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Analgesic, anti-inflammatory, and antihyperuricemic activities of a Thai herbal remedy

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ABSTRACT

Introduction: Moa Chub's gout formulation (MCHGF), a traditional Thai herbal remedy, is used for the treatment of pain and swelling related to gout. **Objective:** This study aimed to determine MCHGF's analgesic, anti-inflammatory, and antihyperuricemic effects. **Materials and Methods:** The analgesic activity of MCHGF was studied in mice using an acetic acid-induced writhing test and the hot-plate method. The anti-inflammatory activity of MCHGF was evaluated in rats using carrageenan-induced paw edema. The antihyperuricemic activity of MCHGF was tested *in vivo* using the model of potassium oxonate-induced hyperuricemia mice. MCHGF doses of 50, 100, and 200 mg/kg/day were used in this study. The *in vitro* xanthine oxidase (XO) inhibitory activity was carried out to investigate the mechanism of uric acid-lowering effect. **Results:** The results showed that MCHGF extract reduced writhing response in the acetic acid test, while MCHGF doses of 100 and 200 mg/kg/day increased the reaction time in the hot-plate method. In addition, MCHGF significantly inhibited carrageenan-induced paw edema at 3 and 4 h after carrageenan injection. Moreover, all doses of MCHGF significantly reduced the serum uric acid levels. However, MCH GF did not inhibit XO activity in *in vitro* study. **Conclusion:** MCHGF demonstrated analgesic, anti-inflammatory, and antihyperuricemic activities in the experimental animal models.

Key words: Herbal remedy, Analgesic, Antiinflammation, Uric acid, Xanthine oxidase

INTRODUCTION

Gout is a metabolic disorder caused by excess uric acid in the blood, which results in the accumulation of urate crystals in the joints. The depositing of urate crystals in joint cavities initiates the inflammatory process as a result of the joints becoming engulfed by synovial phagocytic cells, leading to the release of lysosomal enzymes and the production of inflammatory chemokines.[1] The management of gout includes both the treatment of gouty arthritis (acute attacks) and the prevention of future attacks. In acute gout attacks, the main drugs used for the relief of inflammation and pain are colchicine, nonsteroidal anti-inflammatory drugs (NSAIDs), and corticosteroids. Other medications include allopurinol, febuxostat, and probenecid, which are taken daily to prevent future gout attacks.[2,3] However, prolonged administration of these drugs can have adverse effects. Herbal medicines are prevalent in developing countries as a form of primary healthcare.[4,5] Herbal drugs are cheaper and have fewer adverse effects than synthetic drugs.[6]

Moa Chub's gout formulation (MCHGF), a Thai herbal remedy, is used for the management of gout. It consists of 12 medicinal herbs: *Khow Yen Thang Song (Smilax spp)*, *Khan Thong Phayabaht (Gelinium multiflorum)*, *Sri SabYang (Smilax peguana)*, *Thong Phan Chang (Rhinacanthus nasutus)*, *Than Mai Sark (Erythrophleum succirubrum)*, *Chumtled Tade (Cassia alata)*, *Som koong noy (Embelia ribes), Som koong yai (Begonia inflate), Tian Dum (Nigella sativa)*, garlic *(Allium sativum)*, pepper *(Piper nigrum)*, and *Ma Dook (Siphonodon celastrineus)*. Conventionally, this formulation is used for relief of pain and swelling related to gout. Previous studies have evaluated the cytotoxicity of MCHGF using human white blood cells and found that there was no toxicity.[7] In a qualitative test, piperine was reported as the basis of this formulation.[8] Some medicinal plants in MCHGF, such as *P. nigrum, Cassia alata*, and *N. sativa*, have analgesic and anti-inflammatory activities.[9-11] *A. sativum* has been reported to have analgesic activity by inhibiting cyclooxygenase (COX) activity.[12,13] However, no scientific evidence on its efficacy to support the traditional use of this formulation in gout

patients. Thus, the present study aimed to assess the analgesic, anti-inflammatory, and hypouricemic effects of MCHGF for gout treatment following oral administration in animal models. In addition, the xanthine oxidase (XO) inhibitory activity test was carried out *in vitro* to determine the mechanism of uric acid-lowering effect.

