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Total alkaloids and anti-inflammatory activity of *Glaucium grandiflorum* wildly grown in Syria: A study on formalin induced paw edema in rats

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ABSTRACT: *Glaucium grandiflorum,* a perennial herb belonging to the Papaveraceae family, exhibits significant biological activities. This study aims to quantitatively determine the alkaloid content in various plant parts and evaluate the anti-inflammatory activity of its hydroethanolic extract from aerial parts during the flowering stage in rats.The total alkaloid content was determined through alkaloid precipitation at pH=10 using concentrated ammonium hydroxide, followed by weighing. The anti-inflammatory activity was assessed by injecting 0.1 mL of 2.5% formalin into the right hind paw to induce acute inflammation. Quantitative analysis revealed the presence of alkaloids in all tested plant parts. The highest alkaloid content was found in fruits (5.07%), followed by aerial parts in the fruiting stage (3.21%) and aerial parts in the flowering stage (1.41%), with the lowest content in the roots (0.66%). The extract exhibited significant inhibition of paw edema ($P < 0.05$). Both oral and intraperitoneal administration of 200 and 400 mg/kg of the extract reduced edema formation, indicating that *G. grandiflorum* possesses anti-inflammatory activity. The maximum anti-inflammatory activity was observed at a dose of 400 mg/kg (95.42%), surpassing indomethacin at 10 mg/kg (89.77%) on oral administration. Our study suggests that the hydroethanolic extract of flowering aerial parts is likely safe in rats up to a dose of 2000 mg/kg and possesses anti-inflammatory activity when administered orally or intraperitoneally. In conclusion, the hydroethanolic extract of *G. grandiflorum* may be a valuable resource for managing disorders associated with inflammation as an anti-inflammatory agent. This activity is likely attributed to the presence of alkaloids, phenols, flavonoids, and saponins.

KEYWORDS: *Glaucium grandiflorum;* Papaveraceae; total alkaloids; anti-inflammatory activity; formalin; paw edma.

1. INTRODUCTION

Inflammation represents the immune system's response to chemical or physical stimuli, pathogens, or irritants [1]. This response manifests through histological changes such as pain, redness, swelling, elevated temperature, and, in some cases, a loss of organ function [2]. Inflammatory responses are commonly triggered in various diseases, including cancer, cardiovascular disease, rheumatoid arthritis, inflammatory bowel disease, certain chronic respiratory disorders (such as asthma), and central nervous system disorders like Alzheimer's and Parkinson's disease [3]. This process involves increased blood flow, vasodilation, the migration of inflammatory cells, including macrophages, edema formation due to fluid accumulation, tissue damage [4], and the release of inflammatory mediators like prostaglandins, histamine, leukotrienes, prostacyclin, lymphokines, interleukins (IL), and tumor necrosis factor-alpha (TNF-α) [5]. Non-steroidal anti-inflammatory drugs (NSAIDs) constitute a cornerstone in the treatment of inflammation [6]. NSAIDs work by inhibiting the activity of the cyclooxygenase enzyme, thereby reducing prostaglandin production [7]. While these drugs are readily available and effective for managing acute and chronic inflammation [6], they often come with serious side effects, such as gastric ulcers, hypertension, renal failure, and bleeding, especially with prolonged or high-dose usage [8]. Consequently, there is growing interest in

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exploring natural sources as safer alternatives with proven potential for treating inflammation and fewer side effects compared to synthetic drugs.

Glaucium grandiflorum Boiss. & Huet, a member of the Papaveraceae family, is distributed across the Middle East, with a presence in the eastern Mediterranean (Turkey, Syria, Iraq) and extending to Iran. It is a perennial wild herb, typically reaching a height of 40 to 50 cm, with flowering occurring in April and ripe fruits observed in June to July. The petals of this herb are typically reddish-orange with a dark basal spot [9,10]. Various species within the genus have been employed in traditional herbal medicine for their laxative, hypnotic, and anti-diabetic properties. The latex extracted from the stem and leaves has been used to treat sores, wounds, dermatitis, and as an antiseptic [11-13]. External application of *G. corniculatum* extract has been considered useful for alleviating joint pain, headaches, and constipation [14]. Capsules of *G. grandiflorum* have been used in Turkey, while the latex in Iran has been employed to treat ophthalmic diseases [15].

