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Antinociceptive and anti-hyperuricemic effects of ethanolic extract from *Homalomena pierreana* Engl., Araceae

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ABSTRACT

Background: Gout is one of arthritis diseases resulting from high levels of plasma uric acid. Screening of medicinal plants for analgesic and anti-hyperuricemic effects is necessary to prevent and treat gout disease. **Objectives:** *Homalomena* has been widely used in traditional medicine for the treatment of bone diseases. *Homalomena pierreana* is a newly discovered rare species found in Vietnam. The study aims to clarify the antinociceptive and anti-hyperuricemic effects of 45% ethanolic extract from *H. pierreana* rhizome (*H. pierreana* extract) in male Swiss albino mice. **Methods:** Acetic acid-induced writhing and thermal stimulus-induced pain (hot plate) assays were applied to investigate antinociceptive effects. Model of potassium oxonate-induced acute hyperuricemia in mice was used to examine anti-hyperuricemic effects. **Results:** The results revealed that 5-day pretreatment with *H. pierreana* extract at the oral doses of 390 mg/kg and 780 mg/kg, as well as a reference drug diclofenac sodium, decreased the number of acetic acid-induced writhing in mice. Administration of the extract at doses of 390 mg/kg and 780 mg/kg also significantly delayed the reaction time of mice to pain (or an increase in the latency to licking/jumping) caused by thermal stimulus in hot plate test but the effect was weaker than those of morphine (10 mg/kg, i.p.). Moreover, *H. pierreana* extract as well as a reference drug allopurinol, significantly reduced plasma uric acid levels of hyperuricemic mice and restored to the baseline levels. **Conclusion:** *H. pierreana* extract possesses antinociceptive and anti-hyperuricemic effects which confirm its usefulness of the gout management.

Keywords: *Homalomena pierreana* rhizome, antinociceptive effect, anti-hyperuricemic effect

1. INTRODUCTION

In Vietnam, according to the Community Oriented Program for the Control of Rheumatic Diseases, gout prevalence is about 0.14% in Hanoi [1]. Gout is one of arthritis disease resulting from high levels of plasma uric acid. Plasma uric acid levels are likely to reflect current dietary habits. Uric acid is the end product of purine metabolism, and eating a lot of purine-riched foods contributes to total uric acid levels [2]. Pain relief and uric acid-lowering monitoring are first-line treatment for management of gout and improving the quality of life for people with gout.

Besides drug therapy, adjusting dietary habits and using natural products from medicinal plants help to prevent and minimize the risk of gout.

Homalomena has been widely used in traditional medicine for the treatment of rheumatism and bone diseases. *Homalomena pierreana* Engl., Araceae is a newly discovered rare species that has been referenced in studies conducted in Vietnam recently. *H. pierreana* [another name: *Homalomena griffithii* (Schott) Hook. f.] was identified by Van Hong Thien et al. by DNA barcode sequence in 2020 [3]. This plant had some research publications on its

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phytochemistry and pharmacological effects including: Analysis of the chemical composition of the essential oils [4], sesquiterpene compounds isolated from *H. pierreana* rhizome and its antibacterial activity against six tested bacterial strains [5, 6]. Recently, Huong et al. reported that 45% ethanolic extract from *H. pierreana* rhizome suppresses the inflammatory response via inhibition of lipopolysaccharide-induced nitric oxide production in murine macrophage cell line RAW 264.7 and reducing carrageenan-induced paw edema in mice [7]. Moreover, successful clonal propagation of *H. pierreana* has made this species more attractive to study for their chemical composition as well as potential for further medicine usages [8].

Notwithstanding, there is no scientific investigation which has been discussed about the antinociceptive effect and anti-hyperuricemic effects of this plant. Based on anti-inflammatory results [7], this study aims to evaluate further outcomes of 45% ethanolic extract from *H. pierreana* rhizome as alternative agent in treating gout by using mouse models of pain and potassium oxonate-induced acute hyperuricemia.

2. MATERIALS AND METHOD

2.1. Plant material and extraction

H. pierreana rhizome was collected in Phu Quoc National Park, Kien Giang province on December, 2022. The plant was identified and scientifically named by Mr. Cao Ngọc Giang of the Research Center of Ginseng and Medicinal Materials in Ho Chi Minh City (sample code: ĐD 05A/2023). Dried powdered material (pass through a sieve 250) was extracted (the ratio was 1: 15 of raw material: solvent, w/v) with 45% ethanol at room temperature for 48 h in a percolator apparatus. The extract was collected at a rate of 1 mL/min and concentrated using a rotary evaporator at 60 °C under reduced pressure to obtain crude condensed extract (*H. pierreana* extract) with moisture content of 18.1%. The yield of extraction was 31%. *H. pierreana* extract at oral doses of 390 mg/kg and 780 mg/kg mouse body weight (equivalent to 1.25 and 2.5 g raw materials) were selected for pharmacological study according to previous study [7].

