared to those of PD control rats, respectively. Especially all three different dosages of GJBRHe administered rats showed clear dose-dependent favorable inhibitory effects on PD-induced body weight gain decreases, but lesser than those of IND 5 mg/kg, in this study (Table 5).

Table 5. Body Weight Gains after 10 Days of Treatment of Test Substances in Intact Vehicle or Primary Dysmenorrhea Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weights (g)</th>
<th>Body weight gains [B-A]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At initiation of test article treatment [Day 0, A]</td>
<td>At sacrifice [Day 9, B]</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact vehicle</td>
<td>169.30±4.64</td>
<td>189.90±9.43</td>
</tr>
<tr>
<td>PD*</td>
<td>169.00±9.04</td>
<td>164.60±9.62</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>168.70±7.47</td>
<td>175.20±10.20b</td>
</tr>
<tr>
<td>GJBRHe*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>169.00±6.78</td>
<td>173.10±9.02c</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>169.30±6.88</td>
<td>171.70±5.93d</td>
</tr>
<tr>
<td>125 mg/kg</td>
<td>169.20±6.68</td>
<td>170.30±5.95d</td>
</tr>
</tbody>
</table>

Values are expressed mean±S.D.
a : p<0.01 as compared with intact vehicle control by LSD test
b : p<0.01 and c : p<0.05 as compared with PD control by LSD test
*PD : primary dysmenorrhea, GJBRHe : Gyejibokryeong-hwan aqueous extracts

2. Changes on the uterus weights and gross inspections

Marked congestion and enlargement of the uterus were demonstrated in PD control as compared to those of intact vehicle control rats at gross inspections, and related significant (p<0.01) increases of the uterus absolute and relative weights were also demonstrated in PD control as compared with those of intact vehicle control, respectively. However, noticeable decreases of uterus congestions and enlargements, and related significant (p<0.01) decreases of uterus weights were observed in all test substance administered rats including IND 5 mg/kg as compared to those of PD control, respectively. Especially all three different dosages of GJBRHe administered rats showed clear dose-dependent favorable inhibitory effects on PD-induced uterus congestions and enlargements, and related uterus weight increases, but lesser than those of IND 5 mg/kg, in the present experiment (Fig. 2 and 3).
3. Analgesic activities on the abdominal writhing test

The numbers of abdominal writhing responses were significantly (p<0.01) increased. It means increases of pains were detected in PD control as compared to those of intact vehicle control. However, significant (p<0.01) decreases of the numbers of abdominal writhing responses were detected in all test material administrated rats including GJBRHe 500 mg/kg (oral administration) as compared with those of PD control. All three different dosages of GJBRHe administered rats showed clear dose-dependent favorable inhibitory effects on PD related abdominal pains, but lesser than those of IND 5 mg/kg, in the current experiment (Fig. 4).

4. Changes on the uterus lipid peroxidation

Significant (p<0.01) increases of the left uterine horn MDA contents, means increases of lipid peroxidation were detected.
in PD control as compared with intact vehicle control, but significant (p<0.01) decreases of the uterus lipid peroxidation were detected in all test materials administered rats including GJBRHe 250 mg/kg as compared with those of PD control, respectively. Especially, all three different dosages of GJBRHe administered rats showed clear dose-dependent favorable inhibitory effects on PD-induced uterus MDA content increases, the lipid peroxidation, but lesser than those of IND 5 mg/kg in this experiment (Table 6).