Phytochemical analysis has suggested that *G. grandiflorum* is rich in isoquinoline alkaloids as the primary nucleus, which can be further categorized into several types, including Aporphine (e.g., glaucine, isocorydine, corydine, isocorytuberine), Protopine type (e.g., protopine, cryptopine, allocryptopine), Benzophenanthridine type (e.g., dihydrochelerythrine, (-)-norchelidonine, (-)-8-acetonyldihydrochelerythrin), and Tetrahydroprotoberines (e.g., isocorypalmine) [16-18]. However, limited research has been conducted on *G. grandiflorum* regarding its pharmacological and medicinal activities, including anti-microbial, anti-bacterial, anti-inflammatory, analgesic, anti-tumoral, and anti-acetylcholinesterase properties [11,12, 15-19].

Although the anti-inflammatory activity and acute toxicity of its methanolic extract were previously evaluated in Turkey in 2002 [20], the types and concentrations of active plant components, particularly phenols, flavonoids, alkaloids, saponins, and their biological roles, can be influenced by environmental factors such as temperature, humidity, and soil quality [21].

However, the content of alkaloids in various plant parts of *G. grandiflorum* wildly grown in Syria and its anti-inflammatory potential have not yet been thoroughly investigated. To comprehend the source of this effect, it is crucial to identify the chemical composition of the extract. Thus, this study aims to investigate the anti-inflammatory activity of *G. grandiflorum* through both oral and intraperitoneal administration and to elucidate the relationship between this effect and the active ingredients in the extract. The results presented in this paper are a step toward discovering new, safe, and potent herbal anti-inflammatory compounds.

2. RESULTS

Syrian *G. grandiflorum* can be morphologically distinguished from the species found in Iran and Turkey, with variations in flower color and size between the flowering and fruiting stages. This characteristic distinction has been recorded for the first time in the Syrian species (Figure 1). Therefore, it was imperative to determine the differences in alkaloid content in various aerial parts (flowers, leaves, stems) during flowering and fruiting stages, roots, and capsules.

A B

Figure 1. A: Aerial parts in flowering stage (red flowers, stems, leaves, buds).
B: Aerial parts in fruiting stage (orange flowers, stems, leaves, capsules).

The total alkaloid content was found to be the highest in the fruits (capsules) at 5.07%, followed by aerial parts in the fruiting stage at 3.21%, flowering aerial parts at 1.41%, and the lowest in the roots at 0.66%. The results showen in Table 1 and Figure 2.

Table 1. Total content of alkaloids in different plant parts.

Sample	mg of curd alkaloid / g dry plant*	% Alkaloid
Flowering aerial part	14.13 ±2.25	%1.41
Fruiting aerial parts	30.40 ±0.11	%3.04
Fruit	50.76 ±0.65	% 5.07
Root	6.61 ±1.71	% 0.66

* Values are showed as the mean± SD

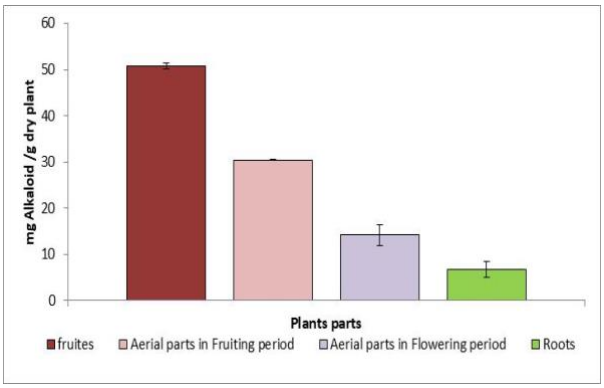


Figure 2. Graph illustrating the total content of alkaloid in different plant parts of *G. grandiflorum*.

Our results indicate that the oral and intraperitoneal administration of the plant extract at graded doses did not result in mortality or toxic effects in rats, except for some behavioral changes observed at higher doses (≥ 800 mg/kg), such as drowsiness, lethargy, and reduced appetite within the first 4 hours.

The results of the effect of *G. grandiflorum* hydroethanolic extract of the flowering aerial parts on formalin-induced paw edema in rats are presented in Table 2 and 3.

