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Harpagophytum procumbens (Devil's Claw): A Possible Natural Anti-Inflammatory Agent (An Experimental Study)

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ABSTRACT

Extract of *Harpagophytum procumbens* (devil's claw) has become the focus of research as a potential therapeutic agent in the treatment of rheumatic arthritis and pain due to its favorable side effects profile compared to synthetic alternatives. This superior safety of treatment is very valuable, especially in view of that in mandatory long duration of therapy in chronic diseases. None of NSAIDs is ideal in controlling or modifying the signs and symptoms of inflammation, particularly in the common inflammatory joint diseases. Many studies evaluated the anti-inflammatory and analgesic effects of *Harpagophytum procumbens* with inconsistent and contradictory results. The aim of this study was to investigate the effect of *Harpagophytum procumbens* on both acute and chronic inflammatory processes in rats and pain responses in mice. In addition, its safety on gastric and duodenal mucosa was evaluated histopathologically. Eighty rats of both sexes weighing 150-200 g each and twenty-four mice of both sexes weighing 25-30 grams each, were used in this work. For a pharmacological study, these animals were classified for induction of the different experimental models. The acute model of inflammation includes Carrageenan-induced rat back-paw edema test. The chronic models of inflammation include Complete Freund's adjuvant-induced arthritis test and cotton pellet-induced granuloma test. The analgesic model includes writhing test in mice. A biochemical study was done on the Complete Freund's adjuvant-induced arthritis test group. Blood samples were taken for measuring acute phase proteins; C-reactive protein & serum albumin, and serum cortisol. Histopathological assessment of gastric and duodenal mucosa for the effect of *Harpagophytum procumbens* in comparison with the effect of indomethacin was done in the Complete Freund's adjuvant-induced arthritis test group. In Carrageenan-induced rat back-paw edema test; Carrageenan sub-plantar injection in right back-paw in rats induced highly significant increase in paw thickness ($p \leq 0.001$). *Harpagophytum procumbens* pre-treatment induced highly significant reduction ($p \leq 0.001$) in right back-paw thickness, an effect similar to indomethacin. In Complete Freund's adjuvant-induced arthritis test; Freund`s adjuvant-induced arthritis in rats induced highly significant increase in paw thickness of rats ($p \leq 0.001$), significant decrease in serum cortisol ($p \leq 0.05$), highly significant decrease in serum albumin ($p \leq 0.001$) and significant increase in C-reactive protein ($p \leq 0.05$). *Harpagophytum procumbens* and indomethacin administration caused insignificant effects on these parameters and caused only significant reduction of paw thickness ($p \leq 0.05$). In cotton pellet-induced, granuloma test; *Harpagophytum procumbens* and indomethacin intra-peritoneal administration in cotton pellet-induced granuloma in rats caused a reduction of inflammation manifested by marked and highly significant decrease of cotton pellet weight ($p \leq 0.001$). In Writhing test in mice, *Harpagophytum procumbens* and acetyl salicylic acid had an analgesic effect manifested by highly significant reduction in the number of writhing reactions ($p \leq 0.001$). The results of the histopathological study revealed the greater safety of *Harpagophytum procumbens* on GIT mucosa in comparison to the more injurious effect of indomethacin as a NSAID.

Keywords: *Harpagophytum procumbens, NSAIDs, Arthritis, Pain*

Rheumatoid arthritis is a highly variable, chronic inflammatory condition affecting mostly diarthrodial (hinge-like) joints but often with articular and systemic involvement. The available data indicate that 76% of patients with RA are taking NSAIDs [1, 2]. Apart from treating the underlying disease, it is necessary to relieve

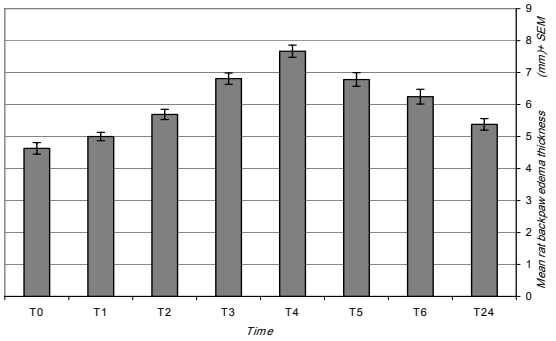

Fig 2. Effect of tested drugs on carrageenan-induced backpaw edema thickness in rats (Group I-a).

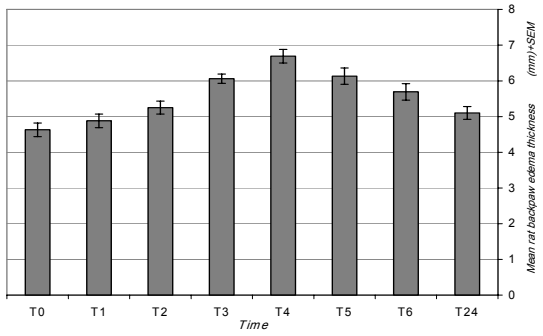

Fig 1. Effect of tested drugs on carrageenan-induced backpaw edema thickness in rats (Group I-b).

patient's pain. This has led to major improvements in the treatment of acute and chronic pain. In the pharmacological treatment of acute pain, aspirin-like and morphine-like drugs still form the cornerstone of most therapies [3]. There was a controversy about the anti-inflammatory effect of *Harpagophytum procumbens* (devil's claw), a herbal product marketed in Canada and Europe, as a home remedy for relief of arthritic diseases. Recent studies suggest that *Harpagophytum procumbens* has anti-inflammatory and analgesic effect. Extract of *Harpagophytum procumbens* have become the focus of research as a potential therapeutic agent in the treatment of rheumatic arthritis and pain due to its favorable side effects profile compared to synthetic alternatives [4]. *Harpagophytum procumbens* was effective in the treatment of osteoarthritis and reduced the need for analgesic and NSAIDs therapy [5]. Treatment, (800 mg of extract, three times daily with total dose of not more than 2400 mg per day), has been as accompanied by a reduction of use of analgesics [6]. *Harpagophytum procumbens* can probably help many of those who suffer low back pain with fewer side effects than NSAIDs treatment that are troublesome in the elderly, at a cost that is certainly not excessive [7]. Devil's claw-treated patients had low incidence of side effects. This superior safety of treatment is very valuable, especially in view of long duration of therapy in chronic diseases mandatory [8].

