Lee et al. Applied Biological Chemistry (2023) 66:52
https://doi.org/10.1186/s13765-023-00802-5

REVIEW

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Mechanism-based biomarkers for the quality control of Dangkwisoo-san: a scoping review

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Abstract

Dangkwisoo-san (DS) is a traditional Korean herbal medicine used to treat traumatic diseases, including pulmonary contusions, traumatic pneumothorax, bruising, and ankle sprain. Quality control (QC) biomarkers for DS can help ensure its safety and efficacy. Although chemical quality assessments are performed to ensure consistent efficacy of DS, the identity and quantity of the compounds contained within a given natural product is a frequent complication. We conducted a literature review to identify biological assays that support the chemical QC of DS. The results of our investigation confirmed that in vitro experiments with aqueous and alcoholic extracts of DS exhibited positive effects on many aspects of treatment. With 80% EtOH extraction, a low concentration of DS (1 μg/ml) significantly diminished the expression of inflammatory factors, such as nitric oxide (NO), TNF-α, IL-1β, and IL-6, in the Raw264.7 cell line. MeOH extracts activated NRF2 and antioxidant activities in response to the inflammatory inducer LPS, and water extracts of DS remarkably reduced proinflammatory cytokine levels compared to dexamethasone and cyclosporin treatments. Aqueous extracts of DS at a moderate dose of 125 μg/ml supported bone regeneration, recovered ischemic injury in an eNOS-dependent manner, and prevented metabolic disorders (TRPM7 channel inhibition). Cytokines, NO, and immunoglobulins are potential biological QC biomarkers to assess the anti-inflammation and immune response to DS. Future quality evaluation studies of herbal medicines (herbal prescriptions) should aim to select the mechanism-based in vitro efficacy evaluation methods that can estimate consistent clinical effects.

Keywords Dangkwisoo-san, Efficacy, Quality evaluation, Biological method, Herbal medicine

Introduction

The use of complementary and alternative medicines, including herbal medicines, has been increasing worldwide [1, 2]. Distinguished from synthetic drugs, which are targeted toward specific acute disease symptoms and pathologies, botanical drugs target a wide range of physiological processes for long-term therapies [3, 4]. Many traditional therapy systems have recorded theoretical knowledge and practical applications, such as prevention, diagnosis, and management of physical and mental disorders. Overall, 80% of the world's population residing in developing countries relies on herbal products as a primary source of pharmaceutical drugs [5–7].

Although significantly consumed worldwide, herbal medicines are facing difficulties to reach the new drug application (NDA) step of the Food and Drug

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Administration (FDA) [8]. To put this into perspective, the key issue of botanical products is to guarantee "therapeutic consistency" and present clinical evidence consistent with standard treatments in phase III clinical trials [9]. Since the FDA approval of Veregen, a green tea-derived drug, for genital wart treatment [8], few botanical drugs have met these criteria. Traditional herbal medicines contain certain extracts and compounds that affect multiple targets [10, 11], and many of them may not be identified. Typical pharmacokinetic studies cannot cover the active combinations and conflicts among them. To address this difficulty, the co-treatment of herbal medicines with commercialized drugs has been established. Recently, the combination of nelfinavir, an FDA-approved drug that effectively inhibits SARS-CoV-2, and the natural origin drug cepharanthine showed enhanced efficacy [12, 13]. Another line of research is the study of the active components of herbal remedies to eliminate potential risks. Exelon and Razadyne are herb-derived compounds that are FDA-approved for clinical trials against Alzheimer's disease [14].

For the widespread use of natural origin prescriptions, the mode of action of these drugs demands supporting scientific rationale. Unlike the vertebrate immune system, plants use phytochemical metabolites as a defense mechanism. They are rich in phenolic compounds, such as alkaloids, coumarins, flavonoids, polyphenols, and tannins, which exhibit efficacy against health disorders and pathogenic organisms [15]. These herbal substances stimulate secondary messenger systems, resulting in the activation of transcription factors of certain genes in response to specific stimulants against illness or diseases [16]. Secondary messengers, including cyclic nucleotides, lipid derivatives, ions, and intracellular signals, are small and rapidly broadcasting molecules present during signal transduction [17]. Among them, the agonist–G-protein-coupled receptor (GPCR) complex activates the adenosine 3′,5′-cyclic monophosphate (cAMP) pathway [18] and leads to the formation of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) in response to growth factors and receptor tyrosine kinase (RTK) binding [19], forming a notable messenger system that amplifies the signal following intracellular and extracellular stimulation.

Chemical quality control (QC) refers to the assessment of the chemical composition and purity of a drug or herbal medicine. Although chemical QC is important for ensuring the safety and efficacy of drugs, it has some limitations [20]. Chemical analysis does not always reflect biological activity: a compound may be present in high concentrations in a sample, but it may not be the active ingredient responsible for the biological effects of the drug. Therefore, relying solely on chemical analysis may not provide an accurate assessment of the biological activity of a drug. Chemical analysis may miss important components; some active ingredients or biomolecules may be present in small quantities or may not be amenable to detection by chemical methods. Therefore, relying solely on chemical analysis may miss the important components of the drug that contribute to its biological activity [20, 21]. Chemical analysis may not detect contaminants or adulterants, particularly if they are structurally similar to the active ingredients of the drug. Therefore, relying solely on chemical analysis may not provide an accurate assessment of drug safety and quality.

To overcome these limitations, biological QC can be performed in addition to chemical QC. Biological QC refers to the assessment of the biological activity of a drug or herbal medicine using in vitro or in vivo methods [22]. It can provide a more accurate assessment of the biological activity of a drug and can help detect potential contaminants or adulterants that may not be detected by chemical analysis. Therefore, the mechanism-based biological QC is an important complement to chemical QC for ensuring the safety and efficacy of drugs and herbal medicines.

Dangkwisoo-san (DS) is a herbal medicine used to treat various pathological conditions, such as qi movement stagnation and static blood primarily caused by trauma, such as contusions or falls [23]. This prescription was first described in Introduction to Medicine by Li Chan, a Confucian scholar of the Ming Dynasty of China. According to the composition of the prescription, *Angelicae Gigantis Radix Palva* had the highest dose among the nine herbs, followed by *Paeoniae Radix*, *Linderae Radix*, *Cyperi Rhizoma*, and *Sappan Lignum* in equal amounts, and *Carthami Flos*, *Persicae Semen*, *Cinnamomi Cortex*, and *Glycyrrhizae Radix et Rhizoma*. Clinically, these herbs are boiled with water and medicated wine at a ratio of 1:1 and provided in the form of decoction preparations; however, they are also used in various formulations, such as powder preparations [24]. DS exerts various pharmacological effects on the cardiovascular system [25], inflammatory diseases [26, 27] and cancers [28, 29]. Regarding the potential benefits and safety of natural origin medicines, including DS, in human and animal diseases, bioactive components have been verified, followed by probable modes of action and adverse reactions. To eliminate the undesired effects of the extracts, chemical modifications are employed to optimize and expand their scope of therapeutic use. In addition, dereplication methods are applied to serve proper biological functions and exclude the known active constituents of complex natural products [30].