Table 2. Anti-inflammatory effect of *G. grandiflorum* hydroethanolic extract and Indomethacin on formalin-induced paw edema.

	Paw thickness (mm) at various time interval± S.D				
	0h	1h	2h	3h	4h
Group I	4.24±0.5	5.63±0.69	5.77±0.81	5.76±0.86	5.87±0.76
Group II	4.39±0.30	5.32±0.44	5.08±0.44	4.97±0.43	4.86±0.41
Group III	4.27±0.08	5.32±0.17	5.22±0.25	5.05±0.16	4.95±0.24
Group IV	4.07±0.19	4.89±0.30	4.68±0.16	4.62±0.14	4.50±0.13
Group V	4.59±0.20	5.55±0.22	5.26±0.29	4.91±0.14	4.77±0.22
Group VI	4.58±0.35	5.42±0.21	5.36±0.17	5.14±0.27	5.05±0.35
Group VII	3.81±0.49	4.48±0.51	4.32±0.45	4.09±0.47	3.88±0.47

Values are showed as the mean± SD.

Table 3. Anti-inflammatory effect of *G. grandiflorum* hydroethanolic extract and ındomethacin on ınhibition of paw edema ınduced by formalin

	Percentage of Swelling (Edema inhibition (%))			
	1h	2h	3h	4h
Group I	32.81±0.75	35.83±2.63	36.35±3.15	38.33±1.18
Group II	20.94±2.25* (36.17%)	15.61±2.04* (56.43%)	12.80±2.60*[a] (64.80%)	10.47±1.80*[a] (72.68%)
Group III	24.39±1.75* (25.66%)	22.11±3.71* (38.29%)	18.08±1.76* (50.27%)	15.72±3.47* (58.99%)
Group IV	20.13±1.84* (38.66%)	15.04±1.40* (58.03%)	13.70±1.86*b (62.32%)	10.62±1.89*b (72.28%)
Group V	20.93±0.53* (36.22%)	14.65±2.83* (59.11%)	6.94±1.52*a (80.90%)	3.92±1.38*a (89.77%)
Group VI	18.31±3.30* (44.19%)	17.09±3.87* (52.29%)	12.13±2.14* (66.64%)	10.24±1.61* (75.12%)
Group VII	17.71±1.93* (46.03%)	13.04±2.81* (63.60%)	27.32±1.51*b (79.88%)	1.76±0.791*b (95.42%)

Values are showed as the mean± SD
*All test groups showed significant differences from the negative control group ($p<0.05$), as determined by one-way ANOVA followed by the Tukey test.
[a] The Groups treated with indomethacin (oral or injection) were statistically significant at the 3rd and 4th hours ($p<0.05$).
[b] The groups treated with the extract at a dosage of 400 mg/kg (oral or intraperitoneal) were statistically significant at the 3rd and 4th hours ($p<0.05$).

All test groups showed significant differences from the negative control group ($p<0.05$), as determined by one-way ANOVA followed by the Tukey test. Groups treated with indomethacin (oral or injection) were statistically significant at the 3rd and 4th hours ($p<0.05$). The groups treated with the extract at a dosage of 400 mg/kg (oral or intraperitoneal) were statistically significant at the 3rd and 4th hours ($p<0.05$).

The extract exhibited significant anti-inflammatory activity when administered both intraperitoneally and orally at treatment doses of 200 and 400 mg/kg. Paw thickness initially increased in the first hour but gradually decreased thereafter. Oral administration of the extract resulted in inhibition rates of 75.12% and 95.42% at doses of 200 mg/kg and 400 mg/kg, respectively, while intraperitoneal administration led to inhibition rates of 58.99% and 72.28% at the 4th hour (Figures 4 and 5).

3. DISCUSSION

G. grandiflorum is a wild plant endemic to the Syrian region that has not been previously investigated. This study marks the first attempt to determine the total alkaloid content in different plant parts. In accordance with a previous report from Aleppo University, it was found that the total phenol and flavonoid content of the Syrian species were superior to the Turkish species. Given this, it became necessary to thoroughly investigate the anti-inflammatory activity of the Syrian species in comparison to its Turkish counterpart. Furthermore, we sought to evaluate the effects of the extract when administered orally compared to intraperitoneally and assess the efficacy of higher doses beyond 200 mg/kg.