MATERIALS AND METHODS

Drugs and Chemicals Used

- Complete Freund's adjuvant (Sigma Chemical CO., USA).
- Croton oil obtained from local market.
- P-Benzoquinone (phenyl-isoquinone) (Eastman Organic Chemical Co., Michigan ,USA).
- Sodium Carrageenan (Sigma Chemical CO., USA).
- Acetyl salicylic acid (Emic Pharmaceutical Co., Egypt).
- Dried aqueous extract of *Harpagophytum procumbens* (Atos Pharmaceutical Co., Egypt).
- Indomethacin (Merk, Sharp and Dohme, USA).

Animals Used and Experimental Design

Eighty albino rats of local strains, of both sexes, weighing 150-200 g, were used for testing the anti-inflammatory activities of the tested drugs. Twenty-four mice of local strains, of both sexes, weighing 25-30 gm, were used for testing the analgesic activities of the tested drugs. The animals were maintained under standard conditions of humidity, temperature and light. All animals were conditioned in small experimental cages (8 rats or mice per cage). The animals were fed laboratory balanced diet, had free access to drinking tap water and were fasted over night before the experiments. Animals were assigned to control and test groups (8 animals for each group).

Pharmacological study

Acute inflammatory model. Rats were exposed to carrageenan-induced back-paw edema. Twenty-four rats were used in this test. They were subdivided into 3 equal groups, 8 rats for each group.

Group I-a: Control saline-treated group.
Group I-b: Treated group given aqueous extract of *Harpagophytum procumbens* in a single dose of 800 mg/kg, intra-peritoneally [9].
Group I-c: Reference group given indomethacin, as a reference anti-inflammatory drug, in a single dose of 10 mg/kg, intra-peritoneally [9].

Thirty minutes after drug administration, each animal received 0.05 ml 1% carrageenan suspension in normal saline (as an inflammatory agent) in its right

Table 1. Effect of carrageenan sub-plantar injection on rat back-paw thickness.

	Mean (mm) ± SEM rat back-paw thickness		
	Before Carrageenan injection	**After Carrageenan injection**	
G I-a	3.50 ± 0.19	4.62 ± 0.18	$p \leq 0.001$
G I-b	3.37 ± 0.18	4.62 ± 0.18**	$p_1 \leq 0.001$
G I-c	3.37 ± 0.16	4.62 ± 0.18**	$p_2 \leq 0.001$

Each group contains 8 rats, G I-a: Saline-treated control group, G I-b: Harpagophytum procumbens-treated group, G I-c: Indomethacin-treated group, p: Comparing rat back-paw thickness in GI-a before & after carrageenan injection, p_1: Comparing rat back-paw thickness in GI-b before & after carrageenan injection, p_2: Comparing rat back-paw thickness in GI-c before & after carrageenan injection, ** highly significant difference.

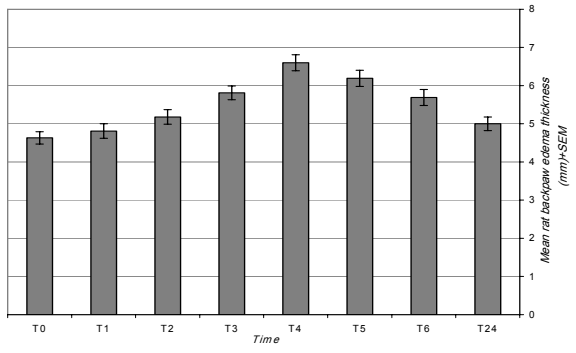

Fig 3. Effect of tested drugs on carrageenan-induced backpaw edema thickness in rats (Group I-c).

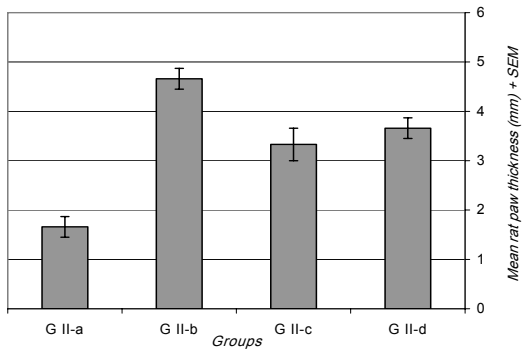

Fig 4. Effect of tested drugs on paw thichness in Freund's adjuvant-induced arthritis in rats.

back-paw through a sub-plantar injection (under planter aponeurosis) [10]. Right back-paw thickness for each rat was measured from ventral to dorsal surfaces with dial calipers, just before injection of carrageenan and 1, 2, 3, 4, 5, 6 and 24 hours after carrageenan injection.

Chronic inflammatory models. Animals were assigned into two groups:

(A) Group II; rats were exposed to adjuvant arthritis test. Thirty-two rats were used in this test. They were subdivided into 4 equal groups, 8 rats for each group.

Group II-a: Control non-arthritic saline-treated group.

Group II-b: Control arthritic saline-treated group, received saline for 2 weeks after development of arthritis.

Group II-c: Treated group given aqueous extract of *Harpagophytum procumbens* in a dose of 800 mg/kg, 3 times/week, every other day, for 2 weeks after development of arthritis [11].

Group II-d: Reference group given indomethacin, as a reference anti-inflammatory drug, in a dose of 2 mg/kg, intra-peritoneally, 3 times/week, every other day, for 2 weeks after development of arthritis [11].

Adjuvant arthritis was induced by single intradermal injection of 0.1 ml of Complete Freund's adjuvant into the base of each rat's tail. Systemic arthritis developed 14 days after the adjuvant injection [12-14].