Because obtaining a renewable supply of active compounds from biological sources can be challenging, we need a general and complete knowledge of DS component properties. This scoping review aimed to confirm how the effects of DS were evaluated in various diseases in addition to extending the knowledge of DS component properties. This was achieved through the process of identifying and comprehensively summarizing and analyzing in vitro, in vivo, and ex vivo experiments and clinical studies on DS through a systematic methodology. Our research can be expected to suggest biological assays to assess biomarkers that support chemical QC.

Search strategy and selection criteria

This review introduces the current state of cell-, animal-based, and clinical studies on target diseases and efficacy of DS by collecting, identifying, and classifying the literature with a pilot search-based search strategy. Considering that DS prescription is known by various scientific names, the following search terms were set through related reviews and databases: "dangguisoo," "dangguisu," "dangguixu," "danggwisoo," "danggwisu," "dangkisoo," "dangkisu," "dangkwisoo," "dangkwisu," "当帰須散," and "当归须散." A literature search was conducted on six databases: EMBASE, CNKI, ScienceON, KISS, RISS, and OASIS up to August 8, 2022, and there were no restrictions on language during the search process. These classification criteria were included in the review of clinical and basic laboratory studies evaluating effects based on the original regimen of DS only without other components. Studies were excluded for the following reasons. (1) research unrelated to traditional East Asian medicine; (2) studies not related to DS; (3) studies in which it is difficult to confirm the effect of DS alone (e.g., case reports in which DS and other interventions were applied simultaneously); (4) studies that included only chemical analysis without data on the effectiveness of DS; (5) non-original research (e.g. conference abstracts, editorials, letters). As for the search strategy, researchers independently reviewed and evaluated the title, abstract, and full text qualifications of the thesis, extracted data on certain items from the literature, and other researchers independently verified the results. Disagreements that occurred in the process of literature selection and data extraction were resolved through discussions among researchers. Afterward, the extracted data was summarized and presented in a table with a narrative description. Among a total of 298 articles, 35 articles (6 clinical studies, 2 ex vivo studies, 15 in vivo studies, 10 in vitro studies, and 2 in vivo and in vitro studies) were selected and included in the analysis using mentioned search strategy (Fig. 1).

Clinical studies on DS

Six clinical studies [27, 31–35] on DS were analyzed, including three randomized controlled trials (RCTs) [27, 31, 35] and three case reports [32–34] (Table 1). DS was used as a treatment for traumatic diseases, including pulmonary contusion, traumatic pneumothorax, bruising, and ankle sprain, in all studies except for one case series of peptic ulcers. In the RCT for pulmonary contusion [31], the use of DS in the form of a decoction in addition to usual care ameliorated inflammation-related indicators and reduced hospitalization times significantly compared to the control group. In two RCTs on ankle sprains, DS powder capsules were [27, 35] more effective in alleviating pain, functional abnormalities, and edema and improving quality of life than the placebo control group [27].

The preparation form of DS used in the six clinical studies was primarily a decoction of medicinal herbs (n = 3) or powder encapsulation (n = 2), and one study did not describe the form of the prepared medicine [34]. In the case of decoction, only one case mentioned preparing the herbal decoction using alcohol and water together as in the original prescription [33]. In another study using decoction [31, 32] and two studies using commercialized powdered capsules, water extraction was performed [27, 35].

The composition of the DS prescriptions used in the six clinical studies was slightly different. The content of *Angelicae Gigantis Radix Palva* was the highest, consistent with the composition of the original prescription. However, the proportions of the remaining herbs, including the four herbs listed as the second-highest proportions in the original prescription (*Paeoniae Radix*, *Linderae Radix*, *Sappan Lignum*, and *Cyperi Rhizoma)*, were heterogeneous in the literature. When the content of *Angelicae Gigantis Radix Palva* in the prescription was set as 100%, the proportion of other herbs in each study and the original prescription were calculated and are presented in Table 2.

In vivo and ex vivo studies on DS

Between 1982 and 2021, 18 studies on the efficacy of DS have been published. The efficacy of DS has been studied for various target diseases, such as bruises, hematomas, toxin coagulation, blood pressure control, fractures, wound healing and inflammation, brain damage, and liver toxicity. The details of in vivo and ex vivo studies on DS are summarized in Tables 3 and 4.

Two articles on bruises were published in 2002, and the same author evaluated the efficacy of DS by measuring the levels of lactate dehydrogenase (LDH) in the blood and a water maze test for studying the recovery of endurance exercise capacity and dehydration enzymes [36]. In another study [37], the researchers evaluated the effect of DS on blood enzyme activity in bruised rats by measuring blood levels of glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), leucine aminopeptidase (LAP), and alkaline phosphatase (ALP).