5. Changes on the uterus antioxidant defense systems

Significant (p<0.01) decreases of the left uterine horn endogenous antioxidant - GSH contents and endogenous antioxidant enzymes - SOD and CAT activities were demonstrated in PD control as compared with intact vehicle control, respectively. However, they were significantly (p<0.01) decreased by 10 days continuous administration of all test materials including GJBRHe 125 mg/kg as compared with those of PD control, respectively. Especially, all three different dosages of GJBRHe administered rats showed clear dose-dependent favorable antioxidant activities - increases of GSH contents, SOD and CAT activities on the left uterine horn tissues in PD rats, but lesser than those of IND 5 mg/kg, in this analysis (Table 6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Items (unit)</th>
<th>MDA* contents (μM/mg protein)</th>
<th>GSH* contents (μM/mg protein)</th>
<th>SOD† activities (U/mg protein)</th>
<th>CAT† activities (μU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact vehicle</td>
<td></td>
<td>2.66±1.79</td>
<td>12.95±4.13</td>
<td>434.76±121.47</td>
<td>130.47±48.85</td>
</tr>
<tr>
<td>PD§</td>
<td></td>
<td>2.35±1.30</td>
<td>4.16±1.33</td>
<td>157.50±25.81</td>
<td>39.60±14.31*</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td>9.85±1.53ab</td>
<td>8.47±1.73ab</td>
<td>299.30±68.62ab</td>
<td>77.95±15.61ab</td>
</tr>
<tr>
<td>GJBRHe†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mg/kg</td>
<td></td>
<td>14.57±4.25ab</td>
<td>7.44±1.44ab</td>
<td>280.20±69.03ab</td>
<td>71.75±10.59ab</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td></td>
<td>17.97±2.80ab</td>
<td>7.02±1.36ab</td>
<td>251.40±41.77ab</td>
<td>64.39±10.53ab</td>
</tr>
<tr>
<td>125 mg/kg</td>
<td></td>
<td>26.21±4.85ab</td>
<td>6.22±1.53ab</td>
<td>224.60±39.04ab</td>
<td>58.93±12.05ab</td>
</tr>
</tbody>
</table>

Values are expressed mean±S.D.

a : p<0.01 as compared with intact vehicle control by MW test
b : p<0.01 as compared with PD control by MW test

†PD : primary dysmenorrhea, †GJBRHe : Gyejibokryeong-hwan aqueous extracts
6. Changes on the uterus NF-κB and COX-2 mRNA expressions

Significant (p<0.01) increases of left uterine horn NF-κB and COX-2 mRNA expressions were demonstrated in PD control as compared with intact vehicle control, respectively. But, significant (p<0.01 or p<0.05) decreases of the uterus NF-κB and COX-2 mRNA expressions were detected in all test substance treated rats including IND 5 mg/kg as compared with those of PD control, respectively. Especially, all three different dosages of GJBRHe administered rats showed clear dose-dependent favorable inhibitory activities on the PD related uterus NF-κB and COX-2 up-regulations, but lesser than those of IND 5 mg/kg, in this analysis (Table 7).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Times</th>
<th>Left uterine horn tissues realtime RT-PCR analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NF-κB* COX-2*</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact vehicle</td>
<td></td>
<td>1.01±0.08 1.00±0.05</td>
</tr>
<tr>
<td>PD</td>
<td></td>
<td>4.88±1.36 3.61±0.41</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td>2.23±0.48ab 1.54±0.26ab</td>
</tr>
<tr>
<td>GJBRHe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mg/kg</td>
<td></td>
<td>2.70±0.55ab 2.00±0.14ab</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td></td>
<td>3.23±0.46ab 2.31±0.39ab</td>
</tr>
<tr>
<td>125 mg/kg</td>
<td></td>
<td>3.54±0.56ac 3.06±0.32ab</td>
</tr>
</tbody>
</table>

Values are expressed mean±S.D., relative to control/GAPDH mRNA expressions
a: p<0.01 as compared with intact vehicle control by MW test
b: p<0.01 and c: p<0.05 as compared with PD control by MW test

7. Histopathological inspections on the uterus

Classic inflammatory responses – edematous changes with mucosal inflammatory cell infiltrations were observed in the right uterine horn tissues after estradiol benzoate and oxytocin treatment in PD control, and consequently significant (p<0.01) increases of total and mucosal thicknesses of right uterine horn, the mean numbers of inflammatory cells infiltrated on the mucosa were demonstrated in PD control rats as compared to those of intact vehicle control rats, respectively. However, these microscopic inflammatory responses on the right uterine horn tissues were markedly inhibited by 10 days of continuous oral treatment of all test substances including GJBRHe 500 mg/kg, respectively. Especially, all three different dosages of GJBRHe administered rats showed clear dose-dependent favorable inhibitory activities.
on the PD related uterus inflammatory responses at histopathological levels, but lesser than those of IND 5 mg/kg, in the present inspections (Table 8, Fig. 5).

