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Study of Anti-hyperuricemia, Anti-inflammatory, and Anti-nociceptive Effects of *Hylocereus undatus* stem bark Extract in Animal Models

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ABSTRACT

Dragon fruit, known as *Hylocereus undantus* (HU), has traditionally been utilized in Banyuwangi, East Java, Indonesia, for addressing gout and rheumatism. Despite its empirical use, there is a lack of reported pharmacological research on this plant. This study aimed to explore the properties of HU extract in treating hyperuricemia, inflammation, and pain. Adenine-potassium oxonate-induced mice were employed to evaluate anti-hyperuricemia activity, measuring uric acid, blood urea nitrogen (BUN), and creatinine serum levels. Carrageenan-induced paw edema in rats was used to assess anti-inflammatory activity, while acetic acid-induced abdominal writhing and hot plate tests were conducted to investigate anti-nociceptive effects. The results revealed that administering HU extracts at 400 mg/kg notably reduced uric acid levels ($p<0.01$). Moreover, doses of 100, 200, and 400 mg/kg showed significant reductions in BUN and creatinine serum levels ($p<0.0001$). At a dose of 400 mg/kg, HU extract exhibited a significant anti-inflammatory effect two hours post-administration, manifesting a 15% and 26% reduction in paw edema for male and female mice, respectively ($p<0.005$, $p<0.001$). Additionally, doses of 200 and 400 mg/kg demonstrated anti-nociceptive effects in acetic acid-induced abdominal constriction. Furthermore, at 400 mg/kg, the extract exhibited anti-nociceptive activity ($p<0.001$) three hours post-administration using the hot plate method. This study underscores the potent anti-hyperuricemia, anti-inflammatory, and anti-nociceptive properties of HU. The implications of these findings contribute significantly to comprehending the therapeutic potential of HU.

Keywords: *Hylocereus undatus; dragon fruit; anti-hyperuricemic activity; anti-inflammatory activity; anti-nociceptive activity*

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INTRODUCTION

Gout, an inflammatory arthritis condition linked to hyperuricemia, is characterized by joint swelling and pain, significantly impacting individuals' quality of life. As per the Global Burden of Disease (GBD) report in 2021, gout affected approximately 41 million individuals worldwide (Danve et al., 2021; Yin et al., 2020). Hyperuricemia, indicated by elevated uric acid levels surpassing the normal concentrations (6.0 mg/dL in women and 7.4 mg/dL in men), stands as a metabolic disorder. Research has correlated excessive uric acid levels with various conditions, including gout, cardiovascular diseases, kidney diseases, metabolic syndrome, and rheumatoid arthritis (Chang et al., 2021; Ragab et al., 2017; Riaz et al., 2022). Moreover, hyperuricemia triggers the crystallization, aggregation, and deposition of monosodium urate crystals within joints or soft tissues, inciting an intense inflammatory response involving leucocyte recruitment, local cytokine release, reactive oxygen species, and proteolytic enzymes (Chang et al., 2021; Pascual et al., 2015).

Currently, the medications for gout or inflammatory arthritis involve anti-gout, non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, and corticosteroids, markedly enhancing therapy outcomes. However, prolonged use of these drugs might lead to undesirable side effects and incur a relatively high treatment cost (Lin et al., 2021). Hence, alternative therapies for gout or inflammatory arthritis are still required to be explored.

Hylocereus undatus (HU), commonly known as dragon fruit, thrives in Southeast Asian countries, including Indonesia. Its stem bark has been traditionally used by the local communities in Banyuwangi, East Java, Indonesia, for treating rheumatism and gout by drying and grinding it into tea. Phytochemical investigation of *Hylocereus undatus* revealed the presence of bioactive compounds, including phenolics, flavonoids, and vitamins. Several identified flavonoids in the glycosidic and free aglycone forms, including epicatechin, quercetin, and rutin (Pang et al., 2021; Salam et al., 2022; Wu et al., 2011) exhibited potential antioxidative, anticancer, and anti-diabetic properties (Tang et al., 2021).

Studies by Lioa also indicated that HU's extract from its flower diminished the key factors contributing to allergic asthma, including inflammatory cell accumulation, pro-inflammatory molecules, airway inflammation, etc. This extract also acts as an anti-inflammatory that significantly decreases the expression of p-38 mitogen-activated protein kinases (MAPKs)- and NF-κB (Liao et al., 2022). This suggests the potential dual impact of HU extract in treating hyperuricemia disorders linked with inflammatory activity. Given HU's historical success in gout and rheumatism treatment, this study aims to assess its anti-hyperuricemia, anti-inflammatory, and anti-nociceptive activity in animal models.

