Research article

Saengmaeksan, a traditional herbal formulation consisting of *Panax ginseng*, ameliorates hyperuricemia by inhibiting xanthine oxidase activity and enhancing urate excretion in rats

Yoon-Young Sung, Heung Joo Yuk, Dong-Seon Kim*

Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea

**ARTICLE INFO**

**Abstract**

**Background:** Saengmaeksan (SMS) is a traditional Korean medicine composed of three herbs, *Panax ginseng, Schisandra chinensis,* and *Liriope platyphylla*. SMS is used to treat respiratory and cardiovascular disorders. However, whether SMS exerts antihyperuricemic effects is unknown.

**Methods:** Effects of the SMS extract in water (SMS-W) and 30% ethanol (SMS-E) were studied in a rat model of potassium oxonate-induced hyperuricemia. Uric acid concentrations and xanthine oxidase (XO) activities were evaluated in the serum, urine, and hepatic tissue. Using renal histopathology to assess kidney function and uric acid excretion, we investigated serum creatinine and blood urea nitrogen concentrations, as well as protein levels of renal urate transporter 1 (URAT1), glucose transporter 9 (GLUT9), and organic anion transporter 1 (OAT1). The effects of SMS on in vitro XO activity and uric acid uptake were also evaluated. The components of SMS were identified using Ultra Performance Liquid Chromatography (UPLC).

**Results:** SMS-E reduced serum uric acid and creatinine concentrations, and elevated urine uric acid excretion. SMS-E lowered XO activities in both the serum and liver, and downregulated the expression of renal URAT1 and GLUT9 proteins. SMS-E reduced renal inflammation and IL-1β levels in both the serum and kidneys. SMS-E inhibited both in vitro XO activity and urate uptake in URAT1-expressing oocytes. Using UPLC, 25 ginsenosides were identified, all of which were present in higher levels in SMS-E than in SMS-W.

**Conclusion:** SMS-E exhibited antihyperuricemic effects by regulating XO activity and renal urate transporters, providing the first evidence of its applicability in the treatment of hyperuricemia and gout.

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1. Introduction

Purine degradation via xanthine oxidase (XO) catalysis produces uric acid as its final oxidation product in humans; increased amounts of uric acid in the blood result in hyperuricemia [1]. Hyperuricemia arises as a consequence of elevated uric acid production, decreased renal uric acid excretion, or a combination of the two [2]. Hyperuricemia is a crucial risk factor for the development of kidney disease, gout, atherosclerosis, hyperlipidemia, and cardiovascular disease [3–5]. Thus, a urate-lowering therapy that reduces uric acid production but enhances urate excretion would benefit the management of hyperuricemia and hyperuricemia-associated disorders [6]. Commonly used urate-lowering medications, such as allopurinol and febuxostat (XO inhibitors), are extensively used for the treatment of gout; however, these XO inhibitors may have undesired or serious adverse effects, such as allopurinol hypersensitivity syndrome [7–9]. Under these circumstances, new antihyperuricemic agents are needed, with lower (or no) toxicity, which are more effective for the prevention of these hyperuricemia-associated disorders over time.

