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Original Article

# Analysis of Biological Experiments on the Anti-inflammatory and Antipyretic Effects of Hwangryeonhaedok-tang

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**Objectives:** To establish scientific and objective evidence for the use of a Korean medicine, articles regarding Hwangryeonhaedok-tang (HRHDT), a herbal medicine frequently used in Korean medical clinics and hospitals, were gathered and analyzed.
**Methods**: The articles were classified as being from domestic or international journals, and by their year of publication. The mechanisms of the anti-inflammatory and antipyretic effects of HRHDT were investigated.
**Results:** Of the 25 articles analyzed, 7 were published from Korea, 7 were from China, and 11 were from Japan. HRHDT showed anti-inflammatory and antipyretic effects through the regulation of the expression of Th1 cytokines including interleukin-2 (IL-2), IL-8, interferon-$\gamma$ (IFN-$\gamma$), and tumor necrosis factor-$\alpha$ (TNF-$\alpha$); and Th2 cytokines including IL-4, IL-6, and IL-12, which inhibit leukotriene B4 (LTB4), cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), and inflammatory cells. It also lowered preprodynorphin (PPD), and corticotropin-releasing factor (CRF) in the peripheral nerve system and hypothalamus.
**Conclusions:** We speculate that the anti-inflammatory and antipyretic effects could be related to the therapeutic efficacy of HRHDT in removing pathogenic fire and heat.

***Key Words*** : Hwangryeonhaedok-tang (HRHDT), evidence, anti-inflammatory, antipyretic, therapeutic efficacy

## Introduction

A biological experiment is a method that uses an animal or cell model to investigate the efficacy of a treatment or medicine of interest. Numerous investigators of Korean medicine have conducted biological experiments to confirm and investigate the therapeutic effect of herbal medicines or herbal formulas. However, the controversy remained regarding whether modern and scientific experimental methods could be used to investigate the therapeutic effects of traditional Korean medicine. Despite this, finding a relationship between modern biological experiments and Korean medicine would be helpful to establish foundation for the scientific and objective interpretation of Korean medicine.

Hwangryeonhaedok-tang (HRHDT) is a traditional herbal formula consisting of 4 herbal medicines i.e. Coptis rhizome, Scutellariae radix, Phellodendri cortex and Gardeniae fructus. HRHDT has been used to treat symptoms related to pathogenic heat and fire such as agitation, dry mouth, thirst, disordered speech, insomnia,

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hematemesis, epistaxis, purpura, dysentery and jaundice as it is used as a therapeutic agent to remove the pathogenic fire and heat by using 4 bitter-cold herbal medicines[1,2].

Fire and heat, pathogenic concepts used in Korean medicine, evoke a variety of symptoms and diseases when they are transformed into toxins. In the skin and subcutaneous tissue, pathogenic heat and fire cause symptoms marked by local redness, swelling, and burning pain, followed by abscess formation, fever, thirst, reddened tongue with yellow coating, and rapid pulse. When they penetrate internal organs, aggravated symptoms are produced, which are accompanied by high fever with thirst, delirium, constipation, dark-colored urine, crimson tongue with yellow coating, and rapid sunken pulse[2].

Such symptoms in Korean medicine can be related to the pyretic diseases of modern Western medicine such as inflammation. Inflammatory disease is caused by injury or infection, and is characterized by fever, swelling, redness, pain, and loss of function. The process includes increased blood flow with an influx of white blood cells and other chemical substances that facilitate healing[3]. Therefore, HRHDT could be used to treat inflammatory and pyretic diseases as well as symptoms caused by pathogenic heat and fire.

In present study, we investigate articles regarding the biological effects of HRHDT. Articles on anti-inflammatory and antipyretic effects were further analyzed to ascertain the relationship between the biological and therapeutic effects of HRHDT and its mechanisms of action.

