Antihypertrophic Scar Effect of Iraqi Plantago major Extracts

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Abstract :

Background: Plantago major, historically renowned for its medicinal attributes across diverse cultures, has recently been under the research spotlight for its antihypertrophic scar effects.

Aim: To investigate the efficacy of Plantago major extracts in the treatment of hypertrophic scars, particularly comparing the effects of methanol and ethyl acetate extracts.

Methodology: Samples of the plant, procured from Baghdad's Al-Salihiya Neighbourhood in November 2021, underwent authentication at the Iraqi local Herbarium in the Al-Razi centre for alternative medicine. Using the Soxhlet apparatus, the dried plant material was extracted with methanol and subsequently partitioned with ethyl acetate. Both the ethyl acetate and methanol extracts were later formulated into ointments.

Results: The ointments were tested on hypertrophic scars induced in rats. Three groups of 12 rats each were used—ethyl acetate, methanol, and a control group using only Vaseline. Both extracts demonstrated efficacy in reducing scars, with the methanol extract showing more pronounced results.

Conclusion: The methanol extract displayed superior outcomes, potentially attributable to its richer phytochemical content compared to the ethyl acetate extract.

Keywords: antihypertrophic scar, ethyl acetate extract, hypertrophic scars, methanol extract, ointments, phytochemical content, Plantago major, Soxhlet apparatus

تأثير الندبة المضادة للتضخم للمستخلصات الرئيسية من لسان الحمل العراقي

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الخلاصة

الخلفية: تُعتبر نبات لسان الحمل الكبير (Plantago major) المعروف منذ القدم بفضل خصائصه الطبية عبر ثقافات متنوعة، وأخيرًا أصبح محل اهتمام البحوث بفضل تأثيره في علاج الندبات التضخمية.

الهدف: دراسة فعالية مستخلصات نبات لسان الحمل الكبير في علاج الندبات التضخمية، ولا سيما بالمقارنة بين تأثيرات مستخلصات الميثانول والأسيتات الإيثيلي.

طريقة البحث: تم تحصيل عينات من النبات من منطقة الصالحية في بغداد في نوفمبر 2021، وتم توثيقها في مركز الاعشاب المحلي العراقي (مركز الرازي للطب البديل). باستخدام جهاز سوكسليت، تم استخلاص المواد النباتية المجففة بالميثانول ثم تم تقسيمها بواسطة الأسيتات الإيثيلي. تم تحضير كل من مستخلصات الأسيتات الإيثيلي والميثانول على شكل مراهم للاستعمال الخارجي.

النتائج: تم اختبار المراهم المحضرة على ندبات تضخمية تم استحداثها في الجرذان. تم استخدام ثلاث مجموعات، كل مجموعة تحتوي على 12 جرذان - مجموعة بأسيتات إيثيلي، مجموعة بالميثانول، ومجموعة مراقبة باستخدام الفازلين فقط. أظهرت كل من المستخلصات فعالية في تقليل الندبات، مع تحقيق المستخلص بالميثانول نتائج أكثر وضوحًا.
الاستنتاج: أظهر المستخلص بالميثانول نتائج متفوقة، وربما يعزى ذلك إلى احتوائه على مزيد من المواد النباتية الفيتوكيميائية مقارنة بالمستخلص بالأسيتات الإيثيلي

كلمات مفتاحية: مضادات الندب التضخمية، مستخلص الاسيتات الاثيلي, الندب التضخمية, مستخلص الميثانول, مرهم, المحتوى الكيمونباتي, لسان الحمل الكبير, جهاز السوكسليت.