MATERIALS AND METHODS

Animal Study

The animal procedures strictly adhered to the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" as published by the National Institutes of Health. Ethical considerations for the care of laboratory animals were meticulously followed, and all experimental protocols received approval from the Scientific and Ethical Committee of the Faculty of Medicine, Universitas Indonesia, Indonesia, with the ethical clearance number KET-1208/UN2.F1/ETIK/PPM.00.02/2022.

For the experiments assessing the anti-hyperuricemia effect and anti-nociceptive activity, mice were used, while rats were chosen for the anti-inflammatory study to ensure precise measurement of paw edema volume using a plethysmometer. Adult male and female C57BL/6J mice (weighing 20-25 g) were procured from Biofarma, Bandung, West Java. Additionally, adult male and female Sprague Dawley rats (weighing 180-200 g) were acquired from Animal Facilities, Faculty of Veterinary Medicine, IPB University, Indonesia.

All animals were acclimatized for at least seven days to adapt to their new environment before any experimental interventions. They were housed in an animal room at the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Indonesia, under controlled conditions (temperature: 24-28°C, 12/12-hour light/dark cycle) and had ad libitum access to standard laboratory feed throughout the experiments. The animals' body weight was assessed at the commencement of each experiment. The mice used for anti-hyperuricemia and anti-nociceptive activity ranged from 20 to 25 g, while the rats used for anti-inflammatory activity ranged between 200-250 g.

Statistical analysis determined a minimum requirement of four animals for each study. However, to account for any unforeseen casualties during the study, additional animals were included. Specifically, two extra mice were employed in the anti-hyperuricemia study, and one additional mouse was utilized in both the anti-inflammatory and anti-nociceptive studies.

Collection of Plant Material

The whole *Hylocereus undatus* plant was collected from Banyuwangi, East Java, Indonesia. The plant was taxonomically identified by the Centre for Plant Conservation Botanic Garden, Indonesia Institute of Science, Indonesia, with the number 4176/IPH.3. /KS/VII/2015.

Preparation of Extracts

Upon collection, fresh *Hylocereus undatus* plants underwent thorough washing with distilled water. The stem bark, comprising 50 kg of plant material, was separated, chopped into small pieces, and air-dried at room temperature for 14 days. Subsequently, the dried bark underwent grinding to obtain a powdered form, which was then carefully stored in containers.

The aqueous extract was prepared by decocting 100 mg/200 mg/400 mg of the dried stem bark of HU with boiling water 5 ml for 1 hour and then stored at 4°C before administration. This aqueous extract was used in the anti-hyperuricemia study.

The ethanolic extract was derived by macerating 3,000 g of the dried stem bark powder seven times in 1 L of 70% ethanol at room temperature for eight days. The resulting solution was filtered, collected, and evaporated using a rotary evaporator. This meticulous process yielded approximately 102 g of dried ethanolic extract, which was stored at 4°C until further use. This ethanolic extract was utilized in both the anti-inflammatory and anti-nociceptive studies.

Chemical and Reagent

Biological-grade chemicals and reagents, specifically adenine and potassium oxonate (Carbosynth), and carrageenan (Sigma Aldrich), were exclusively utilized in the *in vivo* tests. Adenine and oxonate were precisely suspended in distilled water, achieving a concentration of 80 g/L. The solvents employed for extraction were industrial grade, purchased from a reputable licensed chemical company in Jakarta, Indonesia, and utilized without additional purification processes.

Evaluation of Anti-Hyperuricemia Effect

After a week-long acclimation to a new environment, 36 male mice were divided into six groups, each consisting of six mice: control, hyperuricemia mice model (ade+oxo group), hyperuricemia+allopurinol (allopurinol group) 100 mg/kg, and three HU dosage groups – 100 mg/kg, 200 mg/kg, and 400 mg/kg. Hyperuricemia in mice

was induced by orally administering a combination of adenine (75 mg/kg) and potassium oxonate (150 mg/kg) daily for three weeks, following the combined method outlined by Chen et al. (2019), Guo et al. (2015), and Guan et al. (2020) with minor modifications.

Mice in the control group received oral administration of 0.5% CMC-Na without hyperuricemia induction. From the 14th to the 21st day, allopurinol at 100 mg/kg or HU aqueous extract was orally administered daily, diluted in 0.5% CMC Na. Blood samples were collected from the tail on the 14th and 21st days to measure uric acid levels, while serum obtained by clotting blood at room temperature for approximately 1 hour and subsequent centrifugation at 5,000 rpm for 5 minutes was used for blood urea nitrogen (BUN) and creatinine serum measurement.