## Materials and Methods

### 1. Searching strategy and terms

We searched a variety of Korean and foreign el ectronic bibliographic databases for papers publish ed between 1990 and the present, including the Ko rea Education and Research Information Service (KERIS), National Discovery for Science Leaders (NDSL), Korean Studies Information Service Syste m (KISS), Korean Traditional Knowledge Portal, Oriental Medicine Advanced Searching Integrated System (OASIS), PubMed, ScienceDirect, Google Scholar, China National Knowledge Infrastructure (CNKI), and Citation Information from the Nationa l Institute of Informatics (CiNii) using search term s such as “Hwangryunhaedoktang”, “Hwanglyunha edoktang”, “Hwangnyeonhaedoktang”, “Huanglianji edutang”, “Orengedokuto”, “황련해독탕”, “黃连解毒汤”, “黃連解毒湯”, and “黃連解毒湯” (Table 1).

### 2. Selection criteria and data extraction

Among the papers found, we selected 25

Table 1. Electronic Bibliographic Databases and Search Terms for Hwangryeonhaedok-tang

| Electronic bibliographic databases | Search term |
|---|---|
| Korea Education and Research Information Service http//www.riss4u.net | 황련해독탕 |
| Korean Studies Information Service System http://kiss.kstudy.com | 黃连解毒汤 |
| National Discovery for Science Leaders http://www.ndsl.kr | 黃連解毒湯 |
| Oriental Medicine Advanced Searching Integrated System http://oasis.kiom.re.kr | 黃連解毒湯 |
| Korea Institute of Science and Technology Information http://society.kisti.re.kr/main.html | Hwangryunhaedoktang |
| Korean Traditional Knowledge Portal http://www.koreantk.com | Hwanglyunhaedoktang, |
| PubMed http://www.ncbi.nlm.nih.gov/pubmed | Hwangnyeonhaedoktang |
| Google Scholar http://scholar.google.co.kr | Huanglianjiedutang |
| ScienceDirect http://www.sciencedirect.com/ | Orengedokuto |
| National Institute of Informatics http://ci.nii.ac.jp | |
| China National Knowledge Infrastructure http://www.cnki.net | |

regarding in vivo biological experiments dealing with fever or inflammatory diseases in which experiments were conducted by oral administration of HRHDT. Papers were categorized and the distribution of papers was investigated by their publication year and country of origin. From the selected papers, data were extracted as follows: target disease, animal species, and induction of symptoms, factors of treatment for parts of the body such as serum, organ, or tissue. The treatment factors were further researched to determine the mechanism of the therapeutic effect of HRHDT.

## Results

### 1. The distribution of papers by the publication year and country of origin

Most of the papers regarding the anti-inflammatory and antipyretic effects of HRHDT were published in Japan (11 papers), followed in frequency by papers from Korea and China. In Japan, the number of published papers sharply increased to the highest level in the period from 1996 to 2000 and thereafter the frequency decreased to the average publication level, which has continued up to the present. There has been a steady flow of Chinese papers published throughout the period analyzed, with an increasing frequency after 2006 to the present year. The frequency of publication of Korean papers was higher in the period from 2006 to 2010 than that from 2001 to 2005, representing an increasing publication rate lasting up to recent times (Figure 1).

### 2. Biological effects of HRHDT on inflammatory disease and fever

As shown in Table 2, treatment of inflammation-related diseases by HRHDT, such as colitis, allergic dermatitis, and inflammation induced by toxin and antipyretic effects was investigated.

1) Biological effect on colitis

Rats and mice were used as animal models of colitis. The colitis symptoms were induced mainly by trinitrobenzene-sulfonic acid (TNBS) or dextran sulfate sodium (DSS). After the administration of HRHDT, animal body weight loss was suppressed, and colon weight and intestinal wall thickness were decreased, it also inhibited the shortening of colon length[6-10,12-14].