MATERIALS AND METHODS

Chemicals

Acetic acid, carrageenan, indomethacin, potassium oxonate (PO), allopurinol, xanthine, and XO were purchased from Sigma Aldrich (USA).

Plant materials

MCHGF was prepared by the balanced combination of 12 herbal ingredients, 8.33% (w/w) of each herb, as shown in Table 1. The MCHGF powder was purchased from a licensed traditional medical drug store, Ratchaburi Yathai (Ratchaburi, Thailand). All plant materials were authenticated by Moa Chub pankhumyat, the experienced traditional Thai doctor registered with the Thai Traditional Medicine Council. The specimens of the formulation were deposited in the Department of Pharmacognosy, College of Pharmacy, Rangsit University, Thailand.

Preparation of extract

MCHGF powder was extracted with hot water accordingly in the same manner as used in humans. One hundred grams of the powdered MCHGF decocted with 2 l of boiling water 3 times for 1 h. The final decoction was combined and filtered to remove the residue. A spray-drying process was performed to give a MCHGF dried extract (percent yield 12.98% w/w). Animal doses in this study were calculated from human doses of MCHGF based on body surface area.[14,15] MCHGF extract doses of 50, 100, and 200 mg/kg/day were dissolved in sterile water and used for the experiment in animal model.

Standardization of MCHGF Extract

The standardization of principle compounds found in MCHGF extract was confirmed with the individual 12 active ingredient extracts in the MCHGF recipe. The analytical method for quality control of MCHGF was previously reported by Vchirawongkwin *et al*.[8]

Animals

Wistar rats weighing 200 ± 30 g were used for the anti-inflammation testing. Swiss albino mice weighing 20–25 g were used for the evaluations of analgesic activity and *in vivo* antigout activity. Both rats and mice were obtained from the National Laboratory Animal Center, Salaya, Mahidol University, Nakorn Pathom, Thailand. The animals were housed in standard room which was maintained at a constant temperature of 25 ± 2°C under a 12 h light-and-dark cycle and had free access to food and water. The study protocol was approved by the ethics committee for animal research at Rangsit University (approval number RSEC 03/2559).

Acute Toxicity Test

The acute oral toxicity study was performed according to the Organization for Economic Co-operation and Development guideline no. 420.[16] Sprague Dawley rats were used for the study and orally dosed once with MCHGF extract at a dose of 2000 mg/kg. The treated rats were monitored for general clinical signs and symptoms, as well as mortality, at 1, 2, 4, 6, and 24 h and then once daily for 14 days. MCHGF extract at the high dose of 2000 mg/kg did not produce mortality nor show any signs of toxicity. A previous study also provided critical evidence for the safety of the substance, with LD_{50} >1000 mg/ kg considered practically non-toxic.[17]

Acetic Acid-induced Writhing Effect

An acetic acid-induced writhing effect test was carried out using the method previously described.[18,19] Mice were

Table 1: List of herbal ingredients of MCHGF

Herbal name	Scientific name	Family	Part used	Ratio (% w/w)
Khow Yen Thang Song	*Smilax* spp.	Smilacaceae	Dried rhizome	8.33
Khan Thong Phayabaht	*Gelinium multiflorum*	Euphorbiaceae	Dried stem bark	8.33
Sri SabYang	*Smilax peguana*	Smilacaceae	Dried rhizome	8.33
Thong Phan Chang	*Rhinacanthus nasutus*	Acanthaceae	Dried whole plant	8.33
Than Mai Sark	*Erythrophleum succirubrum*	Fabaceae	Stem charcoal	8.33
Chumtled Tade	*Cassia alata*	Fabaceae	Dried stem	8.33
Som koong noy	*Embelia ribes*	Begoniaceae	Dried root	8.33
Som koong yai	*Begonia inflate*	Begoniaceae	Dried root	8.33
Tian Dum	*Nigella sativa*	Ranunculaceae	Dried seed	8.33
Garlic	*Allium sativum*	Alliaceae	Dried rhizome	8.33
Pepper	*Piper nigrum*	Piperaceae	Dried seed	8.33
Ma Dook	*Siphonodon celastrineus*	Celastraceae	Dried root	8.33