The uric acid level was determined using the Nesco commercial kit (Multicheck, Taiwan), following the manufacturer's instructions. Additionally, the levels of BUN and creatinine in the serum were assessed using the Creatinin FS and Urea FS reagent kits (Diasys, Germany), respectively.

Evaluation of Anti-Inflammatory Activity

The anti-inflammatory activity of HU was assessed using the carrageenan-induced paw edema model in rat hind paws, following the methodologies described by Motevalian et al. (2017), Naz et al. (2022), and Nguyen et al. (2017) with little modifications. Paw measurements were conducted using a plethysmometer (Model 7140, Ugo Basile). Subsequently, 0.1 mL of 1% carrageenan (w/v) was subcutaneously injected into the subplantar region of the right hind paw to induce inflammation.

Rats, randomly divided into five groups (n=5 for each), received different treatments: 0.5% CMC Na diluted in distilled water (control group), the standard anti-inflammatory drug, diclofenac sodium, at 10 mg/kg (diclofenac group), and the HU extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg orally administered 1h before the subplantar injection in each group. The volume of the right hind paw was measured at 1st, 2nd, 3rd, and 4th hours following subplantar carrageenan injection.

Evaluation of Anti-Nociceptive Activity

The anti-nociceptive study aimed to assess the analgesic effects of the HU extract, exploring both peripheral and central analgesic activities. Peripheral analgesic activity was evaluated using the acetic acid-induced writhing response, with acetylsalicylic acid serving as the standard drug for comparison. Additionally, the central analgesic activity of HU was examined through the hot plate test, employing thermal stimuli, and tramadol was utilized as the standard drug for comparative analysis.

Acetic Acid-Induced Abdominal Writhing Test (Chemical Stimuli)

Male and female mice were randomly divided into five groups (n=5 for each). The control group (Group one) received 0.5% CMC Na diluted in distilled water. Group two received the acetylsalicylic acid at 100 mg/kg as the standard drug, while the remaining three groups were administered different doses of the HU extract–100 mg/kg, 200 mg/kg, and 400 mg/kg, orally administered one hour before the injection of acetic acid.

Following the acetic acid injection, the number of abdominal constrictions that happened in each group was observed over a 30-minute period and compared to the control group. Subsequently, the percentage inhibition of writhing was calculated to assess the efficacy of the treatments.

Hot Plate Test (Thermal Stimuli)

The nociceptive response to thermal stimuli was evaluated using a hot-plate test, with the temperature maintained at 51± 1°C, and the reaction time (in seconds) for licking of hind paw or jumping was recorded. The withdrawal latencies of the hind paw were calculated.

For the experiment, mice were randomly divided into five groups (n=5 for each). The control group (Group one) received 0.5% CMC Na diluted in distilled water. Group two was administered with the standard drug drug tramadol at 100 mg/kg, while the remaining three groups received different doses of the HU extract–100 mg/kg, 200 mg/kg, and 400 mg/kg, through an oral delivery. The analgesic effect was assessed by observing the decrease in the change (Δ) in latency, measured in second, at interval of 30, 60, 120 and 360 minutes after the administration of the vehicle, standard drug, and extract treatments.

Statistical Analysis

All data are presented as the mean ±SD, with 'n' representing the number of animals (mice or rats) in each group. Statistical analyses were performed by GraphPad Prism 10 for Mac. A student's t-test was employed to determine significant differences between the two groups. Multiple comparisons were performed using one-way ANOVA, followed by either Tukey–Kramer or Dunnett test. The Tukey-Kramer was utilized to compare each mean with every other means, while the Dunnet was employed to compare each mean with the control mean. Statistical significance was denotated as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

RESULTS AND DISCUSSION

The utilization of traditional medicine for treating various ailments based on empirical history is gaining widespread attention. *Hylocereus undatus* (HU) has

Table 1. Effect of *Hylocereus undatus* extract on carrageenan-induced paw edema in rats