(B) Group III; rats were exposed to cotton pellet-induced granuloma test. Twenty-four (24) rats were used in this test. They were subdivided into 3 equal groups, 8 rats for each group.

Group III-a: Control saline -treated group, received saline for 7 successive days.

Group III-b: Treated group given aqueous extract of *Harpagophytum procumbens* in a dose of 800 mg/kg, intra-peritoneally, once daily, for 7 successive days [11].

Group III-c: Reference group given indomethacin, as a reference anti-inflammatory drug, in a dose of 2 mg/kg, intra-peritoneally, once daily, for 7 successive days [11].

Animals were exposed to cotton pellet granuloma test using cotton pellets prepared from cotton wool. Each pellet weighed approximately 30 mg. They were sterilized by autoclaving for 30-45 minutes [15, 16]. Croton oil was added to each pellet under strict aseptic precautions [17]. Animals were anaesthetized lightly with ether. A toothed forceps grasped the loose skin in the groin region, on either side, and a small incision was made with a pair of sharp scissors. A track was made under the skin by a straight forceps and a pellet was pushed subcutaneously into the track. The incision was closed with one suture. It took 7 days for granuloma to form. Drugs were started on the day of implantation and continued for 7 days. On the eighth day, under ether anesthesia, the cotton pellets along with inflammatory tissue (the surrounding granuloma) were dissected out and cleaned of extraneous tissue. Each pellet was placed in a glass plate and dried in a hot air oven at 60^0C overnight, and in a desiccator to cool and weighed. The weight of the dried granuloma was calculated [15, 16].

Analgesic model. Mice were exposed to Writhing test. Twenty-four (24) mice were used in this test and subdivided into 3 equal groups, 8 mice for each group.

Group IV-a: Control saline-treated group.

Group IV-b: Treated group given aqueous extract of *Harpagophytum procumbens* in a single dose of 400 mg/kg, intra-peritoneally [9].

Group IV-c: Reference group given acetyl salicylic acid, a reference peripheral analgesic drug, in a single dose of 68 mg/kg, intra-peritoneally [9].

After drug administration, animals were isolated, each in an individual cage for 30 minutes before injec-

Table 2. Effect of tested drugs on carrageenan-induced back-paw edema in rats.

	Mean (mm) ± SEM back-paw edema thickness							
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_{24}
G I-a	4.62 ± 0.18	5 ± 0.23	5.69± 0.21	6.81 ± 0.19	7.67 ± 0.18	6.75 ± 0.16	6.25 ± 0.13	5.38 ± 0.18
G I-b	4.62 ± 0.18	4.88 ± 0.23	5.25 ± 0.23	$6.06 \pm 0.19^{*, b}$	$6.69 \pm 0.13^{**, b}$	$6.13 \pm 0.18^{*, b}$	$5.69 \pm 0.19^{*, b}$	5.1 ± 0.19
G I-c	4.62 ± 0.18	4.81 ± 0.21	5.18 ± 0.21	$5.81 \pm 0.2^{*, c}$	$6.60 \pm 0.18^{**, c}$	$6.19 \pm 0.19^{*, c}$	$5.69 \pm 0.19^{*, c}$	5 ± 6.16

Each group contains 8 rats, G I-a: Saline-treated control group, G I-b: Harpagophytum procumbens-treated group, G I-c: Indomethacin-treated group, T: Time after administration of carrageenan by 0,1,2,3,4,5,6 and 24 hours, [b]: Comparing G I-b to G I-a, [c]: Comparing G I-c to G I-a, ** highly significant difference, * significant difference.

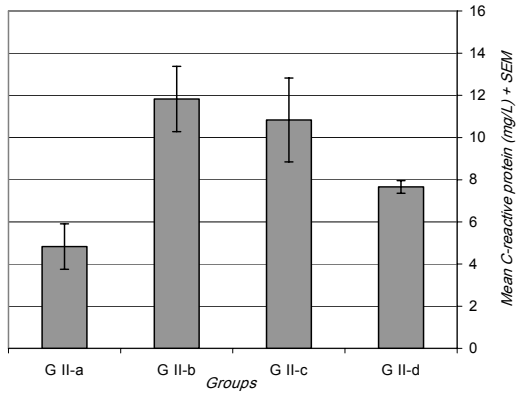

Fig 5. Effect of tested drugs on C-reactive protein in Freund's adjuvant-induced arthritis in rats.

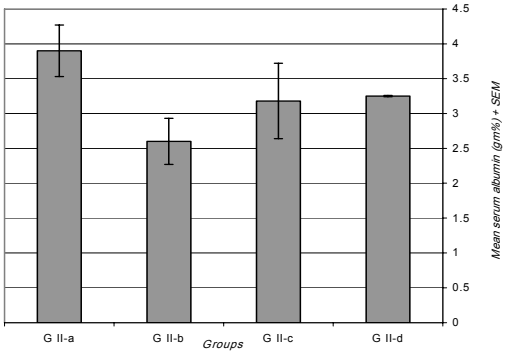

Fig 6. Effect of tested drugs on serum albumin in Freund's adjuvant-induced arthritis in rats.

tion of the writhing agent. The writhing agent was aqueous solution of 0.02% (0.02 mg/dl) P-benzoquinone (phenyl-isoquinone). Phenyl-isoquinone was gently heated and placed in a brown bottle that was kept stoppered during the course of the experiment to protect the solution from deterioration if left exposed to air and light. Each animal received 0.2 ml intra-peritoneally. Writhing response is characterized by abdominal torsion, drawing up of hind limbs to the abdominal wall, marked contractions of the abdominal muscles (guarding) and periodic arching of the back [9, 18]. Number of writhings and stretchings were observed and recorded after injection of the writhing agent for 30 minutes [19].