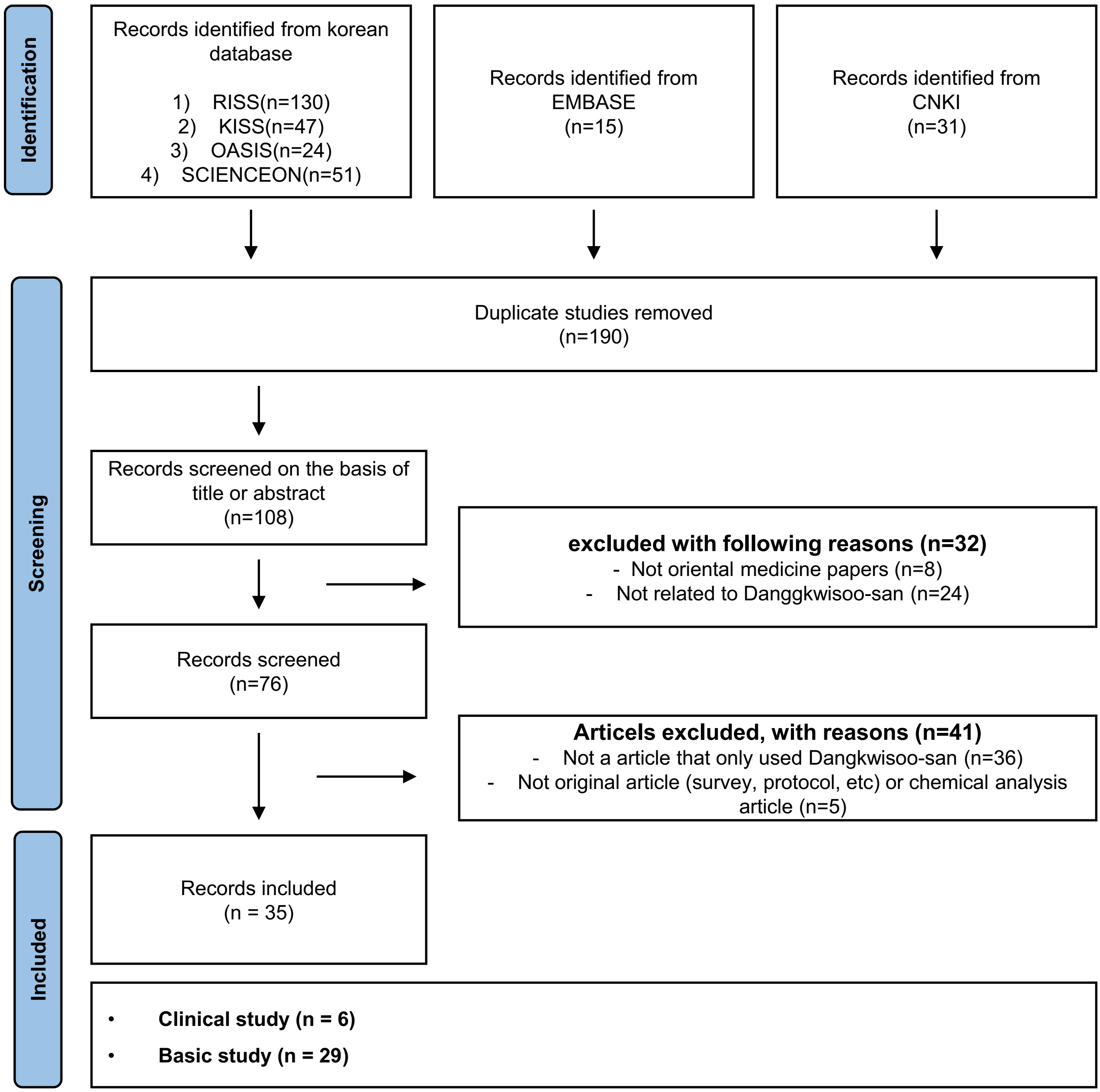

Fig. 1 Flow chart for study selection. The flow diagram indicates the retrieval process of the study

Six articles were published on the efficacy of DS for hemostasis, hematomas, toxin-induced coagulation, and blood pressure. Lee et al. [38] measured the circumference and temperature of the thigh and the levels of platelets, fibrinogen, fibrinogen degradation production (FDP), and prothrombin time, which are factors related to blood coagulation, to evaluate efficacy. In another study on hemostasis [39], the researchers measured the blood levels of white blood cells (WBC), red blood cells (RBC), ALP, LDH, LAP, GOP, and GPT to confirm efficacy. An efficacy study [40] on treating subcutaneous hematomas was histologically analyzed by H&E staining, and it confirmed the efficacy of DS. Efficacy studies on endotoxin-induced coagulation [41, 42] focused on the effect of DS after coagulation induction by measuring the FDP, platelet count, and prothrombin time. In the ex vivo study [43] for blood pressure regulation, New Zealand white rabbit veins were collected, and the efficacy of DS was evaluated by comparing the degree of vascular contraction with that of vessels cultured in atropine, pheniramine, and propranolol.

Table 1 Summary of clinical trials on DS

Target disease	Study design (sample size, n)	Intervention					Control	Effects of *Dangkwisoo-san*		References
		Intervention	Type of preparation form	Extraction method		Duration (frequency)		Outcomes	Results	
Pulmonary contusion	RCT (80:I 40, C 40)	*DS + Usual care*[a]	Decoction	NA	Total 5600 ml (400 ml/day)	2 weeks (2/day)	Usual care[a]	1) Changes in serum TNF-a, IL-6, and CRP levels (1, 3, 7, and 12 days) 2) Incidence of SIRS and ARDS 3) Mortality 4) Average hospital stay	1) p < 0.01 2) I: SIRS (n = 6), ARDS (n = 2); C: SIRS (n = 11), ARDS (n = 5) 3) None (all groups) 4) p < 0.05	Wei, C. H [31]
Acute lateral ankle sprain	RCT (48:I 24, C 24)	*DS + Acupuncture*	Powder capsule	Water	Total 37.8 g (9-capsule/day)	7 days (3/day)	Placebo capsule + Acupuncture	1) VAS 2) FAOS 3) Edema 4) EQ-5D-5L 5) Number of recurrent ankle sprains	1–4) p < 0.01 5) NS	Kim, J. H [27]
Ankle sprain	RCT (45:I 22, C 23)	*DS + Acupuncture*	Powder capsule	Water	Total 37.8 g (9-capsule/day)	7 days (3/day)	Acupuncture	1) FAOS 2) EQ-5D-5L 3) ICER 4) Costs 5) PSA	1, 2, 4, 5) NS 3) -151348 (Korean Won/QALY)	Huang, S. Z [35]
Bruise	Case report (1)	*DS*	NA	NA	NA	10 days (NA)	NA	1) Headache, lower back pain 2) Expiratory dyspnea 3) Groan 4) Tears 5) Food taste 6) Epigastric lumpy stiffness 7) Pressing pain 8) Heart bottom gore	1–4, 7, 8) Disappear 5) Good 6) Soften	NA [34]
Traumatic pneumothorax	Case series (16)	*DS*	Decoction	Water	Total 7~18-pack (1-pack/day)	7~18 days (2/day)	NA	1) Chest pain 2) Choking sensation in the chest 3) Dyspnea 4) CT examination of the right lung	1) Disappear 2–3) Breathe smoothly 4) Clear texture	Chen, M [32]

Table 1 (continued)

Target disease	Study design (sample size, n)	Intervention					Control	Effects of *Dangkwisoo-san*		References
		Intervention	Type of preparation form	Extraction method		Duration (frequency)		Outcomes	Results	
Peptic ulcer	Case series (60)	*DS*	Decoction	Water and alcohol	28~56-pack (1-pack/day)	4~8 weeks (NA)	NA	Effective number of people (%)[b]: significantly effective; effective; or non-effective	38 (63%); 18 (30%); 4 (7%)	Shao, Y. F. [33]

[a] Usual care included oxygenation, endotracheal intubation, rib fixation, closed thoracic drainage, antibiotics, dexamethasone, ventilator, and treating other complications

[b] Effectiveness was evaluated based on the main clinical symptoms, stomach ache, and ulcers