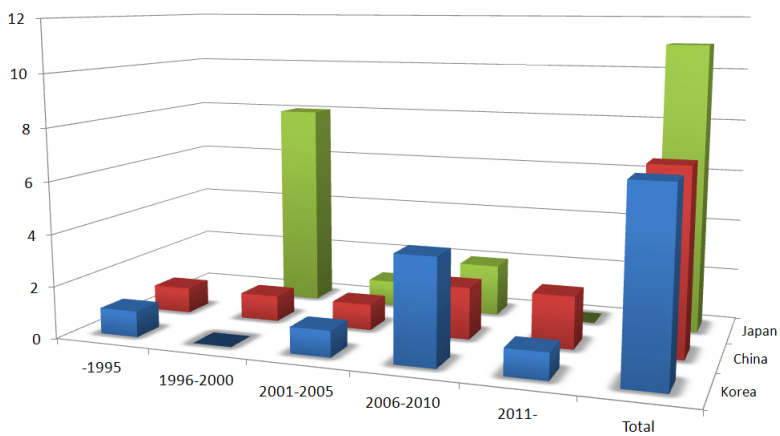


Fig. 1. Distribution of papers classified by year and country of publication

**Table 2.** Biological Effects, Symptoms, Induction Methods, Animal Models and Outcomes (Cytokines and Molecules) of Hwangryeonhaedok-tang

| Biological effect | Symptom / Induction | Animal model | Outcome (Cytokine, molecule) | | |
|---|---|---|---|---|---|
| | | | Organ & Tissue | | Serum |
| Anti-inflammatory disease | ***Colitis***<br>TNBS[5,4,6,8,11]<br>DSS[7,9,10,12-14] | Rat[4,5,8,10,11]<br>Mouse[6,7,9,12-14] | ***Colon tissue***<br>IL-1$\beta$ ↓[14]<br>IL-8 ↓[8]<br>IFN-$\gamma$ ↓[14]<br>LTB4 ↓[8]<br>PGE2 ↓[8]<br>COX-2 ↓[10]<br>MDA ↓[11]<br>SOD ↑[11,13]<br>GPx activity ↑[11,13]<br>Catalase ↑[13]<br>MPO activity ↓[8,11,12,14]<br>CD11a expression ↓[7] | ***Spleen and thymus***<br>CD4+CD8+cell ↑<br>CD4+CD8- and CD4-CD8- cell ↓[6]<br><br>***Splenic lymphocyte***<br>LFA-1 expression on CD3+lymphocyte ↓[9]<br>LFA-1 expression of CD3-lymphocyte ↑[9] | Hb ↑[9]<br>NO ↓[13]<br>PGE2 ↓[13] |
| | ***Inflammation***<br>Acetic acid[5-17]<br>Carrageenin[21,15]<br>LPS[19,22]<br>Dextran[15]<br>CFA[18]<br>D-GALN[19]<br>Dimethyl benzene[20] | Rat[15,17,18]<br>Mouse[16,19-22] | ***Spinal posterior horn neuron***<br>PPD mRNA expression ↓[18] | ***Hypothalamus***<br>CRF mRNA expression ↓[18] | IL-2 ↑[19]<br>IL-8 ↓[17]<br>IL-1$\beta$ ↓[22]<br>IL-6 ↓[22]<br>TNF-$\alpha$ ↓[22] |
| | ***Allergic dermatitis***<br>DNCB[23,25,26]<br>Dermatophagoides farinae[24] | Mouse[23-26] | ***Ear***<br>IL-4 mRNA expression ↓[24]<br>IFN-$\gamma$ ↑[24] | ***Skin***<br>SBA positive response ↑[23]<br>BrdU distribution ↓[23]<br>TUNEL and Fas positive cell ↓[23] | IgE ↓[25,26] |
| Antipyretic disease | ***Fever***<br>Yeast[15,28]<br>Typhoid-paratyphoid A-paratyphoid B triple vaccine[20]<br>Turpentine[27]<br>2,4-Dinitrophenol[28] | Rat[15,20,27,28] | ***Spleen cell***<br>IL-2 ↑[27]<br>CD4+Tcell ↑[27]<br>CD8+Tcell ↓[27]<br>CD4+/CD8+ ↑[27] | | |