8. Immunohistochemical analysis on the uterus

Significant (p<0.01) increases of TNF-α and iNOS immunolabeled cell numbers on the right uterine horn mucosa were demonstrated in PD control rats as compared to those of intact vehicle control rats, respectively. However, significant (p<0.01) decreases of the right uterine horn mucosa TNF-α and iNOS immunostained cell numbers were noticed in all test substance treated PD rats including GJBRHe 250 mg/kg as compared to those of PD control rats, respectively. Especially, all 3 different dosages of GJBRHe administrated rats showed obvious dose-dependent excellent inhibitory activities on the PD related uterus TNF-α and iNOS immunoreactive cell infiltrations at immunohistochemical levels, but lesser than those of IND 5 mg/kg, in the current inspections (Table 8, Fig. 6).

Fig. 5. Representative histological images of right uterine horn tissues, taken from intact or PD rats after 10 days of continuous treatment of test substances.

A = Intact control
B = PD control (Distilled water orally treated PD vehicle control rats)
C = IND (5 mg/kg of IND orally treated PD rats)
D = GJBRHe 500 (500 mg/kg of GJBRHe orally treated PD rats)
E = GJBRHe 250 (250 mg/kg of GJBRHe orally treated PD rats)
F = GJBRHe 125 (125 mg/kg of GJBRHe orally treated PD rats)
LU : lumen, UG : uterine gland, ML : mucosa layer
All Hematoxylin-eosin stain
Dot scale bars = 720 μm. Scale bars = 60 μm.
Table 8. Right Uterine Horn Histomorphometrical Analysis after 10 Days of Continuous Treatment of Test Substances in Intact Vehicle or Primary Dysmenorrhea Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total thickness (μm)</th>
<th>Mucosa thickness (μm)</th>
<th>Inflammatory cells (cells/mm² of mucosa)</th>
<th>TNF-α*+cells (cells/mm² of mucosa)</th>
<th>iNOSⅢ+cells (cells/mm² of mucosa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact vehicle</td>
<td>1864.82±271.77</td>
<td>697.50±72.33</td>
<td>109.60±41.01</td>
<td>25.50±15.17</td>
<td>17.00±7.99</td>
</tr>
<tr>
<td>PD*</td>
<td>3603.97±283.94</td>
<td>1398.97±147.72</td>
<td>803.70±165.41</td>
<td>425.70±67.39</td>
<td>453.70±55.28</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2381.14±248.60</td>
<td>806.11±88.07</td>
<td>195.50±51.70</td>
<td>74.10±17.31</td>
<td>119.00±46.51</td>
</tr>
<tr>
<td>GJBRHe³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>2782.74±218.66</td>
<td>937.10±106.96</td>
<td>349.10±86.93</td>
<td>165.80±30.53</td>
<td>181.40±14.88</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>2964.72±168.88</td>
<td>1035.92±99.49</td>
<td>444.90±62.15</td>
<td>270.20±64.91</td>
<td>306.10±45.22</td>
</tr>
<tr>
<td>125 mg/kg</td>
<td>3107.14±161.96</td>
<td>1130.33±138.97</td>
<td>604.60±111.40</td>
<td>342.00±61.30</td>
<td>363.20±45.74</td>
</tr>
</tbody>
</table>

Values are expressed mean±S.D.

a : p<0.01 as compared with intact vehicle control by LSD test
b : p<0.01 as compared with PD control by LSD test
c : p<0.01 as compared with intact vehicle control by MW test
d : p<0.01 as compared with PD control by MW test

*TNF-α : tumor necrosis factor-α, iNOS : inducible nitric oxide synthase, PD : primary dysmenorrhea, 
³GJBRHe : Gyejibokryeong-hwan aqueous extracts

Fig. 6. Representative TNF-α and iNOS-2 immunohistochemistical images on the right uterine horn tissues, taken from intact or PD rats after 10 days of continuous treatment of test substances.