2.2. Animals

Six-week-old male *Swiss albino* mice (25 ± 2 g)

were purchased from the Institute of Vaccines and Medical Biologicals, Nha Trang, Vietnam. The mice were habituated to the laboratory animal room for at least one week before the experiment. Food and water were available *ad libitum*. Housing was thermostatically maintained at 26 ± 1 °C and a 12-h light-dark cycle (lights on: 07:00 – 19:00). The administered volume was 10 mL/kg mouse body weight. All experiments were conducted according to "Guidelines for preclinical and clinical trials of traditional medicine and herbal drugs" by Vietnam Ministry of Health (under decision No. 141/QĐ-K2ĐT, be valid on 27/10/2015) [9] and followed 3R- principles of Animal testing (Reduction-Replacement-Refinement) [10].

2.3. Acetic acid-induced writhing test

Mice were randomly divided into 4 groups (n = 10 mice/group). Each group was orally administered distilled water (control group), *H. pierreana* extract (test groups at the doses of 390 mg/kg and 780 mg/kg) for 5 consecutive days and diclofenac sodium (reference group) at a single dose of 25 mg/kg (Voltaren® 50 mg diclofenac sodium/tablet, Novartis) on the 5th day.

On the 5th day, after 60 minutes of *H. pierreana* extract or diclofenac administration, 0.6% acetic acid solution was intraperitoneally injected (i.p.). Mice were placed individually into glass box and then observed immediately after the acetic acid injection for a period of 10 minutes. The numbers of writhes were recorded in each animal during 30 minutes. Writhing is defined as a stretch, tension to one side, extension of hind legs, or contraction of the abdomen so that the abdomen of the mice touches the floor, or turning of the trunk (twist) [11].

Analgesic activity of *H. pierreana* extract was inferred from a decrease in the frequency of abdominal writhes. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \{ (W_c - W_t) \times 100 \} / W_c$$

Where, W_c = Average number of writhes in control group, W_t = Average number of writhes in treated group.

2.4. Hot plate test

Mice were randomly divided into 4 groups (n = 8 mice/group). Each group of mice was orally administered distilled water (control group), *H. pierreana* extract (test groups at the doses of 390

mg/kg and 780 mg/kg) for 5 consecutive days and morphine (VIDIPHA Co., injected ampoule 10 mg/mL, reference group) at a single dose of 10 mg/kg, i.p. on the 5th day.

On the 1st day (before administration), the mice were brought into the testing room and a period of acclimation was provided (30-60 min). The surface of the hot plate (Hot Plate Analgesia Meter, Ugo Basil Co., Italy) was cleaned with 70% ethanol prior to use or between the testing of each mouse. The surface of the hot plate was calibrated to a constant temperature of 55°C. The mouse was placed on the testing apparatus and the timer was started. The latency to show a nociceptive response with hind paw lick, hind paw flick, or a jump from the hot plate surface was recorded (baseline latency: T₀). The mouse was immediately removed once this response was observed. If there was no response within 30 seconds (a cut-off time), the test was terminated and the mouse was removed from the hot plate to prevent heat-related injury. Exclusion criteria was removing the mice with rapid reaction (under 8 seconds) or late response (over 30 seconds) [12].

To evaluate the effects of the treatment, the hot plate test was conducted 30 minutes and 90 minutes after the final *H. pierreana* extract or morphine administration on day 5. The procedure was performed similarly on the 5th day to record the latency of mice to thermal stimuli after 30 minutes (T₃₀) and 90 minutes (T₉₀) of *H. pierreana* extract or morphine administration. Statistical comparison was done between T₀, T₃₀ and T₉₀ of *H. pierreana* extract-treated groups and those of control and reference drug morphine. The significant increase of latency of mice to thermal stimuli as compared to untreated group (control) was evaluated as antinociceptive effect.