Male rats					
Group	Paw edema volume (mL) at interval study (h)				
	0	1	2	3	4
Control	0.876 ± 0.052	1.256 ± 0.069	1.421 ± 0.095	1.472 ± 0.064	1.411 ± 0.112
Diclofenac	0.833 ± 0.064	0.872 ± 0.054****	1.022 ± 0.112****	1.063 ± 0.155****	0.941±0.047****
HU 100	0.854 ± 0.052	1.135 ± 0.093	1.361 ± 0.051	1.543 ± 0.061	1.408 ± 0.134
HU 200	0.867 ± 0.039	1.178 ± 0.058	1.406 ± 0.015	1.535 ± 0.054	1.388 ±0.126
HU 400	0.889 ± 0.056	1.085 ± 0.096	1.205 ± 0.022*	1.224 ± 0.076**	1.182 ± 0.089**
Female rats					
Group	Paw edema volume (mL) at interval study (h)				
	0	1	2	3	4
Control	1.005 ± 0.079	1.436 ± 0.103	1.670 ± 0.111	1.576 ± 0.192	1.452 ± 0.127
Diclofenac	1.110 ± 0.154	1.157 ± 0.051	1.100 ± 0.130****	1.155 ± 0.120**	1.093 ± 0.094*
HU 100	0.988 ± 0.139	1.259 ± 0.051	1.4331± 0.128	1.583 ± 0.145	1.481 ± 0.211
HU 200	1.037 ± 0.143	1.174 ± 0.152	1.643 ± 0.264	1.448 ± 0.073	1.323 ± 0.117
HU 400	1.068 ± 0.171	1.194 ± 10.112	1.23 ± 0.199***	1.318 ± 0.137	1.224 ± 0.118

n=5. Values are displayed as mean ± SEM. The values of *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ were considered statistically significant compared to the control.

long been employed as traditional herbal medicine to address gout and rheumatism in Banyuwangi, East Java, Indonesia. Gout, a prevalent form of inflammatory arthritis, stems from a metabolic disorder known as hyperuricemia, characterized by elevated serum uric acid levels. Excess uric acid contributes to monosodium urate deposition in joints and soft tissues, resulting in severe pain, joint swelling, and limited mobility, thereby significantly diminishing patients' quality of life (Ben et al.., 2018; Danve et al., 2021). In the present study, the anti-hyperuricemia, anti-inflammatory, and anti-nociceptive activities of HU extract were assessed using adenine-potassium oxonate, carrageenan, and chemical-thermal as induction methods, respectively.

Firstly, the anti-hyperuricemia effect of HU's aqueous extract was examined in a hyperuricemia mice model induced by the combination of adenine and potassium oxonate (ade+oxo). Potassium oxonate, a recognized selective inhibitor of uricase, is known to elevate uric acid concentration (Benn et al., 2018; Yanai et al., 2021). Hyperuricemia triggers an inflammatory response by stimulating pro-inflammatory mediators and generating oxygen-free radicals, potentially leading to tissue damage and increased strain on the kidneys, which can be evaluated by measuring blood urea nitrogen and serum creatinine levels. The combination of ade+oxo significantly increased not only uric acid (UA) levels but also blood urea nitrogen and serum creatinine levels, indicating the successful establishment of the hyperuricemia mice model.