Introduction:

Plantago major, often referred to as broadleaf plantain, is a perennial herb from the Plantaginaceae family. This family also comprises species with significant medicinal value, such as (Plantago lanceolata[1] and others) This plant is distinguished by its basal arrangement of wide leaves that feature striking parallel veins. For centuries, these leaves have been celebrated for their therapeutic qualities and have found their place in traditional medicinal practices.[2]

Plantago major thrives in numerous areas, spanning continents from Europe to Asia and North America, demonstrating its resilience to diverse climates and terrains. It's commonly seen in places like open fields, grasslands, and adjacent to roads. This vegetation is recognized by its elongated stems bearing understated flowers, often coloured in shades of brown or green.[3]

Plantago major is known to be rich in many phytochemicals, among these, aucubin stands out as a primary bioactive compound known for its anti-inflammatory and wound-healing properties. Additionally, iridoid glycosides like catalpol and plantainoside A, along with tannins, provide anti-inflammatory and antioxidant effects. Flavonoids such as apigenin and luteolin further enhance the plant's therapeutic potential with their antioxidant and anti-inflammatory properties. Plantago major may also contain alkaloids and phytosterols (Beta-sitosterol), which can contribute to its overall health benefits. These phytochemicals collectively make Plantago major a valuable plant in traditional and herbal medicine for addressing various health concerns.[4]

A hypertrophic scar is a raised type of scar that develops during the process of wound healing. It is characterized by an excessive production of collagen, resulting in a thickened and elevated appearance. Hypertrophic scars typically remain within the boundaries of the original wound and can be red, pink, or purple in color. They may cause itching, tenderness, and cosmetic concerns [5]. Unlike keloid scars, which extend beyond the original wound area, hypertrophic scars stay confined to the site of the injury. The exact causes of hypertrophic scarring are not fully understood, but factors such as genetics, wound tension, and inflammation are believed to play a role. [6]

The pathophysiology of hypertrophic scars involves a complex interplay of cellular and molecular processes during the wound healing cascade. When an injury occurs, the body initiates a series of events to repair and regenerate the damaged tissue. However, in the case of hypertrophic scars, this process becomes dysregulated. [7]

Excessive collagen deposition is a key characteristic of hypertrophic scars. Fibroblasts, the cells responsible for collagen production, undergo abnormal activation and proliferation in the wound site. This leads to an overproduction of collagen fibers, which results in the raised and thickened appearance of the scar. [8]

In hypertrophic scars, there is an imbalance between collagen synthesis and degradation. Increased levels of pro-

inflammatory cytokines, such as transforming growth factor-beta (TGF-β), contribute to the excessive collagen production. Furthermore, altered signaling pathways, including the upregulation of certain growth factors and signaling molecules, can disrupt the normal remodeling of the scar tissue. [9]

Other factors that influence the pathophysiology of hypertrophic scars include the duration and intensity of inflammation, genetic predisposition, and mechanical tension on the wound site. These factors can further exacerbate the abnormal collagen deposition and remodeling processes. [10]

In below some of the current therapeutic options for the management of Hypertrophic scars:

1- **Corticosteroids:**
Topical corticosteroids are an effective treatment option for hypertrophic scars. These medications, available as creams, ointments, gels, or sprays, contain corticosteroid compounds that have potent anti-inflammatory and immunosuppressive properties [11].
When applied topically to hypertrophic scars, corticosteroids help reduce inflammation, alleviate symptoms such as itching or tenderness, and promote scar remodeling. They work by suppressing the immune response and modulating collagen synthesis in the scar tissue. This leads to a reduction in scar thickness, improved elasticity, and an overall improvement in the scar's appearance. [12]

2- **Laser Therapy:** Laser treatments, such as pulsed dye laser or fractional laser, can target the blood vessels in hypertrophic scars, reducing redness and promoting scar remodeling. Laser therapy can also stimulate collagen remodeling and improve the texture of the scar. [13]

3- **Cryotherapy:** The application of extreme cold to the scar tissue through techniques like cryosurgery can help flatten and reduce the size of hypertrophic scars. Cryotherapy works by damaging the scar tissue, leading to its gradual remodeling. [14]

4- **Silicone-based Therapies:** Silicone sheets or gels are commonly used as a non-invasive treatment option. They create a protective barrier, hydrate the scar, and modulate collagen production. Silicone therapy can help improve scar texture, color, and overall appearance. [15]

5- **Surgical Interventions:** In cases where hypertrophic scars cause functional limitations or significant distress, surgical excision or revision may be considered. However, surgical interventions carry the risk of potential complications, and the decision to undergo surgery should be made on a case-by-case basis. [16]

Aim of the Study:

To investigate the efficacy of Plantago major extracts in the treatment of hypertrophic scars, particularly comparing the effects of methanol and ethyl acetate extracts.