2.5. Potassium oxonate-induced acute hyperuricemia in mice [13]

Mice were randomly assigned into five groups (n = 8 mice/group) as followed:

- Physiological control group: Mice were administered distilled water for 4 consecutive days and received 0.9% NaCl (i.p.) on 5th day.
- Pathological control group: Mice were administered distilled water for 4 consecutive days. On 5th day, mice were received potassium oxonate (300 mg/kg, i.p.) and administered distilled water one hour later.
- *H. pierreana* extract-treated groups (the dose of 390 mg/kg or 780 mg/kg): Mice were orally administered the extract for 4 consecutive days. On 5th day, mice were received potassium oxonate (300 mg/kg, i.p.) and administered the extract one hour later.
- Allopurinol (10 mg/kg, Sigma-Aldrich, USA)-treated group: Mice were administered distilled water for 4 consecutive days. On 5th day, mice were received potassium oxonate (300 mg/kg, i.p.) and orally administered allopurinol one hour later.

Blood samples (0.15 mL/mouse) were taken from the tail of the mice after 2 hours of potassium oxonate injection into EDTA tubes. Uric acid levels in the plasma were determined according to the instruction of the Human Co. (Germany) test kit by Biochemical Systems Screen Master 3000 Chemistry Analyzer (Italy). The significant uric acid-lowering effect as compared to untreated group (control) was evaluated as anti-hyperuricemic effect.

2.6. Statistical analysis

The data were expressed in terms of mean SEM (Standard error of the mean). Processing data by MS Excel 2016 software. All statistical analyses were conducted using SigmaStat version 3.5 software (USA). One-way analysis of variance (ANOVA) followed by Student-Newman-Keuls or Dunnett's methods were used to examine the significant difference among groups. P values of less than 0.05 were considered statistically significant.

3. RESULT

3.1. The antinociceptive effect of *H. pierreana* extract on acetic acid-induced writhing

Table 1. Number of abdominal writhes of mice counted in a 10 minutes period in the test groups

Group (n = 10)	Doses (mg/kg)	Number of abdominal writhes		
		0 – 10 min	11 – 20 min	21 – 30 min
Control	-	22.90 ± 2.83	25.30 ± 4.75	19.40 ± 4.08
Diclofenac	15	4.80 ± 1.44***	7.70 ± 2.61***	4.60 ± 1.19***
<i>H. pierreana</i> extract	390	9.60 ± 2.66***	12.80 ± 3.15**	8.90 ± 2.69**
	780	8.00 ± 2.57***	10.50 ± 3.14**	5.60 ± 1.60**

** : p < 0.01, *** : p < 0.001 compared to the control group

In the acetic acid-induced writhing test, the number of writhes counted in a 10-minute period and the results have been shown in Table 1. The number of writhes in control group started for 10 minutes and reached a peak in the period of 11 – 20 minutes before slowly dropping for the next 10 minutes. In this test, diclofenac at a dose of 15 mg/kg used as the reference drug showed a significant antinociceptive action ($p < 0.001$) with 74.7% of pain inhibition (Table 2). There was a

significant difference between *H. pierreana* extract at all two doses of 390 and 780 mg/kg with the control group in terms of the number of writhes counted in a 10-minute period (Table 1) and total writhes counted for 30 minutes (Table 2). Compared with the control group, *H. pierreana* extract at all two doses of 390 and 780 mg/kg showed antinociceptive effect (inhibition of 53.70% and 64.34%, respectively) (Table 2) which was similar to diclofenac effect ($p > 0.05$).

Table 2. Number of total writhes of mice counted for 30 minutes of test groups

Group (n=10)	Doses (mg/kg)	Average number of abdominal writhes	Percentage of inhibition (%)
Control	-	67.60 ± 10.66	-
Diclofenac	15	17.10 ± 4.67***	74.70
<i>H. pierreana</i> extract	390	31.30 ± 7.70*	53.70
	780	24.10 ± 7.04*	64.34

*: $p < 0.05$, ***: $p < 0.001$ compared to the control group

3.2. The antinociceptive effect of *H. pierreana* extract on hot plate test

Table 3. The latency of licking/jumping of mice in the test groups

Group (n = 8)	Doses (mg/kg)	Latency of licking/jumping (second)		
		T ₀	T ₃₀	T ₉₀
Control	-	20.75 ± 1.45	14.44 ± 0.88	13.74 ± 0.55
Morphine	10	20.70 ± 1.62	48.05 ± 3.62***	40.63 ± 2.06***
<i>H. pierreana</i> extract	390	20.63 ± 2.63	17.89 ± 0.67*####	15.87 ± 1.49*####
	780	20.53 ± 1.55	17.73 ± 1.03*####	17.58 ± 0.79*####

*: $p < 0.05$, ***: $p < 0.001$ compared to the control group, ####: $p < 0.001$ compared to the reference group (morphine). Baseline latency: T₀, the latency of mice to thermal stimuli after 30 minutes (T₃₀) and 90 minutes (T₉₀) of *H. pierreana* extract or morphine administration.