randomized into five groups of eight animals each. Aqueous extracts of the MCHGF formulation were used at doses of 50, 100, and 200 mg/kg, while indomethacin of 10 mg/kg was used as a standard analgesic drug.[20] The MCHGF extract was administered orally 1 h before the administration of acetic acid (0.75% v/v in water, 0.1 ml/10 gBW, i.p.). The mice were placed individually in a glass box. The number of writhings (a response consisting of contraction of abdominal muscles together with hind-limb extension) in 60 min was recorded.

Hot-plate Test

The hot-plate test was performed to measure latency response according to the method previously described.[21,22] Mice were randomized into five different groups of eight mice each. Mice exhibiting a basal latency time >30 s or <5 s were excluded. MCHGF (50, 100, and 200 mg/kg), morphine (10 mg/kg, as the standard drug), and water (as the negative control) were administered orally, and the mice's reaction times were observed at 0, 15, 30, 60 and 90 min. The hot plate was maintained at 54.0 ± 1°C. The elapsed time in seconds between placing the mice on the hot plate and observing a discomfort reaction (hind-paw licking or jumping) was recorded as the index of latency response. A cutoff period of 25 s was defined as complete analgesia. The experiment was stopped if the cutoff time was exceeded to avoid damage to the paw.

Carrageenan-induced Paw Edema

The anti-inflammatory activity of MCHGF was evaluated using the carrageenan-induced paw edema test in rats.[23] MCHGF (50, 100, and 200 mg/kg) was orally administered 1 h before the injection of 0.05 ml of 1% carrageenan. Paw volume was measured at 0, 1, 2, 3, and 4 h after the carrageenan injection using a plethysmometer (Panlab, s.l.u., Spain). The percentage of inhibition compared to controls was calculated according to the following formula:

$$\text{Percentage of inhibition} = \frac{(PVx - PV0)control \; - \; (PVx - PV0)test\ group}{(PVx - PV0)control} \times 100$$

Where PV_0 = Mean paw size (edema volume) at 0 h after carrageenan injection, and PV_x = mean paw size at x h after carrageenan injection.

In vivo Antigout Activity

Hyperuricemia was induced in mice using PO, a uricase inhibitor, as described previously.[24,25] Mice were randomly divided into six groups of eight mice each. Group 1 (normal control) received only the vehicle (sterile water). Group 2 (the hyperuricemia group) received PO of 250 mg/kg dissolved in normal saline, which was intraperitoneally administered. Both groups 1 and 2 were treated for 14 days In Groups 3–6, each animal was first injected with PO of 250 mg/kg, 1 h before the administration of the test compound; Group 3 received allopurinol of 10 mg/kg as a standard drug. Groups 4–6 received MCHGF doses of 50, 100, and 200 mg/kg, respectively. All samples were administered to the corresponding groups by oral gavage once a day for 14 days. On the past day, 1 h after treatment, blood samples were collected from the abdominal aorta. The blood was allowed to clot for 1 h at room temperature and then centrifuged at 3000 g for 10 min to obtain serum. Serum uric acid concentration was measured using UA2 reagent diagnostic tests on the Cobas Integra 400 Plus analyzer (Roche, Switzerland).

XO Inhibitory Activity Assay

XO inhibitory activity was determined in accordance with the method of Duong *et al*. with minor modification.[26] MCHGF solution was prepared in phosphate buffer. Briefly, 35 µl phosphate buffer (70 mM, pH 7.5) was added in 96-well plate. Then 50 µl of the test solution and 30 µl of freshly prepared XO solution (0.03 U/mL in phosphate buffer) were added and pre-incubated for 15 min at 25°C. The reaction started by 60 µl of xanthine (0.15 mM). The reaction was incubated at 25°C for 15 min. The reaction was stopped by adding 25 µl of HCl solution (1 M). The product as uric acid was measured at 290 nm using Bio Tek Epoch microplate spectrophotometer. Allopurinol (10 µg/ml) was used as a positive control. All experiments were performed in triplicate. The blank well was prepared similarly, but by adding HCl solution before the substrate.