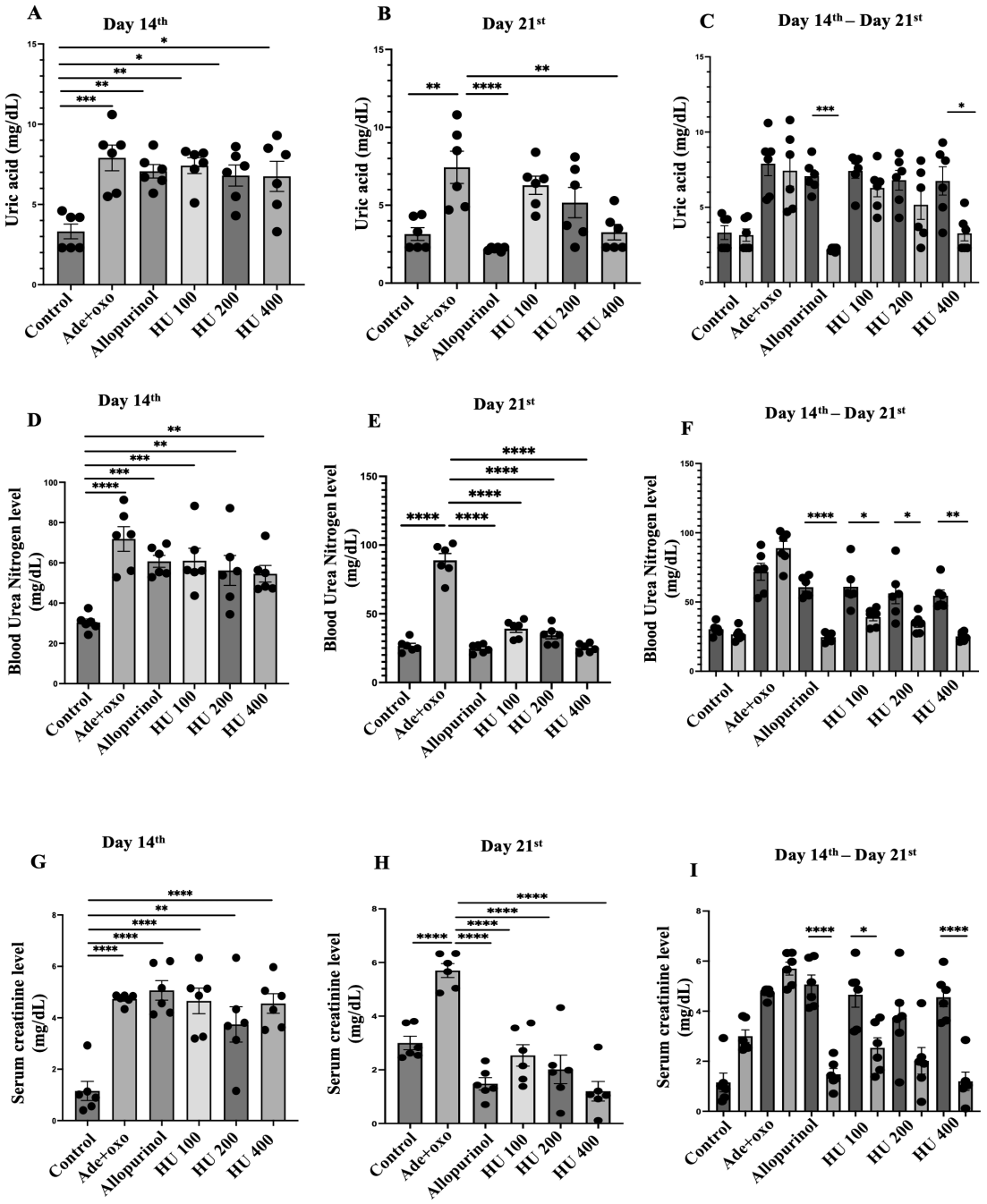

Figure 1. Anti-hyperuricemic effect of *Hylocereus undatus* on uric acid level, blood urea nitrogen (BUN) level, and serum creatinine level in hyperuricemia mice

Mice were administered the combination of adenin and potassium oxonate (ade+oxo) for 21 days, except the control group. Allopurinol or HU aqueous extract was administered orally once a day for seven consecutive days (day 14th - day 21st). Control groups were orally administered with 0.5% CMC Na. Fig A-C Uric acid level in hyperuricemia mice. A. Uric acid level in hyperuricemic mice on day 14th. B. Uric acid level in hyperuricemic mice on day 21st. C. Uric acid level in hyperuricemia mice in each group between day 14th and day 21st. (n=6). D-F Blood urea nitrogen level in hyperuricemia mice. D. Blood Urea Nitrogen (BUN) level in hyperuricemia mice on day 14th. E. Blood Urea Nitrogen (BUN) level in hyperuricemia mice on day 21st. F. Blood Urea Nitrogen (BUN) level in hyperuricemia mice in each group between day 14th and day 21st. (n=6). G-I Serum creatinine level in hyperuricemia mice. G. Serum creatinine level in hyperuricemia mice on day 14th. H. Serum creatinine level in hyperuricemia mice on day 21st. I. Serum creatinine level in hyperuricemia mice in each group between day 14th and day 21st. (n=6). Values are displayed as mean ± SEM. The values of *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 were considered statistically significant.