As shown in Table 3, morphine showed a significant antinociceptive effect at 30 and 90 minutes after injection ($p < 0.001$) and the maximum effect was observed at 30 minutes. *H. pierreana* extract at all two doses of 390 mg/kg and 780 mg/kg showed significant antinociceptive activity when compared with the control group at 30 minutes after administration ($p < 0.05$). *H. pierreana* extract at

the dose of 780 mg/kg provided a better antinociceptive activity than the dose of 390 mg/kg. Mice treated with *H. pierreana* extract at 390 mg/kg had reaction latency times similar to control animals at 90 minutes after administration. However, the antinociceptive effect of *H. pierreana* extract on thermal stimulus-induced pain was weaker than morphine effect ($p < 0.001$).

3.3. The anti-hyperuricemic effect of *H. pierreana* extract on hyperuricemia induced by potassium oxonate

Table 4. Plasma uric acid levels in test groups after 2 hours of potassium oxonate injection

Group (n = 8)	Doses (mg/kg)	Plasma uric acid (mg/dL)	% decrease of uric acid compared to pathological control
Physiological control	-	2.24 ± 0.08	-
Pathological control	-	3.60 ± 0.07***	-

Group (n = 8)	Doses (mg/kg)	Plasma uric acid (mg/dL)	% decrease of uric acid compared to pathological control
Allopurinol	10	2.21 ± 0.10 ^{###}	38.54
<i>H. pierreana</i> extract	390	2.49 ± 0.08 ^{###}	30.90
	780	2.30 ± 0.09 ^{###}	36.11

***: $p < 0.001$ compared to the physiological control group, ^{###}: $p < 0.001$ compared to the pathological control group

Table 4 showed that single injection with potassium oxonate (300 mg/kg) significantly increased plasma levels of uric acid in mice with hyperuricemia (increase of 60.71%) compared with that in normal mice. Allopurinol significantly reduced plasma uric acid levels ($p < 0.001$), accounting for 38.54% decrease as compared to pathological control. Uric acid-lowering effect of *H. pierreana* extract was markedly determined in all two doses of 390 mg/kg and 780 mg/kg (decrease of 30.9% and 36.11%, respectively). Plasma uric acid levels of *H. pierreana* extract-treated group (at dose of 780 mg/kg) as well as allopurinol reached nearly the level of the physiological control.

4. DISCUSSION

Five-day pretreatment with *H. pierreana* extracts at the oral doses of 390 mg/kg and 780 mg/kg showed the antinociceptive effects on acetic acid-induced writhing and thermal stimulus-induced pain. *H. pierreana* extract also reduced plasma uric acid levels of hyperuricemic mice and restored to the baseline levels.

The writhing model represents a chemical nociceptive test based on the induction of peritonitis-like condition in animals by intraperitoneally injecting irritant substances. The acetic acid-induced abdominal writhing test has been used as a screening tool for assessing analgesic or anti-inflammatory agents. When animals are intraperitoneally injected with acetic acid, a painful reaction and acute inflammation emerge in the peritoneal area. Acetic acid triggered the release of arachidonic acid from tissue phospholipids via cyclooxygenase (COX), thereby generating prostaglandins (PG) and instigating a localized inflammatory response [14]. Moreover, the signals transmitted to central nervous system (CNS) in response to pain due to irritation, cause release of mediators such as PG

which contributes to the increased sensitivity to nociceptors [11, 14]. The subsequent activation of nociceptors is sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, a non-selective COX inhibitor. In the study, the antinociceptive effect of *H. pierreana* extract was similar to diclofenac effect. The previous study revealed that *H. pierreana* extract as well as a COX-2 selective inhibitor, celecoxib, showed the anti-inflammatory effect on carrageenan-induced mouse paw edema model [7]. Taking together, further study is necessary to clarify the mechanism of action of *H. pierreana* via COX inhibition.

The hot plate test is a common sensorimotor task that measures thermal nociception in rodent models of CNS disorders. Mice are tested for their baseline latency; then in test conditions, mice are treated with an analgesic agent and evaluated for their sensitivity to pain. The animal will respond to the thermal stimulus by licking or flicking its hind paw or jumping upward (frequently at 55 °C) due to supra-spinal integrated responses. These reflexive behaviors involve both cerebral and spinal mediated circuits [15]. Analgesics can be narcotic or non-narcotic. Narcotic means that the analgesics that act through CNS but do not produce an anti-inflammatory response, such as tramadol and morphine, whereas non-narcotic will act peripherally whilst producing an anti-inflammatory effect such as NSAIDs [16]. In the study, *H. pierreana* extract showed the antinociceptive effects which was clearly weaker than morphine in hot plate test. Also, *H. pierreana* extract exhibited the anti-inflammatory effect in the previous study, suggesting *H. pierreana* extract could be non-narcotic analgesic agent.