In the current study, we investigated whether saengmaeksan (SMS), a medicinal herbal formulation, protects against hyperuricemia and hyperuricemia-induced renal damage in gout and related diseases. SMS is a commonly used traditional Korean medicine that consists of three different medicinal herbs: Ginseng Radix (root of *Panax ginseng* Meyer), Liriopis Tuber (tuber of *Liriope platyphylla*), and Schisandraceae Fructus (fruit of *Schisandra chinensis*). SMS is frequently administered as a summer drink, meant to...
restore the health of the body, or used to prevent various diseases, such as those involving the cardiovascular and respiratory systems [10,11]. Previous studies have indicated that Ginseng Radix and its compounds are involved in numerous biological activities, such as reducing inflammation and protecting against renal damage [12,13]. Schisandrae Fructus has antioxidant, anti-inflammatory, hepatoprotective, and renal-protective activities [14,15]. Liriope Tuber has protective effects against inflammation, hyperlipidemia, and diabetic nephropathy [16,17]. Hyperuricemia promotes renal damage and dysfunction by several pathogenic mechanisms at both the cellular and tissue levels [18,19]. Therefore, we hypothesized that the SMS herbal formulation may exert the beneficial therapeutic effect of preventing kidney injury via antihyperuricemic and anti-inflammatory activities. In this work, we analyzed the anti-hyperuricemic activities of SMS in an animal model of potassium oxonate (PO)-induced hyperuricemia. PO is a selectively competitive uricase inhibitor of uric acid metabolism, which is widely used to block the activity of hepatic uricase and induce hyperuricemia in rodents [20]. PO-induced hyperuricemia in rats can serve as a valuable disease model, not only to examine the pathophysiological mechanism of hyperuricemia but also to assess the efficacy of potential therapeutic agents [21]. To observe the effects and underlying mechanisms of SMS, we examined the dual actions of the suppression of uric acid production and stimulation of urate excretion via the treatment of hyperuricemic rats with SMS.

2. Materials and methods

2.1. Preparation of plant extracts

The roots of L. platyphylla and P. ginseng, and the fruits of S. chinensis were purchased from an oriental herbal market (Omiherb, Korea) that only supplies herbs certified by the Korean Pharmacopoeia. An herbal combination was prepared using L. platyphylla, P. ginseng, and S. chinensis in a 2:1:1 (m/m) ratio and mixed according to the Oriental Medicine Advanced Searching Integrated System (http://oasis.kiom.re.kr) at the Korea Institute of Oriental Medicine (KIOM). The mixed herbs were extracted with distilled water or 30% ethanol using a reflux extractor, filtered, and evaporated. The extract was then dried to produce either a 30% ethanol extract (SMS-E; yield, 34.4%) or water extract (SMS-W; yield, 31.5%).

2.2. Animals

Male, 7-week-old Sprague Dawley rats were obtained from Orient Bio (Seongnam, Korea). They were housed in an air-conditioned animal room (22 ± 1 °C with 50 ± 10% humidity). A standard diet and water were fed to the rats ad libitum. The animal experiments were approved by the Institutional Animal Care and Use Committee of the KIOM and conducted according to the committee’s guidelines (Approval code 19-042).

2.3. Hyperuricemic rats

The uricase inhibitor PO was injected intraperitoneally into rats for the induction of hyperuricemia. PO was first suspended in a 0.5% sodium carboxymethyl cellulose (CMC) solution, and 150 mg/kg PO was injected into the rats. We used allopurinol as a first-line urate-lowering therapy. As allopurinol, an XO inhibitor, is the most widely used agent for the treatment of gout in clinical practice, the effects of SMS were compared with those of the positive control allopurinol group [7]. To investigate the antihyperuricemic effects of SMS (Experiment 1), the animals were split into seven groups (n = 5 each): normal (N), PO-induced hyperuricemia (PO), PO + 10 mg/kg allopurinol, PO + 200 mg/kg SMS-W (SMS-W200), PO + 400 mg/kg SMS-W (SMS-W400), PO + 200 mg/kg SMS-E (SMS-E200), and PO + 400 mg/kg SMS-E (SMS-E400).

Next, for comparisons of SMS and the three individual extracts (Experiment 2), the animals were split into 10 groups (n = 5 each): N, PO, PO + SMS-W400, PO + SMS-E400, PO + 400 mg/kg L. platyphylla water extract (LP-W400), PO + 400 mg/kg L. platyphylla 30% ethanol extract (LP-E400), PO + 400 mg/kg S. chinensis water extract (SC–W400), PO + 400 mg/kg S. chinensis 30% ethanol extract (SC-E400), PO + 400 mg/kg P. ginseng water extract (PG-W400), and PO + 400 mg/kg P. ginseng 30% ethanol extract (PG-E400). In Experiments 1 and 2, the samples were suspended in a 0.5% CMC solution and administered to rats orally 1 h after the PO injection.