Materials and methods

Preparation of topical preparation

100 g of the dried whole plant was extracted using 250 mL of methanol 85% in Soxhlet apparatus for 6 hours, then was partitioned with 100 mL of ethyl acetate for three times. Both methanolic and ethyl acetate fractions were collected then concentrated and dried using rotary evaporator to yield 14g and 8.7g of the plant dried extract respectively.

8 g of both ethyl acetate and methanolic dry extract was incorporated with 72 g of Vaseline as an ointment, to prepare a dose strength of 10% to be used to the rats during the study.

Animal Grouping

36 female rats (10-12) weeks of age, weighing 225-250g. were randomly grouped into 3 groups:

1- 12 rats to be tested with Ethyl acetate extract ointment.
2- 12 rats to be tested with methanolic extract ointment.
3- 11 rats as control group with the application of Vaseline.

All of them were in a good health with no identifiable or obvious signs of any diseases.

Main phytochemical tests

The preliminary tests involved the extraction of 50 grams of shade-dried aerial parts of the plant using a Soxhlet apparatus. The plant material was packed in the thimble of the apparatus and extracted with 250 ml of 85% ethanol until the solution became clear in the Soxhlet chamber. The resulting solution was then filtered, and the solvent was concentrated using a rotary evaporator at a temperature of 45℃. The concentrated extracts were subjected to screening using standard methods to qualitatively investigate the presence of secondary metabolites in the plant's crude extract.

1- **Flavonoids:** 2 mL of Ethanolic KOH was added to 1 mL of the plant extract, yellow color indicate the presence of flavonoids [17]
2- **Tannins:** to test for the presence of tannins, Braymer's test is performed, the ethanolic extract was diluted to 10 times its volume. Subsequently, 1% aqueous ferric chloride was added. A positive result for tannins was indicated by the development of a dark green or dark blue color. [18]
3- **Saponins:** The froth test for saponins involved vigorously shaking a few milliliters of the plant's ethanolic extract with distilled water for 15 minutes. A positive result was determined by the presence of persistent froth after the shaking process. [19]
4- **Iridoids:** The iridoid presence was assessed using a colorimetric method based on the Trim-Hill reaction. In this procedure, extract (1 mL) was combined with 10 mL of Trim-Hill reagent, composed of acetic acid-0.2% CuSO4-conc. HCl in a ratio of 10:1:0.5. The presence of iridoids was indicated by the development of a blue color in the solution. [20]
5- **Alkaloids:** a small quantity of the substance or solution suspected to contain alkaloids is mixed with Dragendorff reagent. The reagent consists of a mixture of bismuth nitrate and potassium iodide in water. The formation of characteristic red-orange color indicates a positive result for the presence of alkaloids. [21]
6- **Terpenoids:** To conduct the test, 0.5 mL of the extract was added to a test tube containing 2 mL of chloroform. Subsequently, 3 mL of concentrated sulfuric acid (H2SO4) was added, resulting in the formation of a distinct layer. The presence of terpenoids was indicated by a reddish-brown coloration at the interface between the chloroform and the sulfuric acid layer. [22]
7- **Phytosterols:**

Add 3 mL of the extract to a test tube containing 5 mL of acetic anhydride was placed in an ice bath for a duration of 30 minutes. Following this, 0.5 mL of sulfuric acid was cautiously added to the test tube, which was still cold from the ice bath. The appearance of a violet-blue color that subsequently transformed into a green hue serves as an indication of the presence of sterols. [23]

8- **Coumarins:** to investigate the presence of coumarins, 2 mL of the extract was added to 3 mL of NaOH, the development of yellow color indicates

the presence of coumarins in the plant. [24]

Induction of Hypertrophic scar

As shown in figure 1, hypertrophic scars were induced according to the following procedure [25]:

1- Anesthesia is administered to the rat using Ketamine anaesthetic agent in a dose of (90 mg/Kg).
2- A full-thickness incisional or excisional wound is created on the shaved dorsal area of the rat using a sterile surgical blade.
3- Appropriate postoperative care was provided, including wound cleaning, and monitoring for signs of infection or complications.
4- Close monitoring and care were continued for 30 days as a sufficient period for development of noticeable and investigable hypertrophic scar.