A = Intact control
B = PD control (Distilled water orally treated PD vehicle control rats)
C = IND (5 mg/kg of IND orally treated PD rats)
D = GJBRHe 500 (500 mg/kg of GJBRHe orally treated PD rats)
E = GJBRHe 250 (250 mg/kg of GJBRHe orally treated PD rats)
F = GJBRHe 125 (125 mg/kg of GJBRHe orally treated PD rats)
All Avidin-biotin-peroxidase based immunohistochemistical stain
Scale bars =120 μm
IV. Discussion

PD is defined as pain during menstruations without identifiable pathological lesion\(^{12}\). Oxytocin have strong constriction promoting effect on uterine arteries, so it is often used to induce uterine contraction in animal model (in vivo and in vitro)\(^{16}\). PD has been reported to cause an increase in lipid peroxidation, indicating oxidative stress\(^{30}\). There are reports of investigating the role of free oxygen radicals and lipid peroxidation by comparing serum nitric oxide (NO) and MDA levels in women with or without menstrual pain\(^{13}\). These studies showed that both substances can play a role in the pathogenesis of dysmenorrhea\(^{27}\). High levels of NO have been detected in various pathophysiological processes, including oxidative stress. iNOS is responsible for generating a significant amount of NO\(^{32}\). A small group of closely related transcription factors, NF-κB, is known to play an important role in modulating the expression of iNOS and COX-2\(^{33}\). NF-κB has been suggested to have roles in the pathology of PD\(^{7,27,31}\) with hormone imbalances like estrogen and oxytocin\(^{16}\). On these aspects, NSAIDs are usually applied to treat PD\(^{6}\). NSAIDs have rapid effects, but it have many side effects on the kidneys, liver, and digestive tract\(^{13-5}\). Considering these disadvantages, herbal medicine is likely to be a feasible alternative. Rat model of PD has been easily achieved by treatment of estradiol benzoate and oxytocin, and various agent have been evaluated through this animal model of PD\(^{18,27}\). In the present study, therefore, we intended to observe the possibilities that GJBRH has favorable analgesic effects or improvements on the PD in rats.

GJBRH is a well-known traditional herbal formula, comprising five medicinal herbs (40 g) - *Cinnamomi Ramulus* (8 g), *Hoelen* (8 g), *Moutan Cortex Radicis* (8 g), *Persicae Semen* (8 g) and *Paoniae Radix* (8 g), and has been used to treat uterine disorders, gynecological diseases and blood stasis syndrome in Asia\(^{11-3}\). Studies have reported effects of GJBRH on several conditions, including endometriosis\(^{14}\), adenomyosis\(^{15}\), diabetes-mellitus\(^{34}\), cardiovascular disease\(^{35}\), brain ischemia/reperfusion injury\(^{36}\), cervical cancer\(^{37}\), and growth of hepatocellular carcinoma\(^{30}\, depression\(^{37}\), and so on. GJBRH showed favorable pre-clinical toxicological profiles - no genotoxicities\(^{39}\) and the no-observed-adverse-effect level was 2,000 mg/kg/day for both sexes after 13 weeks continuous oral administrations in SD rats\(^{40}\).

There are reports about pharmacokinetic-pharmacodynamic modeling of GJBRH\(^{11}\), integrated metabolomic study\(^{42}\), integrative urinary metabolomic study\(^{43}\) of the therapeutic effect of GJBRH on the rat model of PD. And Sun L et al reported that GJBRH capsule had a significant spasmolytic effect on uterine contraction (mouse model of PD, in vivo and in vitro)\(^{44}\). Their study is similar to our study in that they induced uterine contractions with oxytocin exposure after
estradiol benzoate pretreatment, but different from our study in that they focused on uterine tissue contraction and assessed oxytocin receptors, Ca2+ level and so on. We assessed factors including antioxidant effect, anti-inflammatory effect, histopathological change and immunohistochemical change, etc.