To study the dose-dependent effects and the mechanism of action of SMS (Experiment 3), the animals were split into six groups (n = 5 each): N, PO, PO + 5 mg/kg allopurinol, and PO + SMS-E (at 100, 200, or 400 mg/kg doses of SMS-E). The samples were administered orally to the rats 1 h after the PO injection for 5 consecutive days.

2.4. Blood, urine, and tissue sample collection

Blood was drawn 2 h after the final administration of drug, and the serum was obtained by centrifugation at 2,500 × g for 15 min at 4 °C. Urine was collected using a metabolic cage for 2 h following drug administration. At the same time, the kidney and liver tissues were stored separately at −70 °C for further assays. Fractional excretion of uric acid (FEUA) was calculated as follows: FEUA (%) = (Urine uric acid/Serum uric acid)/(Urine creatinine/Serum creatinine) × 100 [22].

2.5. Measurement of uric acid levels

The liver tissues were homogenized using a Precellys Evolution tissue homogenizer (Bertin, Rockville, MD, USA), and the supernatant was obtained by centrifugation at 13,000 × g for 10 min at 4 °C. Uric acid levels from the tissues, urine, and serum were determined using an uric acid assay kit (Biovision, Milpitas, CA, USA).

2.6. XO activity assay

The hepatic and serum XO activities were determined using an XO activity assay kit (Sigma-Aldrich, St. Louis, MO). Briefly, assay mixtures consisting of the XO solution (0.2 U/mL), 100 mM sodium pyrophosphate buffer (pH 7.5), and samples at various concentrations (0–2,000 μg/mL) were incubated at 37 °C. The reactions were induced by adding the substrate (0.5 mM xanthine). The XO inhibitor allopurinol was used as a reference.

2.7. Renal histopathological examination

Kidneys were excised and immediately fixed in formalin and embedded in paraffin. Each specimen was cut in 5-μm-thick sections and stained with hematoxylin and eosin. The sections were then imaged under light microscopy.

2.8. Determination of proinflammatory cytokine concentrations

Serum interleukin (IL)-1β concentrations were determined using an ELISA assay kit (R&D Systems, Minneapolis, MN, USA).
2.9. Western blot analysis

Kidneys were homogenized in a Pro-Prep protein extraction solution (Intron, Seoul, Korea) and centrifuged at 12,000 \( \times g \) at 4 °C. After 15 min, the supernatant was analyzed to determine the expression levels of the targeted proteins. Protein concentrations were measured using a DC protein assay (Bio-Rad, Hercules, CA, USA). The primary antibodies included urate anion transporter 1 (URAT1, MyBioSource, San Diego, CA, USA), organic anion transporter 1 (OAT1), \( \beta \)-actin (Santa Cruz, Dallas, TX, USA), and IL-1β (Abcam, Cambridge, UK). The bands were visualized using the LAS-4000 (GE Healthcare, Seoul, Korea). The band density was determined by densitometry using Image J1.49 software. The target protein levels were normalized to those of \( \beta \)-actin.

2.10. In vitro urate uptake analysis

The URAT1-overexpressing oocytes system was derived from Xenopus laevis and established as previously described [21]. The URAT1 inhibitor benz bromarone (TCI, Tokyo, Japan) was used as a reference. Fifty nanograms of URAT1 cloned RNA were injected into a Xenopus oocyte. After incubation for 2 days at 18 °C, the oocytes were preincubated for 1 h in ND96 buffer containing 1 mM pyrazinecarboxylic acid (Sigma). Then, the oocytes were further incubated in a solution of 50 \( \mu \)M [\(^{14}\)C]-urate with various concentrations of the drugs (0.1–100 \( \mu \)g/mL) for 60 min. The reactions were stopped by the addition of ice-cold ND96 buffer. The oocytes were then lysed in 1 N NaOH, and the lysate radioactivity was measured using a liquid scintillation counter.