ARDS acute respiratory distress syndrome; *C* control group; *CRP* C-reactive protein; *CT* computed tomography; *DS* Dangkwisoo-san; *EQ-5D-5L* European Quality of Life five-dimension-five-level scale; *FAOS* foot and ankle outcome scores; *ICER* incremental cost-effectiveness ratio; *I* intervention group; *IL-6* interleukin-6; *NA* not available; *NS* not significant; *PSA* probabilistic sensitivity analysis; *RCT* randomized controlled trial; *SIRS* systemic inflammatory response syndrome; *TNF* tumor necrosis factor; *VAS* visual analog scale

Table 2 Ratio of each herb to *Angelicae Gigantis Radix Palva* in the Dangkwisoo-san prescription used in the analyzed clinical studies

Study	Disease	*Angelicae Gigantis Radix Palva*		*Linderae Radix*		*Cyperi Rhizoma*		*Paeoniae Radix*		*Sappan Lignum*		*Carthami Flos*		*Persicae Semen*		*Cinnamomi Cortex*	
		Weight (g)	%	Weight (g)	%	Weight (g)	%	Weight (g)	%	Weight (g)	%	Weight (g)	%	Weight (g)	%	Weight (g)	%
Original prescription*		6	100	4	67	4	67	4	67	4	67	3.2	53	2.8	47	2.4	40
Wei (2014)	Pulmonary contusion	15	100	12	80	12	80	12	80	9	60	9	60	10	67	6	40
Kim (2021)	Ankle sprain	0.63	100	0.42	67	0.42	67	0.42	67	0.42	67	0.33	52	0.29	46	0.25	40
Huang (2021)	Ankle sprain	0.63	100	0.42	67	0.42	67	0.42	67	0.42	67	0.33	52	0.29	46	0.25	40
NA (1964)	Bruise	5	100	3	60	3	60	3	60	3	60	2	40	3	60	2	40
Chen (1993)	Traumatic pneumothorax	15	100	12	80	12	80	12	80	9	60	9	60	10	67	6	40
Shao (2000)	Peptic ulcer	5	100	3	60	3	60	3	60	3	60	3	60	3	60	2	40

* The original prescription referred to the Introduction to Medicine written by Li Chan during the Ming Dynasty in China

Table 3 Summary of in vivo studies on DS

Target Study	Animal (sex, age, body weight)	Inducer	Type of extracts	Administration (Frequency/period)	Experimental group	Positive control	Biomarker & outcome	Reference
Bruise	Wistar rat (male, 180–220 g)	–	Aq	5 ml/200 g* (Oral, 2/day, 1 day)	Normal Control (bruised) DS decoction extracts	-	Water maze test, LDH	Yeo, N. H. et al. [36]
Bruise	Wistar rat (male, 180–220 g)	–	Aq	5 ml/200 g* (Oral, 2/day, 1 day)	Normal Control (bruised) DS decoction DS extracts	-	GPT, GOT, LAP, ALP	Yeo, N. H. et al. [37]
Blood stasis	Wistar rat (male, 250 g)	–	Aq	200 mg/individual* (Oral, daily, 1 day)	Normal Control (blood stasis induced by compression for 2 h or 4 h) DS (1 h before compression)	-	Thigh circumference Thigh dermal temperature Platelet Fibrinogen Prothrombin time FDP	Lee, G. H. et al. [38]
Hemostasis (Bruise)	Wistar rat (male, 200 g)		Aq	Decoction: 5 ml/200 g* Extract: 150 mg/200 g* (Oral, 2/day, 1 day)	Normal Control (hemostasis) DS decoction DS extract		WBC, RBC, Total protein, ALP, LDH, LAP, s-GOT, s-GPT	Lee, H. Y. [39]
Subcutaneous hematoma	Wistar rat (male, 250–300 g)	Autologous blood	Aq	125 mg/100 g* (Oral, daily, 7 days)	Normal Control (hematoma) DS		Histological analysis (H&E staining)	Kim, K. H. [40]
Coagulation induced by endotoxin	SD rat (male, 270–290 g)	Endotoxin	Aq	0.1 ml/individual* (SC, daily, 3 days)	Normal Control (endotoxin) TGSS ACUP ACUP control	-	Platelets, RBC, fibrinogen, FDP, prothrombin time	Nam, S. S. et al. [41]
Coagulation induced by endotoxin	Wistar rat (male, 200–220 g)	Endotoxin	Aq	163.2 mg/200 g* (Oral, 4 h after endotoxin injection, 1 day)	Normal Control (endotoxin) DS Dodamtang	-	Platelets, Prothrombin, FDP (fibrinogen degradation products)	Kim, T. S. et al. [42]
Blood pressure reduction effect	New Zealand white rabbit vein	Atropine Pheniramine Propranolol	Aq	0.5 ml/kg*, 1 ml/kg*	Control (stimulation) DS decoction		Blood pressure measurement	Lew, J. H. [43]
Fracture	C57BL/6 J (male, 7w)	-	Aq	300 mg/kg (Oral, daily, 4 weeks)	Normal (no femur fracture) Control Tramadol DS	Tramadol (20 mg/kg)	BMP2, COX2, Col2a1, Sox9, Runx2, osterix, histological analysis	Jeon, D. H. et al. [46, 47]

Table 3 (continued)

Target Study	Animal (sex, age, body weight)	Inducer	Type of extracts	Administration (Frequency/period)	Experimental group	Positive control	Biomarker & outcome	Reference
Fracture	SD rat (male, 8–10w)	-	Aq	500 mg/kg* (Oral, daily, 14 days)	Normal Control (fracture) DS Native copper DS + Native copper	-	ALP, ALT, AST, creatinine, histological analysis, TGF-β1	Jung, I. M. [44]
Fracture	SD rat (male, 8–10w)	-	Aq	500 mg/kg* (Oral, daily, 14 days)	Normal Control (fracture) DS ACUP DS + ACUP	-	Osteocalcin, T-ALT, histological analysis, TGF-β1	Ahn, H. L. et al. [45]
Wound healing	Wistar rat (male, 6w)	-	80% EtOH	200 mg/kg* (Oral, daily, 3 weeks)	Control DS Terramycin DS + Terramycin	Terramycin	Wound healing, TNF-α, IL-1β, IL-6, MMP-1, MMP-2, MMP-9, Histological analysis	Bak, J. W. et al. [48]
Acute lung inflammation	C57BL/6 J (male, 7w)	LPS	Aq	1 g /kg (Oral, daily, 15 days)	Normal Control (LPS) LPS + DS	-	Body weight, BAL fluid	Lyu, J. H. et al. [26]
Analgesic, anti-inflammation, muscle relaxation	ICR mouse (18–20 g) or SD rat (180–200 g)	Acetic acid Carrageenin	50% EtOH	0.1*, 0.3*, 1* ml/20 g or 200 g 0.2, 0.4 ml/20 g	Control (stimulation) DS decoction		Physiological response (Paw licking time, escape time, paw edema, muscle relaxation, body temperature)	Ko, W. S. [49]
Traumatic brain injury	ICR mouse (male, 7w)	-	Aq	50, 150*, 450* mg/kg (Oral, 2/day, 8 days)	Sham (craniotomy alone) Control DS	-	Beam walking test, grip strength test, NOR test, measurement of neutral injury and brain damage	Jung, J. Y. et al. (2018) [50]
Cerebral ischemic injury	C57BL/6 J (male, 20–25 g)	-	Aq	600 mg/kg* (Oral, 2/day, 3 days)	Veh, L-NIO Sham, Control, DS	-	Blood pressure and resting CBF, Analysis of histology, Rotarod test, eNOS, AKT, iNOS, nNOS	Kim, J. H. et al. [51]
Liver toxicity	Wistar rat (male, 180–200 g)	Carbon tetrachloride	Aq	Decoction: 8 mg* or 16 mg*/kg Extract: 720 mg* or 1440 mg*/kg (Oral, 2/day, 1 day)	Normal Control (hepatoxicity) DS decoction DS extract		ALP, LDH, GOT, GPT, LAP, α-hydroxybutyric dehydrogenase	Lim, J. G. et al. [53]