Individual herbal agent consisted of GJBRH have stupendously various active compounds, therefore more screening of the biological active compounds in GJBRH should be investigated with more detail mechanism studies.

Estradiol has been shown to control eating and body weight mainly via modulating the potency of feedback signals that control meal size. In the current observation, marked decreases of body weights were also demonstrated from 2 days after estradiol benzoate subcutaneous treatment in PD control rats, as compared to those of intact vehicle control rats, and also significant body weight gains during 10 days of experimental periods. All three different dosages of GJBRH administered rats showed clear dose-dependent favorable inhibitory effects on PD-induced abdominal writhing response increases, but lesser than those of IND 5 mg/kg. in the present experiment. These findings are considered as reliable indirect evidences that GJBRH has favorable PD refinement effects, enough to inhibit the hormone imbalance-induced body weight changes, but lesser than those of IND 5 mg/kg, at least in a condition of this study. All rats in intact vehicle control of present study showed body weight gains which are ranged in age-matched normal standard SD rats.

Increases of abdominal writhing responses mean increases of abdominal pains. In the current analysis, significant increases of the numbers of abdominal writhing responses were detected in PD control as compared to those of intact vehicle control during 30 minutes after end of oxytocin treatment. But significant decreases of abdominal writhing responses were observed in all test material administrated rats including IND 5 mg/kg as compared with those of PD control rats, respectively. All three different dosages of GJBRH administered rats showed clear dose-dependent favorable inhibitory effects on PD-induced abdominal writhing response increases, but lesser than those of IND 5 mg/kg. in this analysis. These findings are considered as obvious and reliable evidences that GJBRH has favorable analgesic activities, enough to control PD.

Oxidative stress plays an important role in the pathophysiological process of PD. Lipid peroxidation is an autocatalytic mechanism and it results in oxidative destruction of cell membranes. MDA is the terminal product from lipid peroxidation. Therefore, the content of MDA can be used for estimating the degree of lipid peroxidation, and significant increase in uterine MDA content was observed in PD rats, and also increased in this study after estradiol benzoate and oxytocin treatment. GSH is representative endogenous
antioxidants, which keep the ROS at low levels and certain cellular concentrations to prevents tissue damage. Therefore, GSH is accepted as protective antioxidant factors in tissues\(^\text{49}\). SOD is one of the antioxidant enzyme that contributes to enzymatic defense mechanisms\(^\text{50}\). CAT is an enzyme which catalyzes the conversion of \(\text{H}_2\text{O}_2\) to \(\text{H}_2\text{O}\)\(^\text{\text{50}}\). Reduction of antioxidant enzyme activity such as SOD, CAT and GSH content may indicates failure of compensating the oxidative stress induced by PD\(^\text{27,46}\). In this experiment, PD-induced lipid peroxidation, and depletion of the uterus antioxidant defense system were clearly inhibited by 10 days continuous oral administration of GJBRHe, but lesser than those of IND 5 mg/kg. These findings are suggested that GJBRHe has favorable antioxidative activities, enough to inhibit the PD, but lesser than those of IND 5 mg/kg, at least in a condition of this analysis.