TNBS: trinitrobenzene-sulfonic acid, DSS:dextransulfatesodium, $LTB_4$:leukotrieneB4, $PGE_2$:prostaglandinE2, COX:cyclooxygenase, MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, MPO: myeloperoxidase, LFA: lymphocyte function associated antigen, Hb :hemoglobin, NO: nitric oxide, LPS: lipopolysaccharide, D-GALN: D-galactosamine, CFA: complete Freund's adjuvant, PPD:preprodynorphin, CRF : corticotropin-releasing factor, DNCB: 1-chloro-2,4-dinitrochlorobenzene, SBA: soybean agglutinin, BrdU: bromodeoxyuridine, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling, ASG: acid soluble glycoprotein.

Histological damage to colon, such as edema, ulceration, hemorrhage, hyperemia and surface epithelial exfoliation recovered to normal levels, and the crypts on the colon wall were regenerated by HRHDT[5,7-10,13]. Reduced inflammatory cell infiltration, including that by polymorphonuclear cells, leucocytes, marcophages, eosinophils and lymphocytes was observed in colon tissue[4,8,9,12-14]. There were also reductions of mucosal injury, lymphatic follicle generation, and the edema of submucosa[10-12]. The regeneration of surface epithelial cells and goblet cells producing colon mucus was investigated[10].

The serum level of hemoglobin was increased

and levels of nitric oxide (NO) and prostaglandin E2 (PGE2) were decreased. The production of cytokines such as interleukin (IL)-1$\beta$, IL-8, IFN-$\gamma$ and signaling molecules (leukotriene B4, prostaglandin E2, and cyclooxygenase) in colon was inhibited tissue by administration of HRHDT. Antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx) showed increased activity while malondialdehyde (MDA) and myeloperoxidase (MPO) activities were decreased. A localized Ki-67-positive reaction was also found in colon tissue[10]. Spleen and thymus weights were recovered close to the normal state[5-9]. Proliferation of CD4+CD8+ cells in the spleen and thymus increased, whereas that of CD4+CD8- and CD4-CD8- cells decreased[6]. Lymphocyte-function-associated antigen (LFA)-1 expression on splenic CD3+ lymphocytes was decreased, but increased on CD3- lymphocytes.

2) Biological effect on inflammation

Rats and mice were pretreated with Inflammatory molecules and materials including acetic acid, dextran, dimethyl benzene, carrageenin, lipopolysaccharide (LPS), D-galactosamine (D-GALN), and complete Freund's adjuvant (CFA) to induce inflammatory disease. Administration of HRHDT decreased the swelling of hind paws and ears, and reduced exudates from the abdominal cavity in the different models[15,16,18,20,21]. Pain hypersensitivity was also decreased by HRHDT[18].

The serum level of IL-2 was increased while levels of IL-8, tumor necrosis factor (TNF)-$\alpha$, IL-1$\beta$ and IL-6 were lowered in animals administrated HRHDT. HRHDT inhibited the expression of preprodynorphin (PPD) mRNA and hypothalamic corticotropin-releasing factor (CRF) mRNA in spinal posterior horn neurons and the hypothalamus of animals in which inflammation was induced.

3) Biological effect on allergic dermatitis

Mice were used as an animal model of allergic dermatitis and pretreated with allergens such as *Dermatophagoides farinae*, and 1-chloro-2, 4-dinitrochlorobenzene (DNCB). Administration of HRHDT inhibited the swelling and thickening of the epidermis and fibrosis in the dermis of the ear[24]. It also reduced the accumulation of inflammatory cells including lymphocytes, eosinophils, and neutrophils in ear tissues[24]. Through HRHDT treatment, the thickness and edema as well as hyperplasia were decreased in neck skin epidermis, and nuclear damage and vacuolation were recovered to normal levels[23,25,26]. HRHDT treatment decreased erythematous papules in skin and resulted in the reduction of erythema[23,25], narrowed the intracellular space in skin, and ameliorated inflammatory cell infiltration[23]. Moreover, transepidermal water loss was inhibited in the animal group treated with HRHDT[23].