Biochemical study

This study was applied on group II. Blood samples were collected from retro-orbital venous plexus of rats, after 12 hours fasting, using a fine heparinized capillary tube introduced into the medial epicanthus of the rat's eye. Two millimeters of blood were collected in a clear graduated centrifugation tube, left to clot at room temperature in a water bath for 15 minutes, and then centrifuged at 3000 rpm (rotation per minute). The supernatant serum was collected in a dry clean tube [21]. Samples were sent to a private diagnostic laboratory for measuring acute phase proteins; C-reactive protein [21] and serum albumin [22] and serum cortisol [23].

Histopathological study

This study was applied on group II. Animals were killed by overdose of ether the day after the end of the experiment and stomach as well as duodenum was histopathologically examined for evaluation of the tested drugs on gastric and duodenal mucosa.

Statistical analysis of data

The results were expressed as mean ± SE for each parameter investigated, tabulated and statistically analyzed on an IBM personal computer with SPSS software for windows version 10. The statistical analysis of variance was done using the unpaired `t` test according to Gobel et al.,2000 [24]. Results were considered significant when $p \leq 0.05$ and highly significant when $p \leq 0.001$ all through the study.

RESULTS

Pharmacological Study

Acute inflammatory study

Effect of carrageenan sub-plantar injection on rat back-paw thickness. A single sub-plantar injection of 0.05 ml 1% carrageenan suspension in normal saline (as an inflammatory agent), in right back-paw of each rat, induced a highly significant increase in back-paw thickness from 3.5 mm ± 0.19 to 4.62 mm ± 0.18 in group I-a , from 3.37 mm ± 0.18 to 4.62 mm ± 0.18 in group I-b and from 3.37 mm ± 0.16 to 4.62 mm ± 0.18 in group I-c ($p \leq 0.001$ for all) Table 1.

A single sub-plantar injection of carrageenan in right back-paw of saline-treated control rats (group I-a) induced a progressive increase in back-paw thickness over the first 24 hours later carrageenan injection with maximal increase at the 4th hour (7.67 mm ± 0.18) . A gradual decrease in back-paw thickness reached 5.38 mm ± 0.18 at the 24th hour. Table 2 and Fig 1.

Effect of Harpagophytum procumbens on carrageenan-induced rat back-paw edema. In group I-b, a single intra-peritoneal injection of an aqueous extract of *Harpagophytum procumbens*, in a dose of 800 mg/kg, 30 minutes before carrageenan administration, progressively reduced the rats right back-paw thickness with

Table 3. Effect of tested drugs on Freund's adjuvant-induced arthritis in rats.

Mean ± SEM	G II-a	G II-b	G II-c	GII-d
Average paw thickness (mm)	1.66 ± 0.21	4.66 ± 0.33$^{**, b}$	3.33 ± 0.21$^{*, c}$	3.66 ± 0.21$^{*, d}$
C-reactive protein (mg/L)	4.83 ± 0.30	11.83 ± 1.99$^{*, b}$	10.83 ± 1.55	7.66 ± 1.08
Serum albumin (gm %)	3.90± 0.01	2.60 ± 0.54$^{**, b}$	3.18 ± 0.33	3.25 ± 0.37
Serum cortisol (ng/ml)	4.54 ± 0.08	3.45 ± 0.24$^{*, b}$	3.20 ± 0.23	3.30 ± 0.29

Each group contains 8 rats, II-a: Non-arthritic saline-treated group, G II-b: Arthritic saline-treated group, G II-c: Arthritic harpagophytum procumbens-treated group II-d: Arthritic indomethacin-treated group, ^{b}: Comparing G II-b with G II-a, ^{c}: Comparing G II-c with G II-b, ^{d}: Comparing G II-d with G II-b, ** highly significant difference, * significant difference.

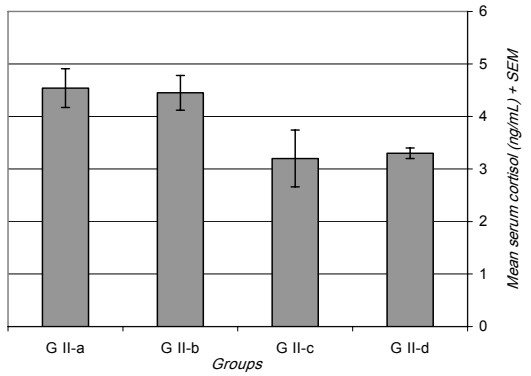

Fig 7. Effect of tested drugs on serum cortisol in Freund's adjuvant-induced arthritis in rats.

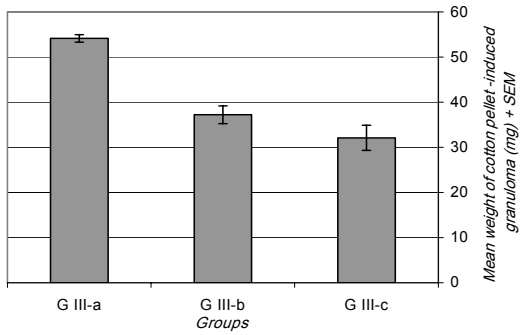

Fig 8. Effect of tested drugs on cotton pellet-induced granuloma weight in rats.

high significant reduction at the 4th hour from 7.67 mm ± 0.18 to 6.69 mm ± 0.13 ($p \leq 0.001$). Table 2 and Fig 2.

Effect of indomethacin on carrageenan-induced rat back-paw edema. In group I-c, a single intra-peritoneal injection of indomethacin, in a dose of 10 mg/kg, 30 minutes before carrageenan administration, progressively reduced the rats right back-paw thickness with a highly significant reduction at the 4th hour from 7.67 mm ± 0.18 to 6.6 mm ± 0.18 ($p_1 \leq 0.001$). Table 2 and Fig 3.

Chronic inflammatory study

Effect of Freund`s adjuvant-induced arthritis in rats. In group II-b, the arthritic saline-treated rats injected with 0.1 ml complete Freund's adjuvant intra-dermally into the base of each rat's tail developed systemic polyarthritis within 14 days manifested by redness and swelling of joints assessed by a high significant increase in average paw thickness from 1.66 mm ± 0.21 to 4.66 mm ± 0.33 when compared to group II-a (non-arthritic saline-treated control group) ($p \leq 0.001$). Table 3 and Fig 4.