2.11. UPLC-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) analysis

An Ultra Performance Liquid Chromatography (UPLC) system equipped with a binary solvent delivery system, an auto-sampler, and a UV detector (Waters, Milford, MA, USA) was used. Aliquots of each sample (2.0 \( \mu \)L) were injected into a 100 \( \times \) 2.1 mm, 1.7 \( \mu \)m BEH C18 column and eluted at a flow rate of 0.4 mL/min using a chromatographic gradient of two mobile phases (A: water containing 0.1% formic acid; B: acetonitrile containing 0.1% formic acid). A linear gradient was optimized as follows: 0 min, 5% B; 0–16 min, 5–45% B; 16–21 min, 45–80% B; 21–22 min, 80–100% B; 22–23.3 min, 100% B; and 23.3–25 min, 5% B. The Q-TOF/MS system (Vion IMS, Waters) was operated in negative-ion mode under the following conditions: a capillary voltage of 2.3 kV, a cone voltage of 50 V, a source temperature set to 110 °C, and a desolvation temperature set to 350 °C. A sprayer with a reference solution of leucine-enkephalin ([M–H]− m/z 554.2615) was used as the lock mass. The full scan data and MS/MS spectra were acquired using MassLynx software.

2.12. Statistical analysis

All results are expressed as the mean ± standard error of the mean. Statistical analysis was performed using GraphPad Prism 7 software (GraphPad, La Jolla, CA, USA). Statistical significance was determined using a one-way ANOVA with a post-hoc multiple comparisons test. Significance was established at a \( P \) value less than 0.05.

3. Results

3.1. Effects of saengmaeksan on serum uric acid concentrations

A single PO injection for a single day significantly increased serum uric acid concentrations in the PO rats compared to those of normal rats (\( P < 0.01 \), Fig. 1A). SMS-W failed to alter the serum uric acid levels, whereas 400 mg/kg SMS-E and allopurinol, as a positive control, significantly decreased the concentrations of serum uric acid (\( P < 0.01 \) and \( P < 0.001 \), respectively). Moreover, oral administration of SMS-E more effectively reduced the concentrations of serum uric acid in the PO rats compared to treatments with single extracts (LP-E, SC-E, or PG-E) at the same dose (Fig. 1B). Thus, the following experiments aimed to identify the activities exerted and the mechanisms used by SMS-E.

Five days of PO injections increased serum uric acid concentrations in the PO rats compared to those in normal rats (Fig. 2A). Administration of 400 mg/kg SMS-E, as well as allopurinol, significantly decreased serum uric acid concentrations (\( P < 0.05 \) and \( P < 0.01 \), respectively). In addition, to determine kidney function, creatinine and blood urea nitrogen (BUN) levels were examined. The administration of SMS-E at doses of 200 and 400 mg/kg restored the serum creatinine concentrations induced by PO injection (\( P < 0.01 \), Fig. 2B). The serum BUN levels increased in PO rats, but these levels were not significantly different among the groups evaluated (Fig. 2C).

Urinary uric acid and creatinine concentrations were examined to investigate the activity of SMS-E on uric acid excretion. The PO rats had decreased urinary uric acid concentrations, which were elevated by the highest dose of SMS-E (\( P < 0.001 \), Fig. 2D). The urine uric acid levels at doses of 100 and 200 \( \mu \)g/mL SMS-E were increased in PO rats; however, these findings were not significant. Urine creatinine levels were not significantly changed among the groups examined (Fig. 2E). However, a notable reduction in FEUA was monitored in the PO rats, which increased in rats administered 400 mg/kg SMS-E (\( P < 0.05 \), Fig. 2F). These data demonstrate that SMS-E might increase urate excretion and decrease the concentration of serum uric acid in PO rats to subsequently improve renal function.

3.2. Effects of saengmaeksan on renal inflammation

As shown in Fig. 3A, mild renal tubular dilatation, swelling, tubular epithelial cell vacuolar degeneration, and slight inflammatory cell infiltration were observed in the PO rats (Fig. 3A). SMS-E effectively improved these histopathological changes in the kidneys. Furthermore, the PO rats exhibited an increase in IL-1β protein levels in both the serum and kidney (Fig. 3B and C). SMS-E at doses of 200 and 400 mg/kg remarkably downregulated serum and renal IL-1β levels (\( P < 0.05 \)).