The results of acute toxicity in this study, using the hydroethanolic extract of flowering aerial parts of *G. grandiflorum*, indicate that the extract is relatively safe and non-toxic to rats, as no mortality was recorded within 72 hours. However, it should be noted that high doses (≥800 mg/kg) did lead to certain behavioral changes, such as drowsiness, lethargy, and reduced appetite, which may limit their practical use.

The extract from the flowering aerial parts of *G. grandiflorum* exhibited anti-edematogenic and anti-inflammatory activity in the acute phase of inflammation induced by formalin. The injection of formalin (2.5%) into the subplantar region of experimental animals typically results in pain associated with significant redness and an increase in paw thickness. It is a commonly used model for studying acute and chronic inflammatory pain, causing paw edema through a biphasic response. The early neurogenic response is stimulated by bradykinin and substance-P, while the late inflammatory response is mediated by histamine, serotonin, bradykinin, and prostaglandins [22-23].

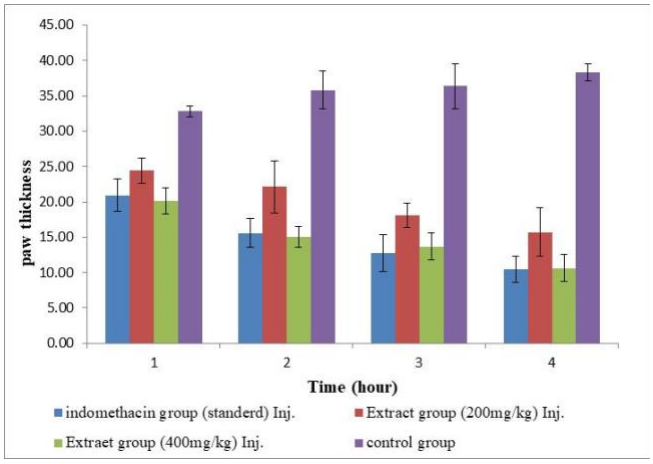


Figure 3. Graph illustrating the inhibition of increasing paw thickness by G. grandiflorum hydroethanolic extract and Indomethacin (intraperitoneal)

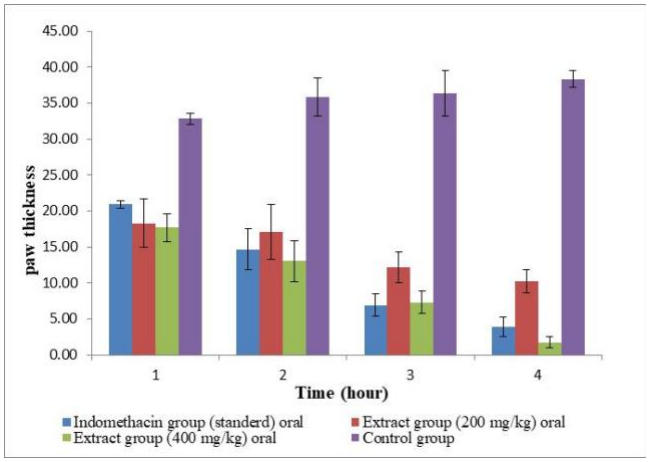
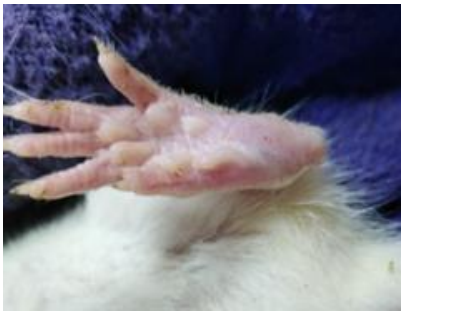


Figure 4. Graph illustrating the inhibition of increasing paw thickness by *G. grandiflorum* hydroethanolic extract and Indomethacin (oral)



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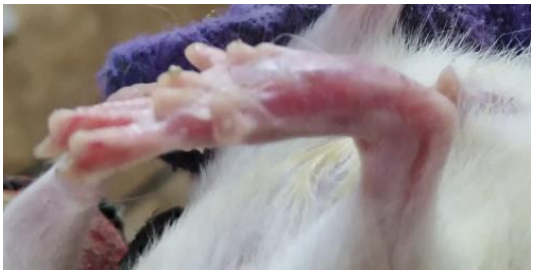