Effect of Harpagophytum procumbens on Freund`s adjuvant- induced arthritis in rats. In group II-c, an intra-peritoneal administration of an aqueous extract of *Harpagophytum procumbens*, at 800 mg/kg, 3 times/week (every other day), for 2 weeks after development of arthritis, produced a significant decease in average paw thickness from 4.66 mm ± 0.33 to 3.33 mm ± 0.21 when compared to group II- b ($p_1 \leq 0.05$). **Table 3** and Fig 4.

Effect of indomethacin on Freund`s adjuvant-induced arthritis in rats. In group II-d, an intra-peritoneal administration of 2 mg/k indomethacin g, 3 times/week (every other day), for 2 weeks after development of arthritis, produced a significant decrease in paw thickness from 4.66 mm ± 0.33 to 3.66 mm ± 0.21 when compared to group II-b ($p_2 \leq 0.05$). **Table 3** and Fig 4.

Cotton pellet implantation-induced granuloma in rats. In group III-a, sub-cutaneous implantation of a sterile cotton pellet weighing 30 mg (the initial weight in all groups) for 7 days produced a granulomatous mass weighing 54.16 mg. **Table 4** and Fig 8.

Effect of Harpagophytum procumbens on cotton pellet granuloma weight in rats. In group III-b, intra-peritoneal administration of aqueous extract of *Harpagophytum procumbens* at 800 mg/kg, once daily, for 7 successive days, produced a highly significant decrease in the granuloma weights from 54.16 mg ± 2.78 to 37.23 mg ± 1.96 when compared to group III-a (saline-treated control group) ($p_1 \leq 0.001$). **Table 4** and Fig 8.

Effect of indomethacin on cotton pellet granuloma weight in rats. In group III-b, intra-peritoneal administration of indomethacin in a dose of 2 mg/kg, once daily, for 7 successive days, produced a highly significant decrease in granuloma weights from 45.16 mg ± 2.78 to 32.11 mg ± 0.83 when compared to group III-a ($p2 \leq 0.001$). **Table 4** and Fig 8.

Analgesic study

Effect of injection of a writhing agent in mice. A single intra-peritoneal administration of 0.2 ml of an aqueous solution of 0.02% (0.02 mg/dl) P-benzoquinone (phenyl-isoquinone), as a writhing agent, at 0.2 ml produced 38.13 ± 0.71 writhing reactions. **Table 5** and Fig 9.

Effect of Harpagophytum procumbens on writhing reactions in mice. In group IV-b a single intra-peritoneal administration of an aqueous extract of *Harpagophytum procumbens* at 400 mg/kg, produced a high significant reduction in the number of writhing reactions from 38.13 ± 0.71 to 29.25 ± 0.61 when compared to group IV-a (saline-treated group) ($p_1 \leq 0.001$). **Table 5** and Fig 9.

Effect of acetyl salicylic acid on writhing reactions

Table 4. Effect of tested drugs on cotton pellet granuloma weight in rats.

	Weight of cotton pellet granuloma (Mean [mg] ± SEM)	
G III-a	54.16 ± 2.78	
G III-b	37.23 ± 1.96**	$p_1 \leq 0.001$
G III-c	32.11 ± 0.83**	$p_2 \leq 0.001$

Each group contains of 8 rats, G III-a: Saline-treated control group, G III-b: Harpagophytum procumbens-treated group, G III-c: Indomethacin-treated group, p_1: Comparing G III-b with G III–a, p_2: Comparing G III-c with G III–a, ** highly significant difference.

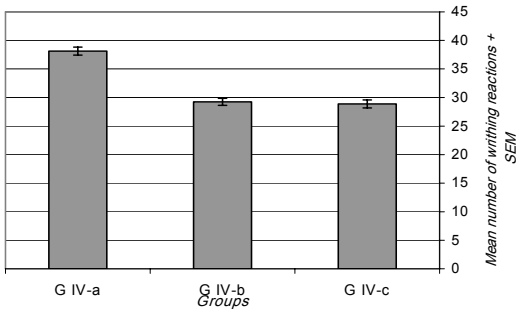

Fig 9. Effect of tested drugs on writhing reactions in mice.

in mice. In group IV-c, a single intra-peritoneal administration of 68 mg/kg acetyl salicylic acid, induced a high significant reduction in the number of writhing reactions from 38.13 ± 0.71 to28.88 ± 0.71 when compared to group IV-a ($p2 \leq 0.001$). **Table 5** and Fig 9.

Biochemical Study

*Effect of Freund`s adjuvant-induced arthritis in rats.* This study was applied only on rats exposed to Freund`s adjuvant-induced arthritis (group II). Group II-b injected with 0.1 ml complete Freund's adjuvant intradermally into the base of each rat's tail developed systemic polyarthritis within 14 days manifested by a significant increase in C-reactive protein from 4.83 mg/L ± 0.3 to 11.83 mg/L ± 1.99 when compared to group II-a (non-arthritic saline-treated control group) ($p \leq 0.05$). Also it caused a highly significant decrease in serum albumin from 3.9 gm% ± 0.01 to 2.6 gm% ± 0.54 ($p \leq 0.001$) and significant decrease in serum cortisol from 4.54 ng/ml ± 0.08 to 3.45 ng/ml ± 0.24 ($p \leq 0.05$) when compared to group II-a. **Table 3** and Fig 5-Fig **7**.

Effect of Harpagophytum procumbens on Freund`s adjuvant-induced arthritis in rats. In group II-c, an intra-peritoneal administration of an aqueous extract of *Harpagophytum procumbens* at 800 mg/kg, 3 times/week (every other day), for 2 weeks after development of arthritis, produced insignificant reduction in C-reactive protein from 11.83 mg/L ± 1.99 to 10.83 mg/L ± 1.55 and serum cortisol from 3.45 ng/ml ± 0.24 to 3.2 ng/ml ± 0.23 when compared to group II-b while produced insignificant elevation in serum albumin when compared to group II-b from 2.6 gm% ± 0.54 to 3.18 gm% ± 0.33. **Table 3** and Fig 5-Fig **7**.