3.3. Effects of saengmaeksan on XO activity

Serum and hepatic XO activities were increased in hyperuricemic rats (Fig. 4A and B). Administration of 400 mg/kg SMS-E and allopurinol significantly lowered XO activities in both the serum and liver (\( P < 0.05 \)). Administration of 400 mg/kg SMS-E and allopurinol remarkably lowered the hepatic levels of uric acid compared to levels observed in the PO group (\( P < 0.05 \), Fig. 4C). Furthermore, the administration of SMS-E at doses of 1,000 and 2,000 \( \mu \)g/mL exhibited inhibitory effects on in vitro XO activity (\( P < 0.001 \)), and the 50% inhibitory concentration (IC\textsubscript{50} value) was 1,221 \( \mu \)g/mL (Fig. 4D and E).
3.4. Effects of saengmaeksan on urate extraction

To identify the mechanism by which SMS-E affects urate excretion, the protein expression of urate transporters (i.e., URAT1, GLUT9, and OAT1) were examined in the kidneys of hyperuricemic rats (Fig. 5A). Administration of SMS-E lowered the protein ratio of the urate reabsorption transporters URAT1 and GLUT9 in the kidney (P < 0.05, Fig. 5B and C). SMS-E did not change the protein ratio of the urate excretion transporter OAT1 (Fig. 5D). In addition, SMS-E exhibited a potent dose-dependent inhibition of URAT1-mediated uric acid uptake in URAT1-expressing oocytes (P < 0.001), and the IC50 value was 0.21 μg/mL (Fig. 5E and F).

3.5. Chemical profiling of saengmaeksan

The 25 ginsenosides in the SMS extracts were tentatively identified using a comparison of HR-MS (accurate mass in negative-ion mode) results with those from an in-house library and compared with published reports (Supplementary Material Table 1) [23,24]. The representative base peak intensity...
chromatograms of SMS-E and SMS-W extracts are shown in Fig. 6A. In negative-ion mode, ginsenosides were identified as [M + COOH]⁻ ions and [M−H]⁻ ions with a high mass accuracy (<4.0 ppm). As shown in Fig. 6B, the 25 identified peaks had different relative levels, depending on the extraction solvent, with SMS-W being detected in a trace or significantly lower amount than SMS-E. In particular, peak 3, containing ginsenoside Rg1, which is the main ingredient of SMS-E extracts, was 32.5% higher in its relative intensity compared with the SMS-W extract.

4. Discussion

XO is a necessary enzyme that breaks down purine nucleotides to produce uric acid. Allopurinol, the most commonly used urate-lowering drug with XO inhibitory activity, decreases serum uric acid levels. XO inhibitors also help limit the overproduction of reactive oxygen species that cause injury to the vascular endothelium by the overactivation of XO, which contributes to the pathophysiology of various diseases, such as metabolic syndrome and renal and cardiovascular diseases [25]. Thus, the inhibition of XO...
overactivation appears to be an attractive therapeutic target to restrict the extent of damage associated with excess uric acid. Our study showed that SMS-E inhibited serum and hepatic XO activities. It also decreased hepatic uric acid concentrations in hyperuricemic rats and inhibited in vitro XO activity. These findings indicate that the inhibition of XO activity by SMS-E may result in reduced uric acid production.