ACUP acupuncture; *Aq* aqueous; *eNOS* endothelial nitric oxide synthase; *iNOS* inducible nitric oxide synthase; *nNOS* neuronal nitric oxide synthase; *BMP2*, bone morphogenetic protein 2; *COX2* cyclooxygenase-2; *Col2a1* collagen type II alpha 1 chain; *DS* Dangkwisoo-san; *Sox9* SRY-Box transcription factor 9; *Runx2* Runt-related transcription factor 2; *GPT* glutamic pyruvic transaminase (*ALT* alanine aminotransferase); *GOT* glutamic oxaloacetic transaminase (*AST* aspartate aminotransferase); *LAP* leukocyte alkaline phosphatase; *ALP* alkaline phosphatase; *LDH* lactate dehydrogenase; *FDP* fibrinogen degradation production; *MMPs* matrix metalloproteinases; decoction, hot water extraction; extracts, processed after hot water extraction and weight possible

* indicates concentrations and biomarkers with statistical significance ($p < 0.05$) in the article

Table 4 Summary of ex vivo studies on DS

Study Target	Experimental model	Inducer	Type of extracts	Concentration	Biomarker and outcome	References
Vasorelaxation	Mouse Aorta	Phenylephrine	Aq	10, 30, 100*, 300* μg/ml	Evaluation of aortic constriction	Kim, J. H. et al. (2011) [25]
Vasorelaxation	New Zealand white rabbit aorta	Phenylephrine	Aq	0.01,0.03,0.1,0.3, 1* mg/ml	Evaluation of aortic constriction	Ko, H. (2019) [52]

* indicates concentrations and biomarkers with statistical significance ($p < 0.05$) in the article

Four studies evaluated the efficacy of DS in treating fractures. The researchers [44, 45] evaluated the efficacy of DS in SD rats by tissue staining and determination of blood levels of total-alanine transaminase (ALT), ALP, aspartate transaminase (AST), and bone formation-related factors osteocalcin and transforming growth factor-beta 1 (TGF-β1) in addition to recent studies [46, 47]. The same research team used C57BL/6 J mice to evaluate the efficacy of DS by measuring blood concentrations of bone morphogenetic protein 2 (BMP2), cyclooxygenase 2 (COX2), collagen type II alpha 1 chain (Col2a1), SRY-box transcription factor 9 (Sox9), runt-related transcription factor 2 (Runx2), and osterix and histological analysis.

Three studies confirming the efficacy of DS for wound healing and inflammation-related diseases have been published. First, wound healing efficacy was evaluated based on the levels of inflammatory factors TNF-α, IL-1β, and IL-6 and wound repair-related factors MMP-1, MMP-2, and MMP-9 [48]. In another study on acute lung inflammation [26], the researchers focused on body weight change, bronchoscopy, and bronchoalveolar lavage (BAL) levels. In a study on analgesia, anti-inflammation, and muscle relaxation [49], the efficacy of DS was confirmed in response to stimuli, such as mouse paw licking time, escape time, foot swelling, body temperature, and muscle relaxation.

Three studies on the efficacy of DS for brain injury and liver toxicity have been published. In a study on traumatic brain injury [50], the efficacy of DS was confirmed by evaluating exercise capacity, including the beam walking test, grip strength test, and NOR test, and measuring the degree of brain damage. In ischemic brain injury research [51], the efficacy of DS was evaluated by measuring blood pressure; resting cerebral blood flow (CBF); damage measurement through tissue staining; levels of inflammatory factors, such as eNOS, AKT, iNOS, nNOS; and exercise capacity. In addition, the efficacy of DS for the contraction and relaxation of vessels was evaluated through an ex vivo experiment using blood vessel collection. In this vascular relaxation study [52], the effect of DS was confirmed using a New Zealand white rabbit aorta. Studies on liver toxicity [53] confirmed the efficacy of DS against carbon tetrachloride-induced hepatotoxicity, and its efficacy was evaluated based on the levels of ALP, LDH, GOT, GPT, LAP, and α-hydroxybutyric dehydrogenase in the blood.

The sample used for evaluating the efficacy of DS was hot water extraction in this study, except for two studies [48, 49]. The difference between decoction and extract is that decoction refers to the state of hot water extraction with herbal medicines, and extracts can be weighed by processing the state after hot water extraction. The oral volume was measured when administering decoction, whereas, in the case of extract, the dry powder was weighed to determine the dose. After preparation, the extract was administered orally.

In vitro studies on DS

Twelve articles investigated the effect of DS using in vitro model. The details of in vitro studies on DS are summarized in Table 5. DS reportedly possesses anti-inflammatory, antioxidant, anticancer, and antimicrobial activities; prevents ischemic injury; and helps in treating metabolic syndromes, such as cardiovascular disease. Studies on DS have primarily focused on anti-inflammatory activity against LPS, an inflammatory inducer in RAW264.7, and DS was primarily extracted with water (92.3%). Generally, DS prescription was boiled with 1L of distilled water by an herb extractor for 2 h, yielding final 200 ml of DS extract. The supernatant was then evaporated and lyophilized under reduced pressure at low temperature. For MeOH extraction, DS was mixed with 3L of methanol at ambient temperature for 24 h. After centrifugation in a sterile condition, the supernatant was collected and lyophilized through evaporation under reduced pressure at − 80 ℃. Another method is using 80% EtOH where DS was boiled in 1L of 80% EtOH for 3 h and then concentrated by rotary vacuum evaporator.