Along with oxidative stresses, inflammations also have been attention to the etiology of PD pathogenesis\(^\text{7,19,27,31}\). At gross inspections, marked congestion and enlargements of the uterus and related increases of uterus weights were demonstrated in PD control rats with noticeable increases of the right uterus horn total and mucosa thicknesses. inflammatory cell infiltrations, increases of TNF-\(\alpha\) and iNOS immunopositive cells, suggesting uterus inflammations, similar to those of previous PD animal models\(^\text{19,27,}\). However, these uterus inflammatory responses induced by estradiol benzoate and oxytocin treatment were dose-dependently and significantly inhibited by 10 days continuous oral administration of GJBRHe, but lesser than those of IND 5 mg/kg. These findings are suggested that GJBRHe has favorable anti-inflammatory activities, enough to inhibit the PD, but lesser than those of IND 5 mg/kg, at least in a condition of the present gross and histopathological inspections. Cytokines TNF-\(\alpha\) can enhance immune responses in vivo at much lower doses than causing weight loss or tissue toxicity. TNF-\(\alpha\) enhances the increase of B and T cells and promotes the production of cytotoxic T cells. Also, it improves interleukin (IL) -2-induced immunoglobulin production and increases IL-2 stimulation natural killer cell activity and proliferation of monocytes\(^\text{51}\). Enhanced NO formation following iNOS induction is associated with the pathogenesis of shock and inflammation\(^\text{52}\). NF-\(\kappa\)B coordinates the expression of proinflammatory enzymes including iNOS and COX-2\(^\text{27,33}\). It has been shown that COX-2 is partially controlled by NF-\(\kappa\)B, and inhibition of NF-\(\kappa\)B activation was related to the down regulation of the expression and synthesis of COX-2\(^\text{33}\). Well corresponded to immunohistochemical analysis to TNF-\(\alpha\) and iNOS, marked increases of NF-\(\kappa\)B and COX-2 mRNA expressions were demonstrated in the uterus of PD control rats at realtime RT-PCR analysis in the present experiment. Favorably and expectedly down-regulation of NF-\(\kappa\)B and COX-2 were simultaneously detected by treatment of GJBRHe with constant dose-
dependent patterns, but lesser than those of IND 5 mg/kg. These findings are considered as critical evidences that GJBRHe has favorable refinement activities against PD, may be through anti-inflammatory and antioxidative potentials mediated by NF-κB down-regulation, but lesser than those of IND 5 mg/kg, at least in a condition of the present analysis.

In the current study, the possible analgesic effects or improvements of GJBRHe on PD were observed in a rat model of PD achieved by treatment of estradiol benzoate and oxytocin as compared with IND 5 mg/kg. As results of estradiol benzoate and oxytocin treatment, classic inflammatory and oxidative stress mediated PD are relatively well achieved - noticeable decreases of body weights and gains, uterus GSH contents, SOD and fCAT activities, increases of abdominal writhing responses, uterus lipid peroxidation (MDA level), uterus weights, NF-κB and COX-2 mRNA expressions were observed in PD control rats with increases of TNF-α and iNOS immunolabeled cells, inflammatory cell infiltrations, congestion and enlargement of the uterus at gross and histopathological inspections. However, these inflammatory and oxidative stress mediated PD signs were favorably and dose-dependently inhibited by 10 days continuous oral administration of 3 different dosages of GJBRHe - 500, 250 and 125 mg/kg, but lesser than those of IND 5 mg/kg, at least in a condition of the present PD rat model. These findings clearly and directly suggest that GJBRHe has favorable analgesic and refinement activities on the estradiol benzoate and oxytocin treatment-induced PD signs, may be through anti-inflammatory and antioxidative potentials mediated by NF-κB down-regulation, but lesser than those of IND 5 mg/kg, at least in a condition of the present experiment. Considering the side effects of IND, the nausea and water retention, it is expected that GJBRHe will be promising as a new potent alternative analgesic or refinement agents to control various menstrual pains including PD. Since GJBRH consisted of 5 herbs and each herb includes various active ingredients, more screening of the biological active compounds should be carried out with more detail mechanism studies.

V. Conclusion

Through this study, we found the following conclusion.

1. Significant decrease of body weights and gains, which is induced by estradiol benzoate and oxytocin treatment, was significantly inhibited by all 3 different dosages of GJBRHe, dose-dependently.
2. Significant increase of absolute and relative weight of uterus because of marked congestion and enlargement of the uterus, which is induced by estradiol benzoate and oxytocin treatment, was significantly inhibited by all 3 different...
dosages of GJBRHe, dose-dependently.

3. Significant increase of abdominal writhing response, which is induced by estradiol benzoate and oxytocin treatment, was significantly inhibited by all 3 different dosages of GJBRHe, dose-dependently.

