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Anti-inflammatory activity of *Euphorbia aegyptiaca* extract in rats

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Abstract

Background: There were no studies on the anti-inflammatory activity of *Euphorbia aegyptiaca*, though it is commonly used by Sudanese herbalists in the treatment of rheumatoid arthritis.

Objectives:
1. To determine phytochemical constituents of *Euphorbia aegyptiaca*
2. To investigate the anti-inflammatory activity of *Euphorbia aegyptiaca* in rats.

Methodology: Plant material was extracted by ethanol and phytochemical screening was done according to standard methods. The thickness of Albino rats' paws were measured before injection of 0.1 ml of 1% formalin in the sub planter region and then, 1, 2, 3, 4 and 24 hours after oral dose of ethanolic extract of *Euphorbia aegyptiaca* at a rate of 400mg/kg, 800mg/kg, indomethacin (5mg/kg) and normal saline (5ml/kg). Edema inhibition percentage (EI%) and mean paw thickness (MPT) were measured in the different groups and compared using appropriate statistical methods.

Results: The phytochemical screening revealed the presence of saponins, cumarins, flavonoids, tannins, sterols, triterpenes, and absence of alkaloids, anthraquinones glycosides and cyanogenic glycosides. The mean of EI% of rats treated with indomethacin at a dose of 5 mg/kg over different time intervals (64.0%) was significantly lower compared to those treated with *Euphorbia aegyptiaca* at a dose of 800 mg/kg (75.0%, $P< 0.001$), but higher compared to rats treated at higher dose of 400 mg/kg (57.4%, $P< 0.001$). In contrast, MPT of rats treated with indomethacin at a dose of 5 mg/kg (6.5±1.1 mm) was significantly higher compared to those treated with *Euphorbia aegyptiaca* at a dose of 800 mg/kg (6.1±.7 mm, $P< 0.001$) as well as 400 mg/kg (5.9±.5, $P< 0.001$).

Conclusion: *Euphorbia aegyptiaca* ethanolic extract has a sustained dose-dependent anti-inflammatory activity.

Keywords: anti-inflammatory, edema, *Euphorbia aegyptiaca*, indomethacin.

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Introduction

Euphorbia aegyptiaca belongs to the family: Euphoriaceae. Arabic synonyms are Um Malbeina, Um Lebein and Um Leban. In Sudan, *Euphorbia aegyptiaca* is distributed in the central region especially along the Nile banks in the Gezira and Rahad. Because of the medicinal interest in Euphoriaceae, these herbs have been examined by several investigators. [1, 2] *Euphorbia* species contain several important phytochemical constituents like triterpenoids, flavonoids, lignans, coumarins, and alkaloids, [3, 4] which reflect their therapeutic potentials. Recent studies demonstrated anthelmintic and antimicrobial activity of *Euphorbia helioscopia* extracts, (5) anti-retroviral effect of ingenol derived from *Euphorbia tirucalli* [2] and anti-hepatitis B virus of flavone glucosides isolated from *Euphorbia humifusa.* [6] Anticancer activities of *Euphorbia tirucalli* were demonstrated in a wide variety of cancers including breast [7] and gastric [8] carcinomas. *Euphorbia hirta* attracted the attention of many investigators because of its unique chemical structure, antioxidant contents, potent anti-inflammatory as well as anticancer activities. [9]

Interestingly most of *Euphorbia* species were proved to have anti-inflammatory effects including *Euphorbia hirta,* [1, 10] *Euphorbia fischeriana,* [11] *Euphorbia lacteal* [12] and *Euphorbia neriifolia.* [13] In Sudan, *Euphorbia aegyptiaca* is used by traditional herbalists for the treatment of some inflammatory conditions like rheumatoid arthritis, dermatitis and conjunctivitis; however, phytochemical screening and medicinal effect of this herb, to the best of our knowledge, was not investigated before. The aim of the present study is to determine the phytochemical constituents and the anti-inflammatory activity of *Euphorbia aegyptiaca* in rats.