Figure 1: Rat preparation for wounds

Treatment plan

At day 30 after the excision, the treatment phase begins and lasts for 21 days during which the ointment would be applied in a thin layer to the scar site with very gentle rubbing to the site of application.

Scar monitoring assessment

1- Visual Inspection: The most fundamental form of assessment is employed, whereby the scar is observed for alterations in size, colour, and shape.
2- This method is inherently subjective, yet it has the potential to yield immediate and non-invasive insights. To ensure comparability, standardized photos are employed, and consistent lighting and angles are meticulously maintained.
3- Scar Rating Scales: Using the modified Vancouver scar scoring (VSS)[26] which is a widely used scoring system to assess the hypertrophic scars resulted from wounds and burns [27], table 1 shows the scoring system of this parameter.

Table 1 Scoring system of the modified VSS

No	Parameter	Sub-parameter	Score (0-8)
1	Consistency	Soft	0
		Chewy	1
		Tough	2
2	Color	Similar with surrounding skin	0
		Hypopigmentation	1
		Hyperpigmentation	2

3	**Height**	Similar with surrounding skin	0
		<1 mm	1
		>1 mm	2
4	**Appearance**	Similar with surrounding skin	0
		Not similar	1
5	**Hair growth**	Yes	0
		No	1

4- Mechanical Measurements: This involves using special tools such as a durometer to measure scar hardness, in this study, as shown in figure 2, Gain Express Compact Pocket Size Digital Shore A Hardness Meter Tester 1-100ha Durometer was used.[28]

Figure 2: Durometer instrument used in the study

Results and discussion

Preliminary test: Table 3 shows the results of the main preliminary tests performed on the plant: as follows:

Table 3: Results of preliminary tests

Phytochemical class	Test used	Result
Flavonoids	Ethanolic KOH test	+++ve
Tannins	Braymer's test	+ve
Saponins	Foam test	+ve
Iridoids	Trim-Hill test	++ve
Alkaloids	Dragendorff	+ve
Terpenoids	H2SO4 in Chloroform test	+ve
Phytosterols	Acetic anhydride test	+++ve
Coumarins	NaOH test	++ve

Visual examination:

In this context, each rat was shaved then inspected carefully for the assessment of the effect of the applied ointments on the color, skin texture and overall scar area. These findings reveal good response on both the ethyl acetate and methanolic groups when compared to the control group as shown in figure 3

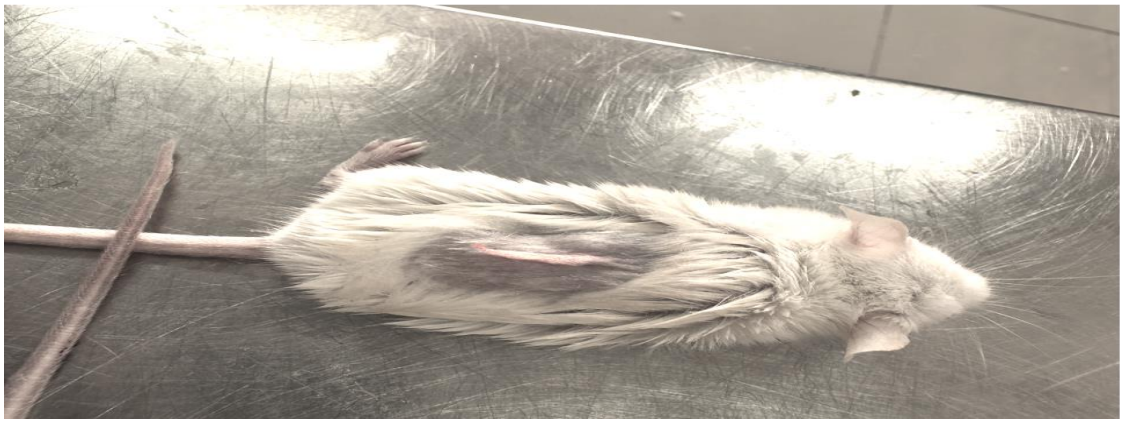

Figure 3 Post treatment inspection of rats (control group)