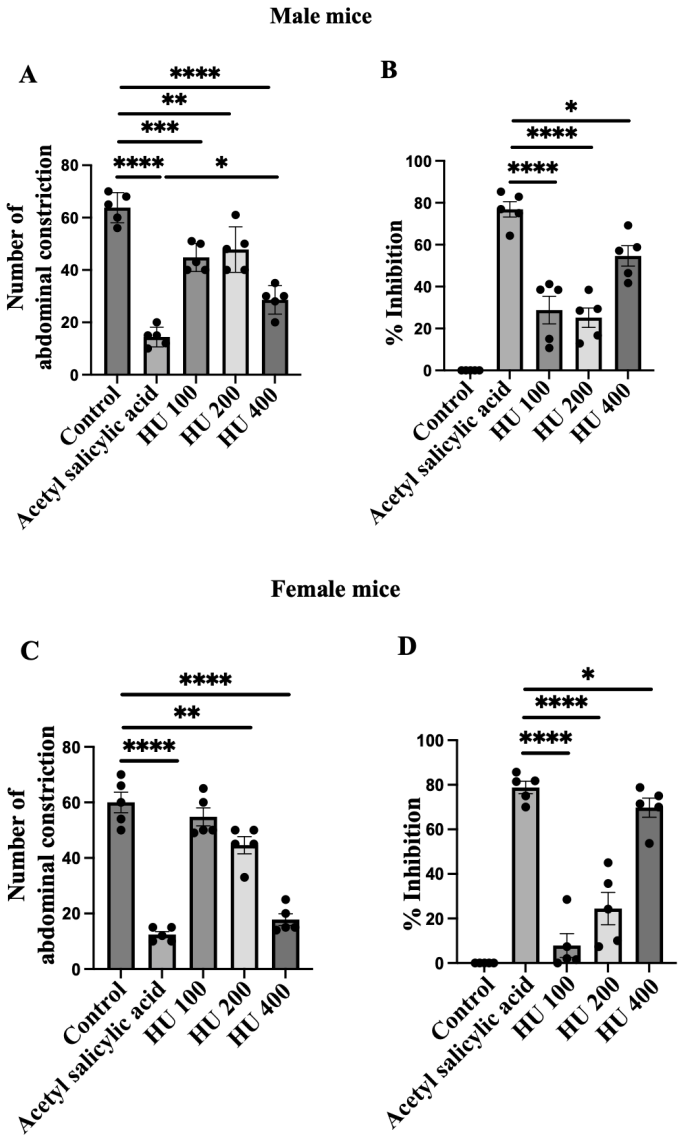

Figure 2. Antinociceptive profile of the *Hylocereus undatus* extract on mechanical stimuli

Mice were divided into five groups (n=5) in the writhing test: control, acetylsalicylic acid, HU 100, HU 200, and HU 400, which was administered for 1 h before injection of acetic acid. The response was calculated 30 min post acetic acid injection and compared with the control group. A. The number of abdominal constrictions produced in each group for 30 min post acetic acid injection in male mice. B. The percentage of inhibition of abdominal constriction in male mice compared to control. C. The number of abdominal constrictions produced in each group for 30 min post acetic acid injection in female mice. D. The percentage of inhibition of abdominal constriction in male mice compared to control. n=5, each male or female. Values are displayed as mean ± SEM. The values of *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$ were considered statistically significant.

The oral administration of HU at doses of 100, 200, and 400 mg/kg was conducted for seven days on adenine and potassium oxonate-induced hyperuricemia mice. Uric acid levels, blood urea nitrogen, and serum creatinine levels were measured using the phosphotungstic acid method. As depicted in Figure 1, the induction groups (ade+oxo, allopurinol, HU 100, HU 200, and HU 400) which were induced orally with ade+oxo for 14 days, exhibited a significant increase in uric acid levels, BUN level, and serum creatinine value (3.316 mg/dL; 30.359 mg/dL; 1.152 mg/dL), compared to the control or non-induced group (Figure 1. A, D, G).

Conversely, administering allopurinol or the HU extract for seven days led to a reduction in uric acid, BUN, and serum creatinine levels compared to the ade+oxo group.

Notably, those parameters in the allopurinol group were significantly lower than those in the HU groups receiving 100/200/400 mg/kg of HU extract (Figure 1. B, E, H). Additionally, comparisons between the 14th and 21st-day results indicated that allopurinol significantly reduced the uric acid levels compared to the HU extract. Intriguingly, no significant difference was observed in BUN and serum creatinine levels between the allopurinol and the HU 400 mg group. The treatment with HU extract at doses of 100, 200, and 400 mg/kg showed a gradual decrease in uric acid, BUN, and serum creatinine levels from day 14 to day 21, suggesting the potential of HU in alleviating orally induced ade+oxo-induced hyperuricemia (Figure 1. C, F, I).

Allopurinol, a xanthine oxidase inhibitor widely used in clinical settings for hyperuricemia treatment (Benn et al., 2018; Yanai et al., 2021), showcased its efficacy in our study. Intriguingly, HU extract doses of 400 mg, 200 mg, and 100 mg exhibited a gradual reduction in uric acid, BUN, and serum creatinine levels in hyperuricemia mice.

HU has been reported to contain phenolics and flavonoids, predominantly chlorogenic and caffeic acids (Jimenez-Garcia et al., 2022; Joshi & Prabhakar, 2020; Salam et al., 2022). One of the major functions of flavonoids in plants is to exhibit antioxidant properties by scavenging free radicals. These compounds inhibit xanthine oxidase (XO) activity, enhance oxidative stress factors like SOD and GSH-Px, and reduce ROS levels in mice with hyperuricemia (Jimenez gracia et al., 2022; Joshi & Prabhakar, 2020; Salam et al., 2022). SOD, an efficient antioxidant enzyme, rapidly converts O_2 and $\bullet O_2^-$ to H_2O_2, which is subsequently converted to H_2O by GSH-Px catalysis within cells. XO, crucial in converting xanthine and hypoxanthine to UA, is responsible for ROS generation (Li et al., 2019). Excessive ROS is produced alongside UA generation. Thus, the suppression of UA in hyperuricemia mice by the HU extract is attributed to the antioxidant compounds present in the extract.