DS exerts anti-inflammatory effects by regulating inflammatory mediators and cytokines. Lyu et al. [26] demonstrated that DS, which was extracted with distilled water, activated the anti-inflammatory factor Nrf2 (nuclear factor erythroid 2-related factor 2) and the expression of Nrf2-related genes, such as glutamate–cysteine ligase catalytic subunit (GCLC), heme oxygenase

Table 5 Summary of in vitro study designs on DS

Study target	Type of cell	Inducer	Type of extracts	Concentration	Positive control	Biomarker	References
Anti-inflammation	Raw 264.7	LPS	Aq	10, 50*, 100* μg/ml	–	Nrf-2, GCLC, HO-1, NQO-1, ROS, MCP-1*, TNF-α*, IL-6*, NF-kB*	Lyu, J. H., et al. [26]
Anti-inflammation	Raw 264.7	LPS	80% EtOH	1*, 10*, 100* μg/ml	–	Nitric oxide*, TNF-α*, IL-1β*, IL-6*	Bak, J. W., et al. [48]
Anti-inflammation	Raw 264.7	LPS	MetOH, Aq	wDS, mDS 10, 50*, 100 μg/ml	–	IκBα*, TNF-α*, IL-1β*, Nrf2*, GCLC, HO-1, NQO-1	Ryu, J. H. et al., [54]
Anti-inflammation	Raw 264.7	LPS	Aq	10, 50*, 100 μg/ml	–	Nrf2*, NF- kB*	Kim, K. H., et al. [64]
Anti-inflammation	Raw 264.7	LPS	Aq	DS-DE/DS-MEP 50*, 100*, 200* μg/ml	Dexamethasone (20 μM)	Nitric oxide*, PGE2*, IL-1β*, IL-6*, TNF-α*, iNOS*, COX-2*	Jeon, Y. H. et al. [55]
Anti-inflammation Antioxidative activity	Raw 264.7	LPS	Aq	10, 20, 50, 100*, 200 μg/ml	–	Nitric oxide, PGE2, IL-1β*, IL-6*, TNF-α*, DPPH	Jo, N. Y. [56]
Anti-inflammation Bone regeneration	MG63 Raw 264.7	LPS	Aq	MG63: 125*, 500*, 1000* μg/ml RAW264.7: 100, 250*, 500* μg/ml	- Calcitriol (MG63) Cyclosporin A (RAW264.7)	Osteocalcin, Runx 2, TNF-α*	Jeon, D. H., et al. [46]
Anticancer activity	AGS	–	Aq	50, 100, 200*, 300*, 400* μg/ml	–	Caspase 9*, Caspase 3*, P38, JNK, TRPM7	Hwang, M. W., et al. [28]
Metabolic syndrome (TRPM7 channel inhibition)	HEK 293	TRPM7 overexpression	Aq	100, 300*, 500* μg/ml	–	TRPM7*	Kim, B. J. [57]
Antimicrobial activity	10 species of microbe	–	Aq	5, 10, 15, 20 mg/disc	–	Antimicrobial activity (*B. cereus*, *L. monocytogenes*, *V. parahaemolyticus*)	Lee, N. et al. [58]
Cerebral ischemic injury (NO-dependent mechanisms)	HBMECs	–	Aq	DS 30* μg/ml	Acetylcholine	Nitric Oxide	Kim, J. H., et al. [25]
Change in cell membrane potential	Interstitial cells of Cajal (ICCs)		Aq	DS: 1, 10, 30* μg/ml	–	GDP-β-S*, Na^{+}*, Ca^{2+}*, 4-DAMP*	Sung, S. K. [59]

Aq aqueous; *DS*, Dangkwisoo-san; *4-DAMP* 4-diphenylacetoxy-N-methyl-piperidine methiodide; *GDP-β-S* a non-hydrolyzable guanosine 5′-diphosphate analog; *IL* interleukin; *TNF* tumor necrosis factor; Runx, Runt-related transcription factor; *Nrf-2* nuclear factor erythroid-2-related factor 2; *GCLC* glutamate–cysteine ligase catalytic subunit; *HO-1* heme oxygenase-1; *NQO-1* quinone oxidoreductase 1; *ROS* reactive oxygen species; *MCP-1* monocyte chemoattractant protein-1; *TRPM7* transient receptor potential cation channel subfamily M member 7

* indicates concentrations and biomarkers with statistical significance ($p < 0.05$) in the article

(HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO-1) in RAW264.7cells. In addition, pre-treatment of NF-κB reporter cells derived from RAW264.7 with DS, followed by LPS treatment, regulated NF-κB, which was confirmed using HEK293 cells transfected with inhibitor of kappa B (IκB) kinase- β (Iκκ-β) and NF-κB. DS treatment suppressed the levels of pro-inflammatory cytokines, including TNF, MCP-1, and IL-6. In addition, analysis of reactive oxygen species (ROS) induction by DS using flow cytometry confirmed that DS did not produce ROS.

Two studies on the anti-inflammatory effects of DS used extraction solvents other than water and revealed significant effects at low DS concentrations. Bak et al. [48] investigated the anti-inflammatory effects of DS extracted using 80% ethanol. DS reduced the production of NO, IL-1β, IL-6, and TNF-α in the LPS-induced RAW264.7 cell line in a dose-dependent manner (1, 10, and 100 µg/ml). Ryu et al. [54] compared the anti-inflammatory effects of methanol (mDS) and water extracts (wDS) of DS. mDS activated Nrf-2 and its dependent protein HO-1 and expressed Nrf-2-related genes, GCLC, HO-1, and NQO1, in RAW264.7cells. The results confirmed that 50 µg/ml mDS was more effective in increasing Nrf2 than wDS, with sulforaphane (SFN; 5 µM), an Nrf-2 activator, as a control. However, mDS was less effective in decreasing NF-κB than wDS in RAW264.7 stably transfected with NF-κB. NF-κB activation by mDS was weakened 30 min after LPS treatment, and the degradation of IκB-α was not suppressed 15 min after LPS induction in murine macrophages. Pro-inflammatory cytokines TNF-α and IL-1β were reduced by mDS. These findings indicated that DS attenuates inflammation.

In a study of the effect of 9 components of DS on Nrf2 and NF-κB, the activation of Nrf2 at 50 µg/ml of DS in LPS-induced RAW264.7cells was confirmed [22]; however, each constituent of DS showed cytotoxicity at 50 µg/ml. Therefore, DS was used at 25 µg/ml, and most components induced the activation of Nrf2 in the Nrf2 reporter cell line derived from RAW264.7, particularly *Carthamus tinctorius L.* (CT). In addition, nine components of DS regulated NF-κB in the NF-κB luciferase reporter cell line using the same method as for Nrf2, particularly *Carthamus tinctorius L.* (CT) and *Cyperus rotundus L.* (CR). These findings suggested that two constituent herbal medicines, CT and CR, in DS serve as the main anti-inflammatory agents.