Scar rating scale

Using the modified Vancouver scar scoring as stated in Table 2, the following (table 3) shows the effect of each extract on the development and healing of scar

Table 3 Post treatment modified VSS score

No	Control (n=11)	Ethyl acetate (n=12)	Methanol (n=12)
1	7	5	4
2	6	4	3
3	7	4	4
4	7	4	5
5	6	4	3
6	5	5	4
7	6	4	2
8	6	5	3
9	5	3	3
10	7	5	5
11	6	6	4
12		4	3
Mean	**6.18**	**4.41**	**3.58**

The results indicate that both ethyl acetate and methanolic extracts have the potential to reduce the severity of hypertrophic scars. *Plantago major* is known for its anti-inflammatory and wound-healing properties, which might contribute to the observed effects on hypertrophic scars.[29]

The lower Modified VSS scores in both the extract groups (ethyl acetate and methanol) compared to the control group provide evidence that the plant extracts could have a positive impact on scar tissue. The reduced Modified VSS scores suggest improvements in several scar characteristics, such as height, color, consistency, and appearance.

However, the more reduction in scar severity observed in the methanol extract

group compared to the ethyl acetate extract group might indicate that different compounds or concentrations of active constituents in each extract could lead to varied therapeutic effects.

Mechanical measurement:

The durometer readings shown in table 4. indicate the hardness of the material, where higher values represent higher hardness. The Control group has the highest mean durometer reading (25.27), indicating that the untreated samples have the highest hardness. On the other hand, the Methanol extract group has the lowest mean durometer reading (15.58), suggesting that the Methanol extract may have caused a decrease in hardness compared to the Control group. The Ethyl acetate extract group (mean durometer reading of 19.17) also has a lower hardness compared to the Control group but is higher than the Methanol extract group.

Table 4 Post treatment results for the durometry

No	Control (n=11)	Ethyl acetate (n=12)	Methanol (n=12)
1	28	21	17
2	25	23	15
3	26	19	16
4	25	22	19
5	26	17	18
6	24	20	14
7	25	16	15
8	26	19	10
9	20	16	16
10	26	17	14
11	27	21	18
12		19	15
Mean	**25.27**	**19.16**	**15.58**

The changes in hardness observed in the treated groups (Methanol and Ethyl acetate extracts) can be attributed to the presence of bioactive compounds in the plant extracts. Plant extracts often contain various chemical constituents, such as secondary metabolites (e.g., alkaloids, flavonoids, phenolic compounds), which can interact with the skin structure at the molecular level. These interactions can lead to changes in the scar properties, such as hardness.

The Methanol extract may have more potent bioactive compounds or a higher concentration of certain compounds that cause a more significant softening effect on the skin, which could be explained be the synergistic effect resulted from the multiple phytochemicals in the extract [30], resulting in the lowest mean durometer reading among the groups.

On the other hand, the Ethyl acetate extract, while still causing a decrease in hardness, may have milder or fewer interactions with the skin, leading to a higher mean durometer reading compared to the Methanol extract group.

These results are matching with other studies that relied on the Soxhlet apparatus for extraction of active phytochemicals from plant materials.[31]

Conclusion

Extracts from *Plantago major* demonstrated efficacy in ameliorating experimentally induced hypertrophic scars in rodent models. Notably, the methanolic extract displayed superior therapeutic

outcomes in addressing these scars compared to the ethyl acetate extract. The latter, although abundant in flavonoids, lacks the presence of phytosterols and other phytochemicals, which are recognized for their beneficial effects on hypertrophic scars.

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