The anti-inflammatory effects of HU extracts were assessed using the carrageenan-induced paw edema model in rats, widely employed in testing non-steroidal anti-inflammatory drugs (NSAIDs). Prostaglandins play a pivotal role in homeostasis and pathogenic mechanisms, particularly in the inflammatory response, where they are synthesized from arachidonate by cyclooxygenase (COX) isoenzymes. Inflammatory responses manifest as heat (calor), pain (dolor), redness (rubor), and swelling (tumor). As presented in Table 1, intraplantar injection of carrageenan (1% w/v) notably increased paw volume in male and female rats within the control groups, reaching peak effects after 3 hours in males and 2 hours in females. Carrageenan induces paw edema in rats by promoting the accumulation of plasma proteins and exudate fluid, leading to neutrophil accumulation at the inflammation site.

Diclofenac sodium, serving as the positive control, notably reduced the maximum paw edema volume in male rats compared to the control (1.063±0.155 mL vs 1.472±0.064 mL), and in female rats compared to the control (1.100±0.130 mL vs 1.670±0.111 mL). Subsequently, the HU extract at a dose of 400 mg/kg exhibited significant anti-inflammatory potential by reducing paw volume in male and female rats at the 2-hour interval during the study (1.205±0.022 and 1.23±0.199, respectively).

Oral administration of HU extracts notably reduced carrageenan-induced paw edema, demonstrating a significant inhibitory effect comparable to diclofenac sodium. Studies have reported the presence of phenolics and flavonoids in HU (Liao et al., 2022), and various compounds found, such as rutin, quercetin, luteolin, hesperidin, and bioflavonoids, exhibit substantial anti-inflammatory activities. These flavonoids operate through molecular mechanisms that involve inhibiting pro-inflammatory enzymes like cyclooxygenase-2 and lipoxygenase and reducing NO production (Bakshi et al., 2022; Joshi & Prabhakar, 2020; Liao et al., 2022; Serafini et al., 2010; Yahfoufi et al., 2018).

To assess the anti-nociceptive effects of the HU extract, an ethanolic extract derived from the stem bark was employed to evaluate its inhibitory effects. Specifically, the anti-nociceptive effects were investigated using the acetic acid-induced writhing test as a chemical stimulus and the hot plate test as a thermal stimulus. The acetic acid-induced writhing test is commonly employed to assess analgesics for peripheral pain. Writhing induced by acetic acid results from the release of free arachidonic acid from tissue phospholipids through cyclooxygenase and subsequent prostaglandin production, associated with inflammatory responses (Uddin et al., 2014). Acetic acid induces an increase in peritoneal fluid levels of prostaglandins, contributing to peritoneal receptor activation and inflammatory pain by enhancing capillary permeability. Furthermore, acetic acid indirectly stimulates the release of endogenous mediators that activate nociceptive neurons (Choi, 2007).

Figure 2 illustrates that the control group induced by acetic acid, receiving no treatment, exhibited the highest abdominal constrictions in both male and female mice. Notably, HU doses (100, 200, 400 mg/kg) displayed significant anti-nociceptive effects against the acetic acid-induced writhing response in male mice compared to the control group ($p<0.001$, $p<0.01$, $p<0.0001$, respectively).

Table 2. Effect of *Hylocereus undatus* extract on pain induced by thermal stimuli through hot plate method

Male mice					
Group	Latency time for licking the hind paw or jumping (sec) at interval study (h)				
	0	0.5	1	2	3
Control	8.6 ± 0.547	8.4 ± 0.547	8.8 ± 0.447	8.6 ± 0.894	9 ± 0.707
Tramadol	8.4 ± 0.547	10.0 ± 0.707	13.8 ± 0.836****	16.8 ± 2.387****	17.2 ± 2.167****
HU 100	8.2 ± 0.447	8.4 ± 0.547	8.6 ± 0.547	8.6 ± 0.547	10.0 ± 1.732
HU 200	8.2 ±0.836	8.8 ± 0.447	9.4 ± 0.547	9.1 ± 1.0	11.6 ± 2.073*
HU 400	9.0 ± 0.707	8.6 ± 0.547	10.0 ± 0.707	11.6 ± 0.547**	13.4 ± 1.140****
Female mice					
Group	Latency time for licking the hind paw or jumping (sec) at interval study (h)				
	0	0.5	1	2	3
Control	5.8 ± 0.447	6.2 ± 0.447	6.6 ± 0.547	7.2 ± 0.836	7.2 ± 0.836
Tramadol	5.4 ± 0.894	7.6 ± 0.547	10.2 ± 1.095****	17.8 ± 3.03****	15.2 ± 0.836****
HU 100	5.4 ± 0.894	5.8 ± 0.836	6.6 ± 0.547	8.4 ± 0.547	9.2 ± 1.303
HU 200	5.6 ± 0.894	5.8 ± 0.836	8.0 ± 0.707	9.4 ± 0.547	12.2 ± 0.836 ****
HU 400	5.2 ± 0.836	6.8 ± 0.447	8.4 ± 0.547	10.8 ± 1.303****	13.2 ± 1.303 ****