In humans, the kidneys play a critical role in maintaining circulating uric acid concentrations, given that more than 70% of its total excretion from the body is performed by the kidneys [26]. However, impaired renal urate excretion leads to hyperuricemia. Uric acid excretion in the kidneys is performed by two types of uric acid transporters: urate reabsorption transporters and urate excretion (secretion) transporters. Three renal urate transporters, URAT1, GLUT9, and ATP-binding cassette transporter G2, are dominant apical and basolateral urate exchangers in the kidney proximal tubule. Their dysfunction causes renal underexcretion hyperuricemia, as well as renal overload hyperuricemia due to the blockage of urate excretion from the kidneys and intestine [27,28]. OAT1 and OAT3, located in the basolateral membrane of proximal tubular epithelial cells, transport urate from the blood to epithelial cells [29]. Therefore, promoting uric acid excretion by regulating these urate transporters remains an attractive therapeutic target for hyperuricemia. SMS-E reduced the protein levels of renal URAT1 and GLUT9 in PO rats. SMS-E increased both urine uric acid excretion and FEUA, thus exhibiting a uricosuric effect. These findings are consistent with those of a previous study [21]. In addition, the IC50 value of SMS-E for the uptake of uric acid by URAT1, using an in vitro system, was 0.21 μg/mL. In the renal urate reabsorption pathway, URAT1 transfers uric acid from the apical membrane to proximal tubular cells, and then GLUT9 exchanges intercellular urate from these cells to the peritubular interstitium [30]. Therefore, a reduction in uric acid reabsorption, via a decrease

Fig. 3. Effects of SMS-E on renal inflammation. (A) Kidney histopathological changes (original magnification × 200), and (B) serum IL-1β and (C) renal IL-1β protein levels. N, normal; PO, potassium oxonate-induced hyperuricemia; Allo, allopurinol; SMS-E, saengmaeksan 30% ethanol extract. #P < 0.05 vs. the N group; *P < 0.05 and **P < 0.01 vs. the PO group.
in URAT1 and GLUT9, may promote renal uric acid excretion in SMS-E-treated hyperuricemic rats.

An increase in uric acid is associated with inflammation, which aggravates renal damage in hyperuricemic rodents [18]. In this work, impaired renal function was characterized by increased creatinine and IL-1β levels in both the serum and kidneys. Furthermore, hyperuricemic rats also exhibited renal inflammation, which was attenuated by SMS-E treatment. SMS is a mixture of crude extracts from three different plant sources. A previous study reported that ginseng, present in SMS-E, reduced renal damage and nephrotoxicity by reducing serum urea, creatinine, and renal XO levels [31]. Our UPLC analysis showed that SMS-E contains high levels of 25 ginsenosides derived from ginseng. Pharmacologically, ginseng and its major active component ginsenosides possess various biological activities, including anti-inflammatory and antioxidant roles, and provide renal protection [32,33]. Recently, it was reported that ginsenosides decrease uric acid levels by reducing renal dysfunction and increasing urate excretion in hyperuricemic mice [34]. These observations indicate that SMS, a

Fig. 4. Effects of SMS-E on xanthine oxidase (XO) activity. (A) Serum XO activity, (B) hepatic XO activity, (C) liver uric acid levels, (D) in vitro XO activity, and (E) XO inhibition (for determining the IC50 value). N, normal; PO, potassium oxonate-induced hyperuricemia; Allo, allopurinol; SMS-E, saengmaeksan 30% ethanol extract. #P < 0.05 and ##P < 0.01 vs. the N group; *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the PO group.
traditional herbal formulation containing ginseng, may exert advantageous effects on hyperuricemia and kidney damage.

In addition, the efficiency of urate excretion and the anti-hyperuricemic effects of SMS were increased using an alternative method to water extraction. These results suggest that a 30% ethanol-based extraction method may be more effective than the conventional water-based extraction method, in terms of improving its extraction efficiency and in vivo effects. However, our study did not examine whether single extracts of compounds from the SMS-E mixture enhanced the production or excretion of uric acid. Therefore, the effects and underlying mechanisms of single extracts and bioactive compounds derived from SMS-E must be further investigated.

5. Conclusions

The experimental findings in this work demonstrate for the first time that a 30% ethanolic extract of SMS reduces hyperuricemia and kidney inflammation. These effects are achieved by inhibiting XO activity and downregulating urate transporters. Therefore, SMS is a potential candidate therapy for the treatment of hyperuricemia and gout.
Fig. 6. (A) Comparison of representative base peak intensity (BPI) chromatograms of SMS extracts. The peaks (1–25) are listed in Supplementary Material Table 1. (B) Comparison of the relative intensity of ginsenosides from SMS-E and SMS-W extracts.
Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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