Jeon et al. [55] examined the anti-inflammatory activity of DS-dry extract (DS-DE) with DS-mix extract powder (DS-MEP) and used dexamethasone (20 µM) as a positive control. DS-DE and DS-MEP had a greater inhibitory effect than dexamethasone (20 µM) on NO production. DS-DE and DS-MEP suppressed the protein and mRNA expression of iNOS, PGE2, and COX-2 in a dose-dependent manner (50, 100, and 200 µg/ml) in LPS-stimulated RAW264.7cells. The secretion of TNF-α, IL-1β, and IL-6 was significantly suppressed by DS-DE and DS-MEP, coinciding with transcriptional levels.

In a study conducted by Jo et al. [56], 100 µg/ml DS with hot aqueous extraction inhibited the production of NO and PGE and suppressed the levels of IL-1β, IL-6, and TNF-α in LPS-activated RAW264.7. In addition, 100 µg/ml DS had more than 50% free-radical scavenging ability, as measured using the DPPH (2,2-diphenyl-1-picrylhydrazy) radical scavenging method. Further, the effect of DS was evaluated for bone repair in osteoblast-like MG63cell line and RAW264.7 macrophages [46]. DS (500 µg/ml)-treated MG63 cells demonstrated increased gene expression of osteocalcin and Runx2, with calcitriol as a positive control. DS increased the secretion of TNF-α in LPS-induced RAW264.7 cells in a dose-dependent manner compared to cyclosporine A (as a positive control), and 250 and 500 g/ml of DS yielded statistically significant results.

Hwang et al. [28] reported the anticancer efficacy of DS. Levels of caspase-3, caspase 9, and subG1 and mitochondrial depolarization increased in AGS gastric adenocarcinoma cells treated with varying concentrations (50, 100, 200, 300, and 400 µg/ml) of DS. Moreover, DS decreased transient receptor potential melastatin (TRPM7) in AGS and TRPM7-transfected HEK293 cells, determined using the patch-clamp technique. These results highlighted that the MAPK (p38 and JNK) signaling pathway is associated with DS through a JNK II inhibitor or SB203580 (p38 MAPK inhibitor).

Another study [57] on TRPM7 focused on the action of DS in HEK293 cells that stably overexpressed TRPM7 using the whole-cell patch clamp technique. DS suppressed the overexpression of TRPM7 current in a dose-dependent manner (100, 300, and 500 µg/ml), thus alleviating metabolic syndromes, such as cardiovascular disease.

Lee et al. revealed the antimicrobial activity of DS [58] Ten pathogenic microorganisms (*Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogens*, *Vibrio parahaemolytixus*, *Escherichia coli DH5α*, *E. coli O157*, *Salmonella nteritidis*, *Yersinia enterocolitica*, *Shigella flexneri*, *and Helicobacter pylori*) were screened for the antimicrobial effects of 51 herbal formulae used in traditional Korean medical prescriptions. Of these, DS showed antibacterial effects against *B. cereus* and antimicrobial activity against *L. monocytogenes (*5, 10, 15, and 20 mg/disc) and *V. parahaemolyticus* (15 and 20 mg/disc).

Kim et al. [25] suggested that human brain microvascular endothelial cells (HBMEC) treated with 30 µg/ml DS exhibited stimulated NO production, with acetylcholine as a positive control. Therefore, DS in ischemic injury regulates vascular function through an eNOS-dependent mechanism.

DS causes pacemaker depolarization of the interstitial cells of Cajal (ICCs) through the M3 receptor. In addition, G-protein, external Na^{+}, and non-selective cation channels are mediated by DS-activated pacemaker depolarization [59]. Moreover, external or internal Ca^{2+} plays an important role in regulating pacemaker potentials in ICCs, and DS elevates Ca^{2+} in ICCs.

In summary, DS appears to have effects on inflammatory factor, bone regeneration, ischemic injury repair and metabolic disorders. Since a small number of in vitro experimental reports on the metabolic disorders, bone regeneration, and research on the recovery from ischemic injury have been published, there are advance studies should be conducted to generate reproducibility of findings. However, we figured out mouse macrophage RAW 246.7 cell model was used for most of the examinations on effects of DS on inflammatory response, while LPS was used as a stimulant in all of experiments. In addition, the concentration range from 1 to 200 μg/mL was applied for evaluating the DS efficacy. It was confirmed that the effective dose range of DS was homogenous. Although there is only two publishes, it was apparently confirmed that the difference in therapeutic efficacy depending on DS extraction methods. Using an organic solvent extracting method achieved better curing effect even at a lower concentration than that of the hot water extracts.

Although chemical quality assessments are being performed to ensure consistent efficacy of DS, the identity and quantity of the compounds contained within a given natural product is a frequent complication of studies. Even when the composition is relatively well understood, the lack of simple and effective analytical methods hampers the ability to extrapolate results beyond quality studies. We conducted a literature review to identify biological assays that support chemical QC.

Implications that could be obtained from the current clinical research of DS are as follows. First, although the use of DS prescriptions in clinical practice is extensive, relatively few clinical studies have been conducted. In particular, based on the insights obtained during the search phase for clinical studies, DS has been used as a representative prescription for trauma in Korea [60] also recently published in the clinical practice guidelines of Korean medicine for traffic injuries and acute ankle sprains (recommendation level/evidence level: C/Very Low, respectively) [61, 62]. DS has been used to cure diverse symptoms in China; however, it is difficult to consider it a representative prescription, and it has rarely been investigated in Japan. The tendency toward DS utilization in clinical practice was not sufficiently reflected in the results of this scoping review because of the small number of clinical studies. Second, it is necessary to discuss standardization of the herb composition ratio in DS prescriptions. We excluded the modified formula to control heterogeneity among the DS prescriptions. The herbs constituting DS prescriptions were the same; however, there were differences in the proportion of herbs used in each study. The DS prescription used in Korean studies was the same as the original prescription; however, in the two Chinese studies on thoracic injuries, the content ratio of *Linderae Radix*, *Cyperi Rhizoma*, *Paeoniae Radix*, *Carthami Flos*, and *Persicae Semen* to *Angelicae Gigantis Radix Palva* was higher than that of the original prescription. Third, there were also rare cases where the "decoction form by boiling herbs with water and medicinal wine" mentioned in the original prescription was used identically. We need to understand the reasons for the extraction of the original recipe through additional research. In addition, alcohol extraction at a certain ratio may be more efficient than water extraction, which is primarily used in clinical studies [63].