4. Significant increase of uterus lipid peroxidation (MDA contents) and significant decreases of antioxidant (GSH) and antioxidant enzymes (SOD and CAT) activities, which is induced by estradiol benzoate and oxytocin treatment, was significantly inhibited by all 3 different dosages of GJBRHe, dose-dependently.

5. Significant increase of NF-κB and COX-2 mRNA expressions by realtime RT-PCR, which is induced by estradiol benzoate and oxytocin treatment, was significantly inhibited by all 3 different dosages of GJBRHe, dose-dependently.

6. Significant increase of total and mucosal thicknesses of uterine horn, and the mean numbers of inflammatory cells infiltrated on the mucosa, TNF-α and iNOS immunolabeled cell numbers on the uterine horn mucosa, which is induced by estradiol benzoate and oxytocin treatment, was significantly inhibited by all 3 different dosages of GJBRHe, dose-dependently.

Therefore, it is considered that GJBRHe has significant analgesic effect on primary dysmenorrhea.

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□ Revised : Jul 20, 2020
□ Accepted : Aug 28, 2020
목적: 이 실험을 통해 연구의 목적은 인도메타신과 비교하여 투여 후의 원발성 월경통에 대한 개지 복통의 염수 추출물의 진통 및 개선 효과를 관찰하는 것이다.

방법: 햄트 PD 모델을 만드기 위해, Estradiol benzoate를 10일간 투여한 다음 마지막 10일 estradiol benzoate 투여 1시간 후 1 U/kg의 oxytocin을 투여하였다. 개지복통화 염수 추출물은 100, 250 및 125 mg/kg 용량으로 매일 1회 10일간 투여하였다. 이 후 채혈 및 실험 기간 동안의 체중 증가량, 자궁 중량 및 유안부경 소견, 진통 활성을 나타내는 abdominal writhing test, 자궁 조직 내 지질 과산화 (Malondialdehyde, MDA 함량) 및 흉산화 방어 시스템 - glutathione (GSH) 함량, superoxide dismutase (SOD) 및 catalase (CAT) 활성, Nuclear factor-κB (NF-κB) 및 cyclooxygenase (COX)-2 mRNA의 발현, 자궁의 조직방사학적 변화, tumor necrosis factor (TNF)-α 및 inducible nitric oxide synthase (iNOS)로 나타나는 면역조직학적 변화를 관찰하였다. 개지복통화 염수 추출물의 결과는 인도메타신 투여 후의 결과와 비교하였다.

결과: Estradiol benzoate 및 oxytocin 투여 결과, 현저한 체중 및 중체량, 자궁 GSH 함량, SOD와 CAT 활성의 감소와 abdominal writhing 반응, 자궁 중량 및 과산화 (MDA 함량), 자궁 중량, NF-κB 및 COX-2 mRNA 발현의 증가가 TNF-α 및 iNOS 면역반응세포와 염증세포 침윤 증가, 자궁의 중량 및 확대와 함께 관찰되었다. 이는 전형적인 염증 및 산화 스트레스성 원발성 월경통이 잘 유도되었음을 의미한다. 한편 이러한 소견은 개지복통화 염수 추출물의 투여에 의해 염증의존적으로 현저히 억제되었으며, 인도메타신의 억제 효과보다는 낮았다.

결론: 이 연구에서 얻은 결과는 개지복통화 염수 추출물이 estradiol benzoate 및 oxytocin으로 유도된 원발성 월경통에 염증의존적으로 유리한 진통 및 개선 활성을 가질 것을 시사한다.

중심단어: 개지복통화, 원발성 월경통, 산화 스트레스, 염증, 인도메타신

References

8. García Rodriguez LA. et al. Acute liver
23. Sedlak J. Lindsay RH. Estimation
of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman’s reagent. Anal Biochem. 1968:25(1) :192-205.


38. Park WH, et al. Tumor initiation inhibition through inhibition COX-1 activity of

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