Effect of indomethacin on Freund`s adjuvant-induced arthritis in rats. In group II-c, an intra-peritoneal administration of indomethacin at 2 mg/kg, 3 times/week (every other day), for 2 weeks after development of arthritis, produced insignificant reduction in C-reactive protein from 11.83 mg/L ± 1.99 to 7.66 mg/L ± 1.08 and serum cortisol from 3.45 ng/ml ± 0.24 to 3.3 ng/ml ± 0.29 when compared to group II-b while produced insignificant elevation in serum albumin from 2.6 gm% ± 0.54 to 3.25 gm% ± 0.37. **Table 3** and Fig 5-Fig **7**.

Histopathological study

This study took place on rats exposed to Freund`s adjuvant-induced arthritis (group II) to evaluate the safety of the tested drugs on gastric and duodenal mucosa.

Non-arthritic saline-treated group (group IV-a). Microscopic view of rat's gastric mucosa showed that the gastric glands are intact as well as the surface epithelium covering (Fig 10-a). In addition, Microscopic view showed normal intact mucosal lining of the duodenum (Fig 10-b).

Arthritic saline-treated group (group IV-b). Microscopic view of rat's gastric mucosa; showing intact lining and normal appearing gastric glands (Fig 10-c). In addition, rat's duodenal mucosa showed the mucosal villi and crypts lined by intact columnar epithelial cells (Fig 10-d).

Arthritic Harpagophytum procumbens-treated group (group IV-c). Microscopic view showing superficial gastritis manifested in the form of distorted mucosal surface, with proteinaceous covering (protein scab). The glands lumens on the superficial zone are distorted (Fig 10-e). In addition, microscopic view showed superficial duodenitis manifested by loss of the surface epithelium of some villi and ulceration (thick arrow) with inflammatory cells within the cores of duodenal villi (thin arrow) (Fig 10-f).

Arthritic indomethacin-treated group (group IV-d). Microscopic view showing extensive superficial gastritis in the form of loss of the surface epithelium (ulceration) with extensive inflammatory cellular infiltrate in the ulcer base (thick arrow) and surrounding the remaining gastric glands (thin arrow) (Fig 10-g). In addition, microscopic view showed extensive superficial duodenitis manifested by extensively denuded surface epithelium & covered by proteinaceous material. The submucosa showed inflammatory infiltrate (Fig 10-h).

DISCUSSION

None of NSAIDs is ideal in controlling the signs and symptoms of inflammation, particularly in the common inflammatory joint diseases [1]. Many studies confirmed that use of devil's claw causes improvement of low back pain [6, 25] and osteoarthritis [8, 26]. Different investigations evaluated the anti-inflammatory and analgesic effects of *Harpagophytum procumbens* in typical experimental models such as the writhing test in mice versus acetyl salicylic acid, and carrageenan-induced edema and adjuvant-induced arthritis in rats

Table 5. Effect of tested drugs on writhing reaction in mice.

	Writhing reactions (Mean number ±SEM)	
G IV-a	38.13 ± 0.71	
G IV-b	29.25 ± 0.61**	$p_1 \leq 0.001$
G IV-c	28.88 ± 0.71**	$p_2 \leq 0.001$

Each group contains of 8 mice, G IV-a: Saline-treated group, G IV-b*:* Harpagophytum procumbens-treated group, G IV-c*:* Acetyl salicylic acid-treated group, p_1: Comparing G IV-b to G IV-a, p_2: Comparing G IV-c to G IV-a, ** highly significant difference.

versus indomethacin with inconsistent and contradictory results [27].

Carrageenan had been found to give results that are more consistent and is widely used as a standard edema-