Based on in vivo and ex vivo studies, a preclinical study on DS reported efficacy verification and methods for various diseases, such as blood coagulation, hepatotoxicity, fractures, brain disease, inflammation-related disease, and bruises. These results could be valid for evaluating efficacy by a simple method that can be used in clinical applications. As the approval of the FDA for herbal medicine for post-marketing is rather laborious, effective clinical outcome demands attention. In fact, in vivo and in vitro preclinical assessments are the initial stages of the evaluation of later steps. However, preclinical research has certain difficulties, such as ethical problems, excessive experimental period, requirement of high-quality human resources, and high cost. These problems make it difficult to maintain efficacy consistency in the same experiment. In addition, it hinders the use of many in vitro methods and their clinical and preclinical relevance. Therefore, the evaluation and consistency of efficacy of various herbal medicines and prescriptions, including DS, should be subjected to evaluation methods by carefully selecting clinical and preclinical related assays. In this study, we aimed to develop a scoping review of DS as an alternative therapy, particularly for in vitro research.

Inflammation is a representative expression of the secondary defense mechanism of the body in response to foreign stimuli. Many reports on aqueous DS extract treatments have shown effective anti-inflammatory effects. Using an LPS-stimulated Raw264.7 cell model, water-extracted DS reduced the expression level of certain proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, as well as the NF-kB signaling pathway [26, 54, 56, 64]. *Carthamus tinctorius* L. (CT) and *Cyperus rotundus* L. (CR) are two active components of DS that have shown anti-inflammatory effects at lower doses than dexamethasone [55]. Moreover, according to Ryu [54] and Bak et al. [48], MeOH and EtOH extraction of DS notably suppress inflammation of stimulated Raw 264.7 cells at concentrations lower than 50 μg/ml. Accounting for 92.3% of the DS extracts, water-extracted DS was effectively involved in several metabolic mechanisms, including cancer, bone regeneration, intestinal phasic contraction, and cell membrane potential change. For instance, anticancer activity was recorded with the induction of certain apoptotic markers (caspase 9 and caspase 3) by Hwang et al. [28] while inhibiting TRPM7 channel activity. Moreover, Lee et al. [58] demonstrated the antimicrobial activity of aqueous DS extract at less than 20 mg/disc. Sung et al. [59] reported DS-induced changes in the membrane Ca^{2+} potential of ICCs, resulting in pacemaker depolarization.

We concluded that DS in vitro was the most effective anti-inflammatory agent at concentrations of 50 and 100 μg/ml, and in vitro reduction of biochemical markers, such as TNF, IL-1β, IL-6, NO, Nrf2, and NF-κB, effectively demonstrated the potential role of DS in the inhibition of inflammation (Table 4). In addition, DS extracts are extracted by hot water as traditional method. However, using 80% EtOH or MeOH as extraction solvents, the treatment of DS showed more effective results at lower doses in vitro. The efficacy might be associated with the higher concentration of active component because of extraction with organic solvents. DS is composed of nine herbal medicines: *Paeoniae Radix*, *Angelicae Gigantis Radix*, *Sappan Lignum*, *Linderae Radix*, *Persicae Semen*, *Cyperi Rhizoma*, *Carthami Flos*, *Glycyrrhizae Radix et Rhizoma*, *and Cinnamomi Cortex. Rhizoma*. However, the identification of active compounds in DS will help understand their molecular mechanisms and targets. However, multiple herbal medicines for multi-target treatment may produce synergistic effects. Although DS is known to be commonly used to relieve headaches, contusions, and external and cerebral ischemic injuries, in vitro tests for DS are limited. High DS concentration has effects similar to those of other natural compounds. Experimental studies can provide insights into maximizing therapeutic efficacy by inhibiting inflammatory mediators and cytokines.

As introduced in mechanism based QC study showing in vivo activities of irinotecan [65], suitable bioassays to identify the biomarkers related to mechanism of action of the clinical efficacy would be a good example of biological QC. For these biomarkers, standard operating procedures (SOPs) should be prepared for the generation of reproducible and accurate results. Therefore, the mechanism-based QC using biomarkers would be useful for the prediction and confirmation of consistent efficacy of DS. Quality control of herbal preparations including DS should be different depending on the pharmacological usages. As chemical evaluation methods are limited in their ability to distinguish irrelevant chemicals, QCs evaluating specific uses and efficacy should be developed concise and appropriate to biological assays based on in vitro mechanisms of action.

The above discussion suggests that future quality evaluation studies of herbal medicines (herbal medicine prescriptions) should be aimed at selecting an optimal in vitro efficacy evaluation method capable of evaluating the mechanism of action for the assessment of consistent clinical effects. This method should evaluate the mechanism of action of the clinical effect, use appropriate positive controls and statistical methods, and prepare reproducible SOPs.

Abbreviations

DS	Dangkwisoo-san
EtOH	Ethanol
MeOH	Methanol
RCT	Randomized controlled trial
eNOS	Endothelial nitric oxide synthase
iNOS	Inducible nitric oxide synthase
nNOS	Neuronal nitric oxide synthase
BMP2	Bone morphogenetic protein 2
COX2	Cyclooxygenase-2
Col2a1	Collagen type II alpha 1 chain
Sox9	SRY-box transcription factor 9
Runx2	Runt-related transcription factor 2
GPT	Glutamic pyruvic transaminase
ALT	Alanine aminotransferase
GOT	Glutamic oxaloacetic transaminase
AST	Aspartate aminotransferase
LAP	Leukocyte alkaline phosphatase
ALP	Alkaline phosphatase
LDH	Lactate dehydrogenase
FDP	Fibrinogen degradation production
MMPs	Matrix metalloproteinases
4-DAMP	4-Diphenylacetoxy-N-methyl-piperidine methiodide
GDP-β-S	A non-hydrolysable guanosine 5′-diphosphate analogue
ILs	Interleukin
TNF	Tumor necrosis factor
Runx	Runt-related transcription factor
Nrf-2	Nuclear factor erythroid-2-related factor 2
GCLC	Glutamate–cysteine ligase catalytic subunit
HO-1	Heme oxygenase-1
NQO-1	Quinone oxidoreductase 1
ROS	Reactive oxygen species
MCP-1	Monocyte chemoattractant protein-1
TRPM7	Transient receptor potential cation channel subfamily M member 7

Acknowledgements
Not applicable.

Author contributions
Conceptualization: S-YK and KSK; methodology, JHL, SJ, MJL and NKT; investigation, JHL and Y-JK; writing—original draft preparation, JHL and SJ; writing—review and editing, KSK; supervision, S-YK and KSK; project administration, KSK. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

Funding
This study was supported by a Grant (21173MFDS56-3) from the Ministry of Food and Drug Safety, 2023.

Availability of data and materials
All data generated or analysed during this study are included in this published article [and its Additional files].

Declarations

Competing interests
The authors declare that they have no competing interests.

Received: 2 April 2023 Accepted: 10 July 2023
Published online: 23 August 2023

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