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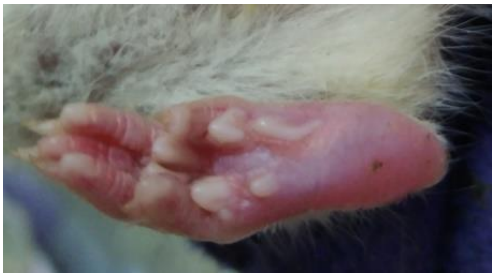

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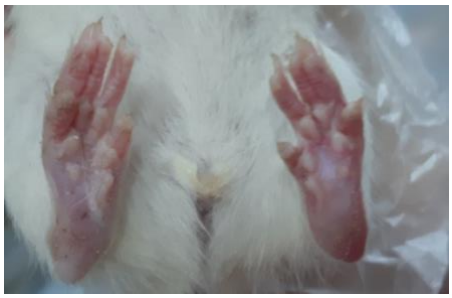

D

Figure 5. A: Rats paw before formalin injection (normal).
B : Negative control
C : Rats paw after formalin injection. (redness and swelling)

D : Rats paw which treatment with the extract. (decrease in edema formation and less redness compared to negative control)

All groups treated with the extract and indomethacin demonstrated significant anti-inflammatory responses throughout the experiment compared to the control group ($p<0.05$). The extract dose-dependently inhibited the development of paw swelling. Paw thickness reduction was observed with the oral administration of the *G. grandiflorum* extract (at a dose of 400 mg/kg) at all measurement time points, similar to the effect of the standard (Indomethacin 10 mg/kg, oral). The maximum inhibition was achieved after 4 hours with the extract (95.42%) at a dose of 400 mg/kg on oral administration, while the standard drug also exhibited its highest inhibition effect after 4 hours (89.77%).

Interestingly, significant anti-inflammatory activity was observed with oral administration at a dose of 400 mg/kg compared to intraperitoneal administration at the same dose ($p<0.05$). This suggests that the extract is more effectively absorbed through the digestive tract than when injected intraperitoneally, emphasizing the importance of sufficient absorption and distribution for pharmacological action [24].

In recent years, there has been a growing focus on secondary metabolites of plants due to their therapeutic potential. Preliminary phytochemical screening of *G. grandiflorum* demonstrated the presence of alkaloids, flavonoids, triterpenoids, sterols, and saponins in the flowering aerial parts, while anthraquinones and cardiac glycosides were absent [25]. Additionally, the total phenol and flavonoid contents in the hydroethanolic extract were determined (16.908 mg GAE/g DW, 14.769 mg Rutin /g DW, respectively), and the antioxidant activity was evaluated through the DPPH assay (IC50= 1.770 mg/ml) [26]. These findings suggest that the anti-inflammatory effect of the extract may be attributed to the presence of these plant chemical constituents.

The exact mechanism by which the Syrian *G. grandiflorum* extract exerts its anti-inflammatory activity remains unclear. Nevertheless, our results and previous literature suggest that phenolic compounds inhibit the production and action of pro-inflammatory mediators [27], flavonoids can inhibit enzymes involved in inflammation, such as phosphodiesterases and phospholipase A2 [28-29], and alkaloids, especially those from the poppy family, have diverse pharmacological activities, including anti-inflammatory and pain-relieving properties. Glaucine is an important alkaloid isolated from Glaucium species. Research suggests that Glaucine may have the ability to reduce edema, which is swelling caused by the accumulation of fluid in tissues. It does so by influencing various inflammatory mediators[30]. Berberine is another alkaloid, and it belongs to the benzylisoquinoline alkaloid group. It has been shown to possess anti-inflammatory effects. Berberine can inhibit swelling by reducing the synthesis of prostaglandin E2 (PGE2), which is an inflammatory mediator [31]. Protopine alkaloids, isolated from Macleaya Cordata and possibly other plants, have demonstrated anti-inflammatory properties. These alkaloids seem to act in a manner similar to non-steroidal anti-inflammatory drugs (NSAIDs) by affecting the activity of the cyclooxygenase enzyme (COX), which is involved in the inflammatory response [32]. Saponins, too, have shown anti-inflammatory effects, particularly in diseases like rheumatoid arthritis [33-34].