HRHDT regulated the cytokine levels by inhibiting the expression of IL-4 mRNA, whereas the level of IFN-$\gamma$ was increased in ear tissue. HRHDT also maintained a soybean agglutinin (SBA)-positive response and lowered bromodeoxyuridine (BrdU) distribution, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), and Fas-positive cells, resulting in a reduction of apoptosis in skin tissue. The serum level of immunoglobulin E (IgE) was reduced by HRHDT.

4) Biological effect on fever

The antipyretic effect of HRHDT has been studied using rat models pretreated with yeast, typhoid-paratyphoid A-paratyphoid B triple vaccine, turpentine and 2, 4-dinitrophenol. Rat body and ileum temperatures were decreased by

administration of HRHDT[15,20,28]. HRHDT increased the production of IL-2 and CD4+ T cells, while decreasing the production of CD+8 T cells; and the ratio of CD4+ T cells/CD8+ T cells was increased by treatment with the HRHDT formula.

## Discussion

Published articles regarding the anti-inflammatory and antipyretic effects of HRHDT were gathered and analyzed to investigate whether the reported those effects could be related to its therapeutic efficacy based of removing the pathogenic heat and fire as proposed by traditional Korean medicine.

DSS induces colonic inflammation associated with thrombus formation in arterioles and IL-1$\beta$ enhances microvascular thromboses in extraintestinal tissues during colonic inflammation by stimulation of colonic macrophages that can activate IL-1-converting enzyme and thus release mature IL-1$\beta$ into the colon mucosa[11,29]. IL-2 is a Th1-type cytokine produced mostly by activated T cells. IL-2 stimulates the proliferation of lymphocytes, macrophages, NK cells, and B cells to induce cytolytic activity or produce antibodies. IL-2 evokes a proinflammatory and anti-inflammatory response[30], and lower levels of IL-2 are observed in pyretic disease such as malignant catarrhal fever of cattle[31]. IL-4 is a critical factor for the development of type 2 immune responses; it stimulates B cells to effect immunoglobulin (Ig) switching to IgE and perform an important role in the development of allergic inflammation[32,33]. IL-6 is a major stimulator of protein produced in acute phase inflammation and its level is elevated in inflammatory diseases such as arthritis and colitis because expression of IL-6 is enhanced at the inflammation site[34]. IL-8, a neutrophil chemoattractant, is elevated in the inflammatory response of ulcerative colitis that predominantly involves IL-8-mediated neutrophil infiltration[35]. DSS also significantly increases IFN-$\gamma$ levels in colitis[36]. IFN-$\gamma$, a type of Th1 cytokine, plays a role in decreasing the endothelial barrier by downregulating intestinal endothelial tight junctions. Production of IFN-$\gamma$ and IFN-$\gamma$ secreting cells is increased in inflammatory bowel disease induced by DSS, although the level of IFN-$\gamma$ is lowered in atopic dermatitis[37-39]. TNF-$\alpha$, related to Th1 type, is a proinflammatory cytokine released by monocytes and macrophages during acute inflammation, and the tissue damage in acute or chronic inflammation responses is attributed to TNF-$\alpha$[40]. HRHDT inhibits the production of IL-1$\beta$, IL-2, IL-6, IL-8, IFN-$\gamma$, and TNF-$\alpha$ while stimulating the expression of IL-2. These findings indicate that the anti-inflammatory and antipyretic effects of HRHDT can be elucidated through the regulation of Th1/Th2 cytokines.