Optical density (OD) was blank-corrected values. The percentage of XO inhibition effect was calculated as using the formula:

Percentage of XO inhibition = $(OD_{control} - OD_{sample})/OD_{control} \times 100$.

Statistical Analysis

The data from the experiment were expressed as mean ± standard error of mean (S.E.M.). Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by least-significant difference test. $P < 0.05$ were considered significantly different.

RESULTS

Acetic Acid-induced Writhing Effect

The analgesic effect of the MCHGF extract on acetic acid-induced writhing is presented in Figure 1. The results showed that the MCHGF extract at doses of 50, 100, and 200 mg/kg significantly reduced the number of abdominal constrictions and writhing when compared to the control group. The analgesic effect of MCHGF was similar to the inhibitory effect of indomethacin at a dose of 10 mg/kg.

Hot-plate Test

In the hot-plate test, the administration of MCHGF at doses of 100 and 200 mg/kg prolonged the latency time compared with the control. No significant analgesic effect was observed for the MCHGF dose of 50 mg/kg. Morphine (10 mg/kg), the positive control used in this study, produced a significant increase in reaction time at all time points. Latency times are shown in Figure 2.

Carrageenan-induced Paw Edema

The MCHGF extract displayed potent anti-inflammatory activity in the models of carrageenan-induced paw edema

in rats. MCHGF at a dose of 50 mg/kg significantly inhibited carrageenan-induced inflammation at 3 and 4 h after carrageenan injection, while MCHGF at doses of 100 and 200 mg/kg significantly inhibited carrageenan-induced inflammation at all time points (1–4 h). Indomethacin at a dose of 10 mg/kg significantly suppressed carrageenan-induced paw edema at 2, 3 and 4 h after carrageenan injection [Figure 3].

In Vivo Antigout Activity

The effect of MCHGF on serum uric acid concentration in PO -induced hyperuricemic mice is shown in Figure 4. PO significantly increased the level of serum uric acid compared to the normal control group. The group receiving both allopurinol and PO had significantly reduced uric levels compared to those receiving only PO. However, the level of uric acid in this group was lower than in the normal control group. MCHGF at doses of 50, 100, and 200 mg/kg significantly reduced serum uric acid levels when compared to the hyperuricemic control group. The level of uric acid for the MCHGF dose of 200 mg/ kg was the lowest among the groups.

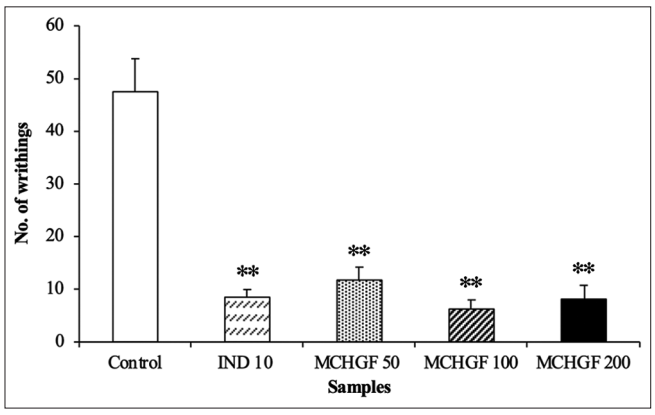

Figure 1: Effect of Moa Chub's gout formulation on acetic acid-induced writhing in mice. Values were expressed as mean ± standard error of mean of the number of contractions registered for 60 min after the acetic acid injection. n = 8/group. All of the values were statistically analyzed by analysis of varianc (**P < 0.01 when compared to the control group)