In previous studies on Glaucium species and their anti-inflammatory properties, it was observed that *G. flavum* ethanol extract demonstrated a reduction in edema upon oral administration in vivo [35]. Additionally, *G. acutidentatum* exhibited anti-inflammatory effects in vitro [36]. Notably, a study conducted in Turkey reported that the methanol extract of *G. grandiflorum*, prepared through maceration, displayed a superior paw edema inhibition effect (98.88%) three hours after carrageenan injection when compared to the percentage of edema inhibition achieved by the hydroethanolic extract of the Syrian species (200 mg/kg and 400 mg/kg), which was 58.99% and 72.28%, respectively, four hours after formalin injection via intraperitoneal administration. However, it is worth noting that the anti-inflammatory efficacy of the Turkish extract was nearly equivalent to that of the Syrian species extract administered orally at a dose of 400 mg/kg (95.42%). The variance in extraction solutions and methods likely accounts for the differences in these observed biological activities. Furthermore, other research has reported the anti-inflammatory action of topically administered *G. grandiflorum* extract. These findings collectively suggest that *G. grandiflorum* exhibits anti-inflammatory properties when administered via intraperitoneal, oral, and topical routes.

4. CONCLUSION

In conclusion, our results indicate that the hydroethanolic extract of Syrian *G. grandiflorum* flowering aerial parts, containing alkaloids, flavonoids, triterpenoids, and saponins, possesses anti-inflammatory

activity. Further studies are required to determine the precise mechanism of action and identify the specific phytochemical compounds responsible for this efficacy.

5. MATERIALS AND METHODS

5.1. Chemicals, Equipment, and Plant materials

Chemicals: The following chemicals were employed in the experiments: Ethanol (Scharlau Chemie, Spain), acetic acid (Panreac, EU), Ammonia solution (Screchem Products LTD., England), formaldehyde solution (Screchem Products LTD., England), and Saline 0.9% (Serda Pharma Solutions, Syria). Indomethacin (a standard drug) was procured from Anhui Biochem United Pharmaceutical, China.

Equipment: The experimental setup included an Ultrasonic apparatus (Hwashin Technology Co., Korea), a Rotary evaporator (Buchi, Switzerland), and an Electronic Digital Caliper (Gilbert, China).

Plant materials**:** *G. grandiflorum* was collected from Qudsaya– Subarb, Damascus, Syria, located in the southern part of the country, in April (flowering stage) and May (fruiting stage) of 2021. The plant was identified by Dr. Thanaa Harammi, Department of Pharmacognosy, Aleppo University, Syria. Various parts of the plant (aerial parts during flowering and fruiting stages, capsules, and roots) were air-dried for two weeks in the shade at room temperature. Subsequently, the dried plant material was powdered and stored in a sealed container.

5.2. Determination of Total Alkaloid Content

For each part (aerial parts in flowering and fruiting stages, capsules, and roots), 5 g of plant material was placed in a flask with 50 mL of 10% acetic acid in ethanol. The mixture was covered and allowed to stand for 4 hours, followed by filtration. This extraction process was repeated three times. The filtrate was then concentrated to one-quarter of the original volume, and concentrated ammonium hydroxide was added dropwise until alkaloid precipitation occurred at pH = 10. The solution was allowed to settle, and the precipitated alkaloids were collected, washed with 0.1M ammonium hydroxide, and filtered. The resulting residue represented the alkaloid content, which was dried in an oven (at 40°C) and weighed. The percentage of alkaloids was expressed as [37-38]:

$$\% \text{ Alkaloid} = (\text{Weight of alkaloid} / \text{Weight of sample}) \times 100$$

5.3. Extraction Procedures

10 g of powdered aerial parts in the flowering stage of *G. grandiflorum* was placed in a flask with 100 mL of 70% ethanol in an ultrasound bath operating at 42 kHz, 70 W, 60 minutes, and 40°C [39]. The extraction process was repeated three times. The resulting extracts were filtered using Whatman No. 2 filter paper and evaporated to dryness in a rotary evaporator under reduced pressure at 40°C. The resulting extract was stored at 4°C until use. The yield of the extract was calculated as the mass of dried extract divided by the mass of 1 g of dried powder, resulting in a yield of 28%.