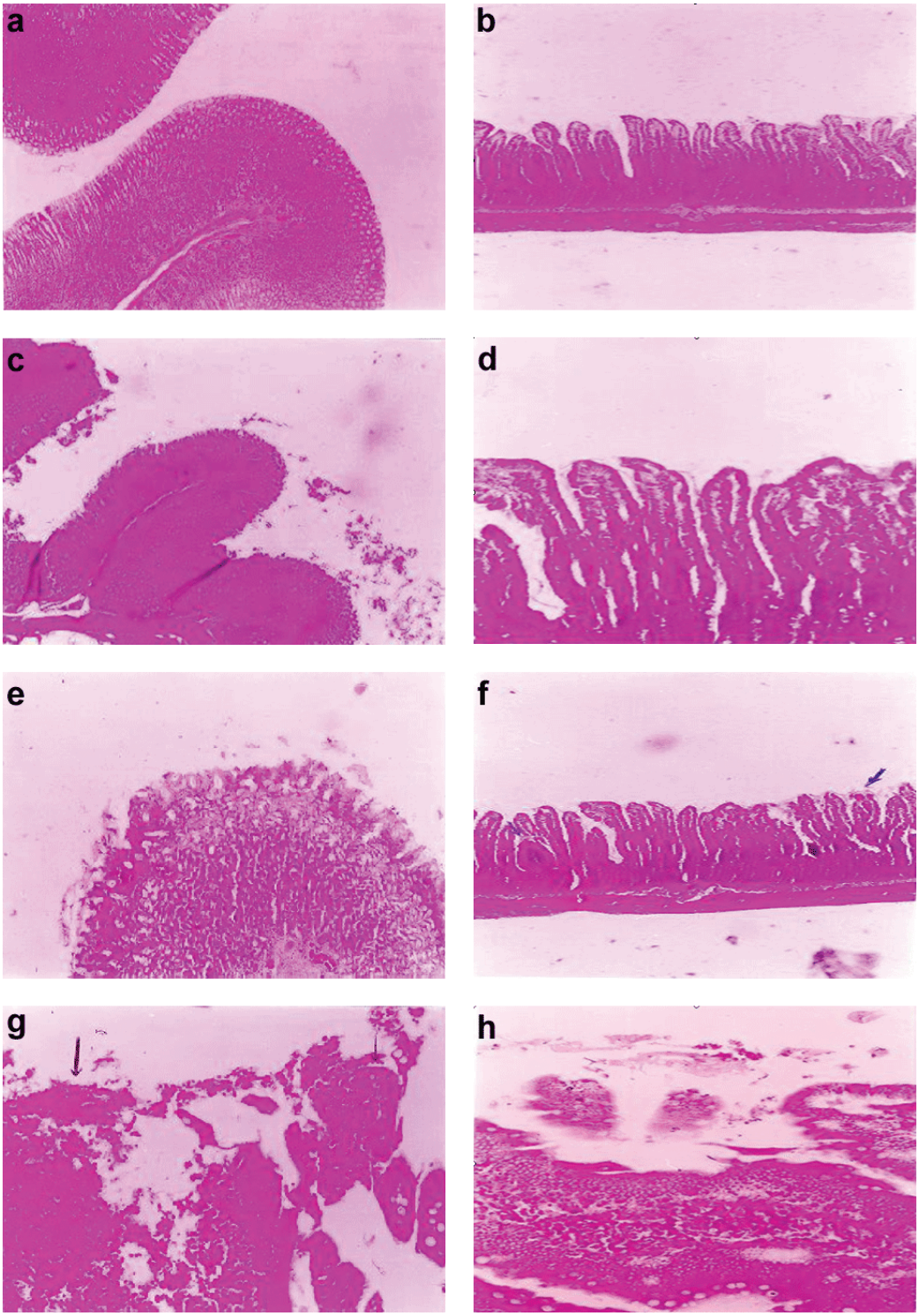

Fig 10. (**a**) Saline-treated non-arthritic group: Microscopic view of normal gastric mucosa; the gastric glands are intact as well as the surface epithelium covering. (Hx. & E., 200×). (**b**) Saline-treated non-arthritic group: Microscopic view; normal intact mucosal lining of the duodenum. (Hx. & E., 40×). (**c**) Saline-treated arthritic group: Microscopic view of normal gastric mucosa; showing intact lining and normal appearing gastric glands. (Hx. & E., 100×) (**d**) Saline-treated arthritic group: Microscopic view of normal duodenal mucosa; showing mucosal villi and crypts, lined by intact columnar epithelial cells. (Hx. & E., 100×). (**e**) Harpagophytum procumbens-treated arthritic group: Microscopic view of superficial gastritis; the mucosal surface is distorted, with proteinaceous covering (protein scab). The glands lumins on the superficial zone are distorted. (Hx. & E., 40×). (**f**) Harpagophytum procumbens-treated arthritic group: Microscopic view of superficial duodenitis, the surface epithelium of some villi is lost; ulceration (thick arrow) with inflamma-tory cells within the cores of duodenal villi (thin arrow). (Hx. & E., 40×). (**g**) Indomethacin-treated arthritic group: Microscopic view of superficial gastric ulceration; the surface epithlium is lost with extensive inflammatory cellular infiltrate in the ulcer base (thick arrow) and surrounding the remaining gastric glands (thin arrow). (Hx. & E., 400×). (**h**) Indomethacin-treated arthritic group: Microscopic view of superficial duodenitis; the surface epithelium is extensively denuded & covered by proteinaceous material. The submucosa showed inflammatory infiltrate. (Hx. & E., 400×).

inducing agent [10]. In the present work, it can be observed that carrageenan sub-plantar injection in right back-paw in rats induced a highly significant increase in paw thickness. *Harpagophytum procumbens* pretreatment induced highly significant reduction in right back-paw thickness, the same effects as indomethacin, and this is parallel with the results by Connolly et al., 1987, Soulimani et al., 1994, Baghdikian et al. 1997 and Schulz et al., 1998 [10, 28-30].

In conformity with the immunological concept of various inflammatory diseases, the need arose for an appropriate design of experimental model of immunological inflammation. This need has been satisfied largely by the development of adjuvant arthritis, largely simulating chronic inflammatory arthritic condition in humans. Adjuvant disease was originally based on the observation of Eichler et al., 1970 [31] and subsequently developed and extended by numerous investigations. This model closely resembles clinical arthritis. It is the most widely used model of experimental arthritis which has been used for screening purposes in the disease produced in the rat by injection of complete Freund`s adjuvant into certain dermal and tissue sites [15].

It can be observed that Freund`s adjuvant-induced arthritis in rats induced highly significant increase in paw thickness of rats, significant decrease in serum cortisol, highly significant decrease in serum albumin and significant increase in C- reactive protein. Similar results have been reported by Farr et al., 1976 [32] and in patients with rheumatoid arthritis by Amos et al., 1977 and Grahame et al., 1981 [33, 34]. *Harpagophytum procumbens* and indomethacin administration in the present study did not affect these parameters but only caused significant reduction of paw thickness. Pearson 1956 [35] Obtained similar findings, who reported that agents like non-steroidal anti-inflammatory agents and glucocorticoids, which provide symptomatic relief of arthritis without altering progression of the disease, did not affect these parameters. But contradictory results were reported in Freund`s adjuvant-induced arthritic rats by Sticher et al., 1985 [36].

In addition, *Harpagophytum procumbens* and indomethacin intra-peritoneal administration in cotton pellet-induced granuloma test in rats caused a reduction of inflammation manifested by high significant decrease of cotton pellet weight. This was reported also by Sigmund 1957 [17].

Assessment of analgesic activity in laboratory animals is difficult but writhing test is considered the simplest chemical method [28]. In the analgesic experimental model, writhing test, the present study revealed that *Harpagophytum procumbens* and acetyl salicylic acid had an analgesic effect and this result was consistent with the results of Sigmund 1957, Soulimani et al., 1994 and ESCOP 1997 [17, 28, 37] who reported that *Harpagophytum procumbens* has analgesic effect.