Hyperuricemia is associated to gout, an inflammatory arthritis caused by deposition of uric acid in joint. In this study, the hyperuricemia model was induced by using potassium oxonate, which had been considered as the uricase inhibitor, this

model based on inhibiting the process of converting uric acid into allantoin (the end product of purine metabolism in rodents), which is more water-soluble than uric acid, leads to an increase in the plasma uric acid in the mice. The reference group used allopurinol, which is a medication used to treat gout by reducing the production of uric acid in the body. It works by inhibiting the enzyme xanthine oxidase, which is involved in the conversion of hypoxanthine and xanthine to uric acid [17]. However, allopurinol may cause many side effects, such as hepatitis, nephropathy, and allergic reactions. The uric acid levels in the *H. pierreana* extract-treated groups showed a marked reduce when compared to the pathological control group. Chemical investigation of *H. pierreana* rhizome confirmed the existence of flavonoids, tannins, saponins, and sesquiterpenes [4, 7]. These compounds reportedly possess the ability to inhibit xanthine oxidase [17]. Therefore, it is necessary to clarify whether *H. pierreana* and its chemical components could inhibit xanthine

oxidase.

Taking together, *H. pierreana* exhibited the anti-inflammatory [7], antinociceptive and uric acid-lowering effects, suggesting this plant may be a potential agent for management of gout.

5. CONCLUSION

In conclusion, the 45% ethanolic extract from *Homalomena pierreana* rhizome at both doses 390 mg/kg and 780 mg/kg (equivalent to 1.25 and 2.5 g raw materials) showed antinociceptive effects on chemical-induced "pain-like" behavior (writhing test) and thermal stimulus-induced pain (hot plate test). The extract also reduced hyperuricemia induced by potassium oxonate in mice.

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Tác dụng giảm đau và hạ acid uric máu của loài thiên niên kiện *Homalomena pierreana* Engl., họ ráy (Araceae)

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ABSTRACT

Đặt vấn đề: Bệnh gút là một trong các bệnh lý xương khớp liên quan đến tình trạng tăng nồng độ acid uric trong máu. Việc tìm kiếm các dược liệu có tác dụng giảm đau và làm hạ acid uric máu có ý nghĩa trong dự phòng và hỗ trợ điều trị bệnh lý này. Mục tiêu nghiên cứu: Chi Thiên niên kiện (*Homalomena*) đã được sử dụng phổ biến trong y học cổ truyền để điều trị bệnh xương khớp. Loài *Homalomena pierreana* mới được phát hiện tại Việt Nam. Nghiên cứu này nhằm đánh giá tác dụng giảm đau và làm hạ acid uric máu của cao chiết cồn 45% từ thân rễ *H. pierreana* (cao *H. pierreana*) trên chuột nhắt trắng đực. Phương pháp: Đánh giá tác dụng giảm đau của cao *H. pierreana* trên các thực nghiệm gây đau xoắn bụng bằng acid acetic và gây đau do kích thích nhiệt (tắm nóng). Mô hình gây tăng acid uric máu cấp bằng kali oxonat được sử dụng để đánh giá tác dụng hạ acid uric máu của cao *H. pierreana*. Kết quả: Sử dụng cao *H. pierreana* trong 5 ngày ở các liều uống 390 mg/kg và 780 mg/kg làm giảm số lần xoắn bụng của chuột nhắt trắng, tương tự như tác dụng của diclofenac. Cao *H. pierreana* ở 2 liều thử cũng làm kéo dài thời gian phản ứng với kích thích đau do nhiệt (hay làm tăng thời gian của phản ứng liếm chân/nhảy) của chuột, tuy nhiên tác dụng yếu hơn so với morphin (10 mg/kg, i.p.). Cao *H. pierreana* ở 2 liều thử, tương tự như allopurinol, làm hạ acid uric máu ở chuột bị tăng acid uric máu và phục hồi về giá trị bình thường. Kết

luận: Cao H. pierreana thể hiện tác dụng giảm đau và làm hạ acid uric máu, cho thấy tiềm năng trong hỗ trợ điều trị bệnh gút.

Từ khóa: *Thân rễ loài Thiên niên kiện Homalomena pierreana, tác dụng giảm đau, tác dụng hạ acid uric máu*

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