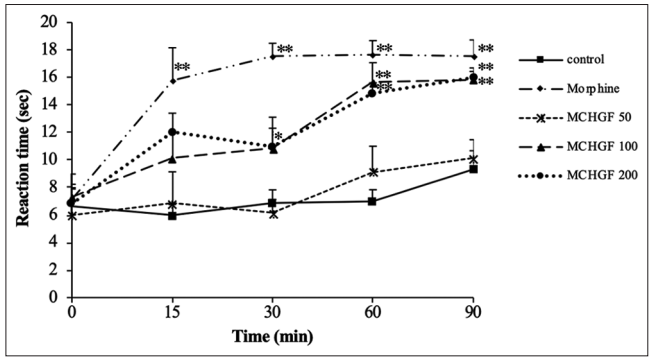

Figure 2: Effect of Moa Chub's gout formulation on the pain threshold of mice in the hot-plate test. Values were expressed as mean ± standard error of mean of the reaction time (seconds) measured at 0, 15, 30, 60 and 90 min after treatment. n = 8/group. All of the values were statistically analyzed by analysis of varianc (**P < 0.01, *P < 0.05 when compared to the control group)

In Vitro XO Inhibition of MCHGF

To investigate the mechanism of the uric acid-lowering effect of MCHGF, MCHGF was determined the inhibitory effect on XO using *in vitro* study. The maximum final concentration was 1 mg/ml due to the brown color of MCHGF, which disturb the absorbance. The results showed that MCHGF at the concentrations of 0.1, 0.5, and 1 mg/ml did not inhibit the activity of XO [Table 2]. Allopurinol used as positive control significantly inhibited XO activity.

DISCUSSION

In recent years, several pharmacological activities of traditional polyherbal formulations related to human health have been reported. The strategy of using multiple herbal combinations in the traditional formulation is to maximize the therapeutic efficacy and minimize toxicity.[27] According to Thai folk medicine practitioners, the MCHGF is used for pain and inflammation in gout. In this study, the analgesic, anti-inflammatory, and anti-hyperuricemic activities of MCHGF were studied in animal models.

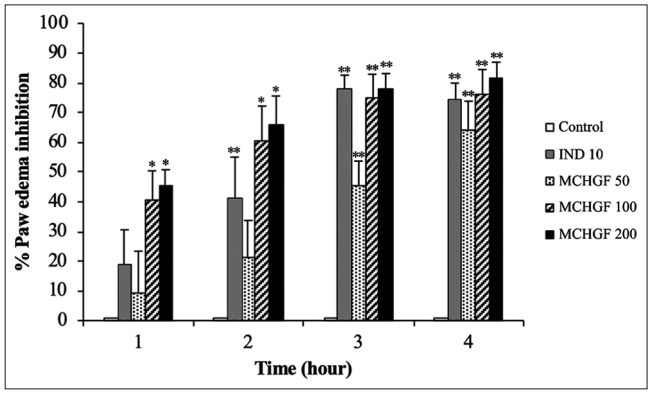

Figure 3: Effect of Moa Chub's gout formulation on carrageenan-induced paw edema in rats. Values were expressed as mean ± standard error of mean of the percentage inhibition of paw edema in each group at 1, 2, 3 and 4 h after the carrageenan injection. n = 6/group. All of the values were statistically analyzed by analysis of varianc (**P < 0.01, *P < 0.05 when compared to the control group)

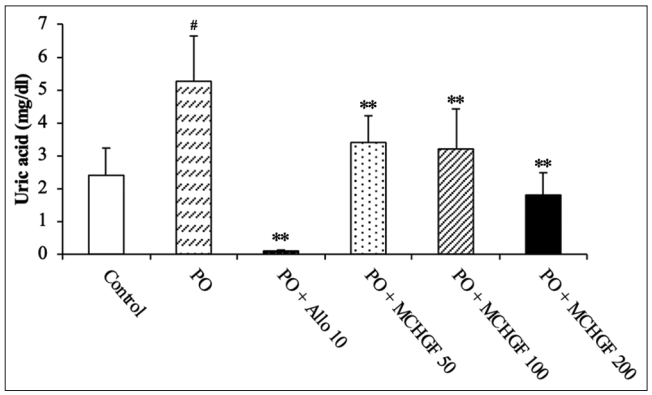

Figure 4: Effect of Moa Chub's gout formulation on serum uric acid levels. Values were expressed as mean ± standard error of mean n = 8/group. All of the values were statistically analyzed by analysis of varianc (#P < 0.01 when compared with control **P < 0.01 when compared with PO) (Allo: allopurinol, PO: potassium oxonate)