n=5. Values are displayed as mean ± SEM. The values of *p < 0.05, **p < 0.01, ***p < 0.00,1, and ****p < 0.0001 were considered statistically significant compared to the control

Acetylsalicylic acid, employed as a standard drug, demonstrated the most potent anti-nociceptive effect (Figure.2.A). It exhibited the highest inhibition of abdominal constriction (76.87±7.33%), followed by HU at doses 400, 100, and 200 mg/kg (54.658±9.719%, 28.784±13.111%, and 25.193±9.275%, respectively) in male mice (Figure 2. B). In female mice, HU at doses of 200 and 400 mg/kg demonstrated significant anti-nociceptive effects compared to the control group ($p<0.01$, $p<0.0001$, respectively) (Figure 2.C). Acetylsalicylic acid, used as the standard drug, exhibited the best inhibitory effect on abdominal constriction (78.802±5.585%), followed by HU extract with doses of 400, 200, and 100 mg/kg (69.784±8.597%, 24.472±14.48%, and 7.898±10.635%, respectively) (Figure 2.D). The HU extract displayed inhibition of abdominal constriction induced by acetic acid, possibly attributed to its rich content of polyphenols, particularly flavonoids (Liao et al., 2022), which are found abundantly in the human diet and are known for their potent anti-inflammatory activity.

The hot plate test, a specific central anti-nociceptive evaluation method, was employed to assess the activity of the HU extract. This method is commonly utilized for demonstrating that opioid compounds exert their analgesic effects through supra-spinal and spinal receptors. Different types of opioid receptors–μ (for morphine), κ (for ketocyclazocine), and δ (first identified in the mouse vas deferens)–play roles in these effects. The μ receptor, primarily responsible for opioid-mediated anti-nociception and tolerance, is thought to be involved. In this test, under constant temperature, behaviors like paw licking and jumping were observed (Orlandi et al., 2011; Nemirovsky et al., 2011).

Table 2 displays that the HU extract notably increased the latency time at a dose of 400 mg/kg after a 2-hour interval study in male mice ($p<0.01$). Similarly, in female mice, the HU extract at doses of 400 mg/kg significantly increased the response latency time at 2 hours compared to the control group ($p<0.0001$). Notably, the administration of the vehicle in the control group did not induce an anti-nociceptive effect. Additionally, the reference drug, tramadol (100 mg/kg), exhibited significant anti-nociceptive activity by markedly prolonging latency time at the 1-hour interval study ($p<0.0001$). The HU extract is expected to contain several groups of flavonoids subclasses, such as flavanones, flavones, flavonols, and flavanols (Serafini et al., 2010), which exhibit anti-nociceptive properties mediated by opioid receptors through several hydroxylated derivatives of flavones (Higgs et al., 2013). This study revealed that the HU extract showed significant anti-nociceptive activity compared to the control group. These findings suggest that the anti-nociceptive effects of the HU extract might be associated, at least partially, with the involvement of the μ-opioid system.

LIMITATION

This study predominantly employed pharmacological approaches to investigate the impact of HU extract in hyperuricemia, inflammatory, and nociceptive animal models. Future investigations should focus on assessing the composition of secondary metabolites present in *Hylocereus undatus* stem bark. Specifically, these studies would aid in determining the relevance of our in vivo findings and understanding the role of specialized secondary metabolites.

CONCLUSION

This study revealed promising outcomes from the aqueous extract derived from the stem bark of *Hylocereus undatus*. This extract notably reduced uric acid, blood urea nitrogen, and serum creatinine levels in the hyperuricemia mice model by substantial margins (40%, 40%, and 60%, respectively). Additionally, the ethanolic extract exhibited significant reductions in paw volume edema induced by carrageenan in a rat-based inflammatory model. Furthermore, our findings indicated an anti-nociceptive effect in mice when exposed to both chemical and thermal stimuli.

The observed potential effects of the HU extract in improving gout pathology and reducing inflammatory pain showcase its potential for further exploration as a viable strategy in gout treatment. These results suggest a promising direction for the development of therapeutic interventions targeting gout and inflammatory conditions, utilizing *Hylocereus undatus* extract.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest in this study.

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