Leukotriene B4 (LTB4), a product of lipoxygenase, acts as one of the most potent chemoattractants and activators of polymorphonuclear leucocytes generated from the cellular and transcellular metabolism of arachidonic acid and its synthesis is increased in the colonic mucosa of patients with ulcerative colitis and Crohn's disease[41,42]. LTB4 production in inflammatory bowel disease may be a secondary event, possibly a consequence of neutrophil infiltration induced by IL-88). Cyclooxygenase-2 (COX-2), one of the two subtypes of COX, is an enzyme essential for the synthesis of prostaglandins, and is induced under inflammatory conditions, for example, in the intestine in a model of inflammatory bowel disease (IBD)[43]. Prostaglandin E2 is a lipid mediator of inflammation and exacerbates the inflammatory process in IBD[44]. These products of arachidonic acid metabolism were inhibited by the

administration of HRHDT. Thus, another anti-inflammatory mechanism of HDHRT occurs by downregulating the arachidonic acid cascade.

Reactive oxygen species (ROS) induce significant oxidative damage to cell structures and macromolecules such as DNA, RNA, proteins, and lipids, resulting in IBD[45]. Superoxide dismutase (SOD), an antioxidant enzyme that is decreased when colitis is induced by DDS or TNBS, ameliorates peroxidation reactions and colonic inflammatory changes in experimental colitis[46]. Other antioxidant enzymes such as catalase and glutathione peroxidase (GPx) are also reduced in colitis induced by TNBS[47]. In contrast, malondialdehyde (MDA), known for its role in leading to release of cell contents, cell death and damage of tissue or organ, is increased in colitis and may cause lipid peroxidation and tissue damage[48,49]. Myeloperoxidase (MPO), an enzyme released from neutrophils, is used as an index of inflammation and neutrophil influx, and is produced excessively in the inflamed mucosa, thus playing a crucial role in the pathobiology of colitis, as does MDA[50]. HRHDT increases the activity of SOD, GPx, and catalase, whereas it decreases MDA and MPO activity. These findings imply that the anti-inflammatory effect of HRHDT is produced by reducing oxidative injury caused by ROS.

LFA-1 has an important role in the activation and recruitment of lymphocytes to sites of tissue inflammation. LFA-1 initiates chronic gut inflammation by promoting naive T-cell priming/activation and expansion within mesenteric lymph nodes (MLNs) and by increasing proinflammatory cytokine production following secondary stimulation by antigen-presenting cells in the colonic interstitium[51]. HRHDT inhibits the adhesion of inflammatory cells such as leukocytes and lymphocytes to the endothelium and thus their transmigration, resulting in a reduction of inflammation.

Spinal preprodynorphin (PPD) mRNA is upregulated by peripheral inflammation in the hind paw of rats induced by complete Freund's adjuvant[52]. Corticotropin-releasing factor (CRF) is a neuropeptide and immunomodulatory factor that regulates the stress and activates the hypothalamic–pituitary–adrenal (HPA) axis[53]. Inhibition of PPD mRNA in spinal posterior horn neurons and CRF mRNA in the hypothalamus indicates that HRHDT can reduce peripheral inflammation through the regulation of spinal nervous system and neural peptide expression by the hypothalamus.

## Conclusions

We surveyed articles regarding the anti-inflammatory and antipyretic effects of HRHDT to investigate the relationship between the biological effect and therapeutic efficacy as defined in traditional Korean medicine. Most papers were published in Japan, followed by Korea and China. Studies of HRHDT have continued up to the present. HRHDT was found to have anti-inflammatory and antipyretic effects through the regulation Th1/Th2 cytokines, inhibition of the arachidonic acid cascade and inflammatory cells, and modulation of the peripheral nervous system and hypothalamus. These anti-inflammatory and antipyretic effects could be associated with the therapeutic efficacy of HRHDT as defined by traditional Korean medicine, namely removing pathogenic fire and heat.

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