Table 2: XO inhibition of MCHGF

Test compounds	% XO inhibition (Mean±S.E.M.)
Allopurinol (10 μg/ml)	91.79 ± 0.25**
MCHGF	
0.1 mg/ml	0.25 ± 0.21
0.5 mg/ml	1.44 ± 0.79
1 mg/ml	1.89 ± 1.54

**$P<0.01$ when compared to the control group. MCHGF: , XO: , SEM:

In the analgesic test, the acetic acid-induced writhing response in mice was used to measure the peripherally analgesic activity, and the hot-plate model was used to measure the centrally acting analgesic activity. All doses of MCHGF extract (50, 100, and 200 mg/kg) significantly inhibited the acetic acid-induced writhing response in mice. The acetic acid-induced writhing effect measures pain by stimulating a localized inflammation response. Intraperitoneal injection of acetic acid leads to the release of free arachidonic acid from tissue phospholipids, causing an increase in the peritoneal fluid level of a variety of mediators such as prostaglandins (PGE_2 and PGF_2^{α}), substance P, and bradykinins.[28] These endogenous mediators stimulate the peritoneal nociceptors involved in inflammatory pain. Prostaglandins, a COX product, are known as a lipid mediator that contributes to inflammatory pain. They are strong hyperalgesic mediators that involve in the nociceptive pathway and enhance the transmission of nociceptive information.[29,30]

NSAIDs, which are inhibitors of COX, suppress inflammatory pain by reducing the generation of prostaglandins.[31] The results of this study showed that indomethacin inhibited the number of acetic acid-induced writhings, thus confirming the analgesic effect of NSAIDs in this model. According to the percentage inhibition of the number of writhings observed after the administration of indomethacin and various doses of the MCHGF extract, the intensity of MCHGF's analgesic effect on mediator production or signal transduction in primary afferent nociceptors is similar to that of indomethacin [Figure 1]. The hot-plate test is widely used to investigate central analgesic activity.[32] This method involves higher brain function, which is a supraspinally organized response.[33] The hot-plate method produces two measurable behavioral components of reaction times to thermal pain. Responses such as paw licking and jumping are supraspinally integrated.[32,34] MCHGF at doses of 100 and 200 mg/kg significantly exhibited analgesic activity by increasing the reaction time when compared to the control group at all time points. This finding indicates that MCHGF at high doses (100 and 200 mg/kg) exhibited analgesic activity via centrally acting at the supraspinal level. Morphine was used as a reference drug, producing the most significant analgesic effect during all observation times. This result confirms that morphine exerts its action by interfering in the pain transmission of the central nervous system. It was found that the analgesic effect of MCHGF was less than that of morphine at 10 mg/kg [Figure 2]. As mentioned earlier, MCHGF contains 12 herbs, and the formulation is used for pain treatment. Its analgesic effect could be correlated with previous studies reporting that *R. nasutus*, *E. ribes*, and *N. sativa*, the component of MCHGF, have analgesic activity. *R. nasutus* was found to inhibit pain in acetic acid-induced writhing and formalin tests.[35] Interestingly, *E. ribes* is the medicinal plant used for the treatment of arthritis in Iranian traditional medicine.[36] In addition, *N. sativa* was found to have analgesic activity in acetic acid-induced writhing test and hot plate test.[9,37,38] This analgesic effect of MCHGF may be due to the presence of piperine in the formulation. It has been reported that piperine has analgesic activity in tail-flick, hot plate, and acetic acid-induced writhing model.[39]