Results recorded from all the experiments done in the present study revealed that the aqueous extract of *Harpagophytum procumbens* (devil's claw) has an anti-inflammatory effect on experimental inflammatory models, whether acute (carrageenan-induced rat back-paw edema) or chronic (cotton pellet-induced granuloma and Freund`s adjuvant-induced arthritis). In addition, it has analgesic effect on pain induced in mice (writhing test). These results are in agreement with the results of Connolly et al., 1987, Soulimani et al., 1994 , ESCOP 1996, Leung & Foster 1996 and ESCOP 1997 [28, 30, 37-39].

A German study found *Harpagophytum procumbens* (devil's claw) root's effects to be equal to the anti-arthritic drug phenylbutazone [40]. In addition, intra-peritoneal administration of *Harpagophytum procumbens* (devil's claw) root extract 100 mg/kg was equal to 2-5 mg/kg indomethacin in anti-inflammatory effects [39].

In a double blind, randomized, multicentre clinical study, the efficacy and tolerance of a herbal medicine product Harpadol (6 capsules/day, each containing 435 mg of powdered cryoground powder *Harpagophytum procumbens*), was used in the treatment, for 4 months, of 122 patients suffering from osteoarthritis of the knee and hip. Spontaneous pain showed a significant improvement during the course of the study. At completion of the study, patients taking Harpadol were using significantly less NSAID and analgesic drugs. The tolerance assessment by patients at the end of treatment favored Harpadol [8].

Both in-vitro and in-vivo tests had resulted in conflicting evidence about *Harpagophytum procumbens*'s therapeutic activity. Pain-reducing and anti-inflammatory properties were observed in guinea pigs, particularly in chronic pain conditions, but when devil's claw root's effects on rats were compared to those of indomethacin and aspirin, significant efficacy was not found [8, 36, 41].

In-vivo experiments with *Harpagophytum procumbens* (devil's claw) had determined that the anti-inflammatory properties differ by dosage method. Intra-peritoneal and intra-duodenal administration appears to be efficient on acute and chronic processes [28, 37]. Oral administration had no effect, regardless of the dose used [11, 16, 36, 37, 42, 43].

Intra-peritoneal pretreatment with an aqueous extract of devil's claw significantly reduced the carrageenan-induced edema. After oral administration, the extracts were inefficient. This result could be attributed to the time in transition in the stomach where the pH is acidic, causing a decrease of the activity of the extract. There was absence of extract activity when it was treated in an environment of pH 1 and 37^{o}C, similar to the physico-chemical conditions found in the stomach. Intra-duodenal pretreatment with the aqueous extract significantly reduced the carrageenan-induced edema. The presence of extract activity after intra-duodenal administration supports the assumption that transition of the extract through the stomach leads to loss of activity [28, 29].

The absence of activity of devil's claw after an acid treatment (0.1 N hydrochloric acid), suggests the use of a suitable galenic preparation in order to protect the active principles from the action of the acid released in

the stomach [28].

The novelty of the present study was histopathological examination of the effect of devil's claw on gastric and duodenal mucosa. The results of this study revealed the greater safety of this product on GIT mucosa in comparison to the more injurious effect of indomethacin, a NSAID. The results favored the opinion suggesting that devil's claw caused lesser interfering, in one way or another, with arachidonic acid metabolism and eicosanoid production than NSAID agents, for the important role of prostaglandins in mucosal protection and preventing development of peptic ulcer and gastritis. So, we can suggest that it is one of the agents which tend to inhibit COX-2 more than COX-1. Whether the extract influenced leukotriene biosynthesis, cyclo-oxygenase pathways, or both, is still open to discussion. Drugs were administrated intra-peritoneally not orally. Therefore, the gastric & duodenal mucosal damaging effect is due to a systemic, not a local irritant effect.

Concerning the mechanism of action of *Harpagophytum procumbens*, [43, 44] adopted the opinion suggesting that devil's claw lacks any inhibitory effect on arachidonic acid metabolism pathways. On the other hand, Lanhers et al., 1992, Tippler et al., 1996 & 1997 and Fiebich et al., 2001 [4, 27, 45-47] suggested that this herb interferes with arachidonic acid metabolism and affects eicosanoid production.

The ulcerogenic effects of NSAIDs result - in part - from an increase in gastric acidity [50]. The variation in GIT side effect profiles of NSAIDs may be a result of the COX selectivity of individual drugs [51]. The very limited risk of *Harpagophytum procumbens*'s gastrointestinal side effects may be attributed to the widely classified mechanisms of action of it in comparison to NSAIDs, which only inhibited the cyclo-oxygenase. So far, it seems not suitable that patients with gastrointestinal ulcers are excluded from the treatment with *Harpagophytum procumbens* (devil's claw). Moreover, this substance represents, due to its favorable risk benefit, a safe alternative in the treatment of rheumatic pain [48]. On the other hand, some authors contraindicate the use of *Harpagophytum procumbens*'s (devil's claw) in patients with gastric and duodenal ulcers [40, 49, 52, 53].

CONCLUSION

It can be concluded that *Harpagophytum procumbens* is a new herbal agent having reasonable anti-inflammatory effect compared to indomethacin, as a standard anti-inflammatory agent and reasonable analgesic effect compared to acetyl salicylic acid, as a standard analgesic agent without harmful effect on gastric and duodenal mucosa. The findings of the present work justified the use of this plant in the treatment of rheumatism and other inflammatory conditions for its anti-inflammatory and analgesic effects.

RECOMMENDATION

At this stage, it is not possible to pin point the exact phytoconstituent(s) responsible for the anti-inflammatory and analgesic activities. Future studies must isolate the active principle(s) and to determine its mechanism of action. In addition, its safety on liver and kidney as well as in-vivo toxicity testing is needed before it can be used in human.

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