5.4. Anti-inflammatory activity of *G.grandiflorum*

5.4.1 Animals

Male and female Albino Wistar rats weighing 125-150 g were used for evaluating anti-inflammatory activity. The animals were kept under controlled conditions with a temperature of 20 ± 2°C, humidity at 50 ± 5%, and a 12-hour light and dark cycle. Prior to the experiment, the rats were fasted for 24 hours with free access to water.

5.4.2 Acute Toxicity

To assess the acute toxicity of the hydroethanolic extract of flowering aerial parts of *G. grandiflorum*, Wistar albino rats were randomly divided into six groups of five rats each. These rats were observed for three days (72 hours) following intraperitoneal (i.p.) and oral administration of hydroethanolic extract at doses of 200, 400, 800, 1200, 1500, and 2000 mg/kg. The study recorded the number of rat deaths during the experiment and monitored signs of acute toxicity, such as behavioral changes.

5.4.3 Experiment

The dosage of the extract and indomethacin was selected based on a previous study [20], which demonstrated their effectiveness as anti-inflammatory agents.

The animals were randomly divided into seven groups of six rats each:

- Group I (Negative Control): Received physiological saline (0.9%).
- Group II (Standard Group): Administered 10 mg/kg of Indomethacin intraperitoneally (i.p.).
- Group III: Administered 200 mg/kg of hydroethanolic extract intraperitoneally.
- Group IV: Administered 400 mg/kg of hydroethanolic extract intraperitoneally.
- Group V (Standard Group): Administered 10 mg/kg of Indomethacin orally.
- Group VI: Administered 200 mg/kg of hydroethanolic extract orally.
- Group VII: Administered 400 mg/kg of hydroethanolic extract orally.

5.4.4 Paw Inflammation Induced by Formalin

In all groups, paw edema was induced by injecting 0.1 mL of 2.5% formalin (freshly prepared from 37% formaldehyde in 0.9% saline) into the right hind paw. One hour prior to formalin injection, indomethacin (10 mg/kg) and the extract (200 mg/kg and 400 mg/kg) were administered orally, while the intraperitoneal injection 30 minutes after formalin injection [40]. The left hind paw received a saline injection to determine the impact of the injection process on paw thickness changes (see Figure 5). This method was approved by the Ethics committee of the faculty of Pharmacy in Aleppo university (certificate Nr. 13/V. 15/5/2022).

5.4.5 Determination of Edema Formation

The increase in paw thickness was measured using a vernier caliper at different time points, including before (0 h) and after formalin injection and drug administration (1, 2, 3, and 4 h) [41].

To calculate the percentage of swelling, the following formula was employed [42]:

$$\text{Swelling}\% = [(V - V_i) / V_i] \times 100$$

Where:

V represents the paw thickness after formalin injection.

V_i represents the paw thickness before formalin injection.

Additionally, the percentage inhibition of edema was determined using the following formula:

$$\text{Inhibition of Edema}\% = [(V_C - V_T) / V_C] \times 100$$

Where:

V_C represents the paw thickness of the negative control group.

V_T represents the paw thickness of the treatment group.

5.5. Statistical analysis

The data were expressed as the mean ± SD (standard deviation). Statistical analysis was carried out using SPSS 26. The values were subjected to analysis of variance (one-way ANOVA), and post hoc Tukey tests were employed for multiple comparisons. In addition, an independent samples T-test was performed when applicable. A significance level of $p < 0.05$ was considered statistically significant.

Author contributions: Concept – N.A., T.H., A.I., A.M.; Design – N.A., T.H., A.I., A.M.; Supervision – N.A., T.H., A.I., A.M.; Resources – N.A., T.H., A.I., A.M.; Materials – T.H., A.I., A.M.; Data Collection and/or Processing – N.A.; Analysis and/or Interpretation – N.A., T.H., A.I., A.M.; Literature Search – N.A.; Writing – N.A.; Critical Reviews – N.A., T.H., A.I., A.M.

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