The model of carrageenan-induced inflammation is the method for screening the orally active anti-inflammatory agents. The mechanism of carrageenan-induced edema is biphasic. The first phase involves the release of histamine, kinin, and serotonin during the first few hours after the injection of carrageenan, and the second phase involves the release of prostaglandins in 2–3 h.[40] The second phase is sensitive to treatment with NSAIDs.[41] From the results, it was clear that MCHGF exhibited an anti-inflammatory effect against acute inflammation. MCHGF inhibited carrageenan-induced paw edema in a concentration-dependent manner. In addition, the anti-inflammatory effect of MCHGF at doses of 100 and 200 mg/kg was comparable with that of indomethacin of 10 mg/kg at 3 and 4 h after the carrageenan injection [Figure 3]. This finding suggests that the intake of MCHGF had anti-inflammatory effects in both the first and second phases of acute inflammation, which is similar to the observed effects of indomethacin. This indicates that the mechanism of the anti-inflammatory effect of MCHGF may be mediated via the inhibition of prostaglandin synthesis. Thus, MCHGF may be active against diseases with pathophysiologies involving inflammation. These effects might be the result of synergistic interactions between the multiple compounds in the MCHGF. The anti-inflammatory activities of the extracts or constituents obtained from *G. multiflorum*,[42] *R. nasutus*,[35,43] *C. alata*,[44,45] *E. ribes*,[46,47] *N. sativa*,[9,48,49] *A. sativum*,[50,51] and *P. nigrum*[52] have been reported. Moreover, these results are consistent with previous studies that piperine, the major fraction of this formulation, has anti-inflammatory effects in monosodium urate crystal-induced inflammatory models.[52] Another research found that piperine inhibits tumor necrosis factor-α, Interleukin (IL-6), IL-1β, and prostaglandin E_2 production in lipopolysaccharide induced inflammation.[53,54] Thus, the anti-inflammatory observed in MCHGF might be attributed to the presence of piperine.

Uric acid is the last metabolite in the purine catabolic pathway formed by XO enzyme. High blood uric acid levels cause the accumulation of urate crystals in the joints, which is an important risk factor for gouty arthritis. This condition can cause inflammation and is painful for gout patients.[55] MCHGF is used in Thai folk medicine for the treatment of pain and swelling related to gout. However, the effects of MCHGF on uric acid levels had not been examined before the current research. In this study, the effect of MCHGF on uric acid levels was studied using the model of PO -induced hyperuricemic acid.[56] The treatment group of PO showed a significant elevation in serum urate levels as compared to the normal group, revealing that this model has been successfully established. Allopurinol, the standard hypouricemic drug, also led to a significant reduction in serum urate levels. Treatment with MCHGF extract at doses of 50, 100, and 200 mg/kg significantly reduced uric acid

levels when compared with the hyperuricemic control group. The results of the current study demonstrated that MCHGF extract has a uric acid-lowering effect. It helped to reduce uric acid accumulation in the joints, and thus might reduce gout attacks. The mechanism of MCHGF that decreases uric acid level was also investigated. In this study, the inhibitory effect of MCHGF on XO was determined. The results revealed that MCHGF did not show XO inhibition although some plants in MCHGF, i.e., *P. nigrum*,[57,58] *A. sativum*[50] and *C. alata*,[59] have been reported to demonstrate *in vitro* XO inhibition. These results suggested that MCHGF may not directly inhibit XO, or there might be other mechanisms that decrease uric acid in rats including increase the uricase activity or decrease uric acid reabsorption to promote uric acid excretion.

MCHGF exhibited significant antinociception activity against both chemical stimuli (acetic acid test) and thermal stimuli (hot-plate test). These results suggest that the mechanism of MCHGF on analgesic activity may be via inhibition of both peripherally and centrally mediated nociception. MCHGF was found to have acute anti-inflammatory effects. In addition, it also had the effect of reducing the level of uric acid in the blood. However, MCHGF did not show any effect on *in vitro* XO activity. Further investigation is required to elucidate the mechanism of MCHGF including prostaglandin inhibition for analgesia and anti-inflammation, and other possible mechanisms for the uric acid-lowering effect.

CONCLUSION

The results of the present study show that MCHGF had strong analgesic, anti-inflammatory, and antihyperuricemic activities in the experimental animal models. All these effects support the wide use of this remedy for the management of pain and inflammation in gout patients.

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