Experimental

Extraction of Plant Materials

The herb was gathered from Kassala province - Eastern Sudan. The plant was authenticated by a taxonomist of MAPRI; a sample was kept at the herbarium of the MAPRI under the code [MAPRI/H/94]. After drying whole plant material at room temperature for three days, the herb was coarsely powdered then extracted using the soxhlet apparatus. The extraction was processed by petroleum ether, chloroform and 70% ethanol to extract the fatty constituents, non-polar and polar compounds respectively. [14, 15] The extract was dried under low pressure and stored into a refrigerator at – 4 ºC until being used.

Phytochemical screening

Sterols and triterpenes

One ml chloroform was added to the extract, then 0.5 ml of acetic acid anhydride and 2 drops of concentrated H_2SO_4. Slow appearance of green to blue color suggested presence of sterols. [14] Alternatively, pink to purple color indicted presence of triterpenes. [15]

Alkaloids

To 0.5 g of the dry extract, 5 ml 2 N hydrochloric acid were added, and stirred while heating in a water bath for 10 minutes, cooled, filtered and separated into 2 test tubes. Few drops of Mayer's and Velser's reagents were added to the two test tubes respectively. A slight turbidity or heavy precipitate in either tube was taken as presumptive evidence for the presence of alkaloids. [14, 15]

Flavonoids

To 0.5 g of the ethanolic extract, one ml of ethanol and one ml of 1%KOH were added. Appearance of dark yellow color was taken as an evidence of the presence flavonoids. [14, 15] For confirmation, appearance of yellow color following addition of one ml of aluminum chloride was ensured. [14, 15]

Saponin

One ml of distilled water was added to the extract in a test tube and shaked. Formation of foam was taken as an evidence of presence of saponin. [14, 15]

Cumarins

Half gram of the dry extract was boiled in 20 ml of distilled water in a test tube. A filter paper was attached to the test tube to be saturated with the vapor, after which a spot of 0.5 N KOH was put on it. Then the filter paper was inspected under UV-light, the presence of cumarins was indicated if the spot adsorbed the UV light. [14, 15]

Anthraquinon

Anthraquinon was detected by boiling 0.5 g of the extract in 10 ml of 0.5 N KOH containing one ml of 3% H_2O_2 solution. Five ml of

benzene was shaken with the mixture and then allowed to form two layers, then 3 ml of 10% $Al(OH)_3$ solution were added. The presence of anthraquinones was indicated if the alkaline layer was changed to pink or red color. [14, 15]

Tannins

To one gram of the extract, 10 ml of hot normal saline were added; allowed to cool then gelatin salt reagent was added to 5 ml of the mixture. Immediate precipitation was considered as an evidence for the presence of tannins. For further confirmation, $FeCl_3$ test reagent was added to the other 5 ml of the mixture. Blue, black or green colors were considered positive for the presence of tannins. [14, 15]

Induced paw edema model

An ethical clearance for this study was received from the College of Veterinary Medicine Research Board - Sudan University of science and technology – Khartoum - Sudan. Twenty rats of Westar albino (weigh 130-200 gram) were divided randomly into four equal groups (N = 5). The rats were housed in colony cages at an ambient temperature of 25±2ºC, 12/12 hours light/dark cycle with free access to food and water. The rats of group I were treated with oral normal saline at a dose of 5 ml/kg and served as a control group. Group II was orally dosed with indomethacin at a dose of 5 mg/kg. Groups III and IV were orally dosed with ethanolic extracts of *Euphorbia aegyptiaca* at dose rates of 400 mg/kg and 800 mg/kg respectively.

Each rat in the different experimental groups was injected subcutaneously with 0.1 ml of 1% formalin in the plantar region of the right hind paw. Paw's thickness of each rat was determined before the formalin injection, and then 1, 2, 3, 4 and 24 hours post-treatment. Edema formation (EF%) and edema inhibition (EI%) were calculated using the formulae:

- Edema formation percentage (EF %) = (Tt-To)/To × 100.
- Edema inhibition percentage (EI %) = (EFc -EFt)/EFt × 100.

Where

- To = the paw thickness before formalin injection (mm).
- Tt = the paw thickness after t hours of formalin (mm).
- EFc = edema formation rate of the control group.
- EFt = edema formation rate of the treated group at t hours.

Using SPSS program (version 18), EI% and MPT were compared in different study groups using analysis of variance and Dennett's tests. [14, 15]

Results

The phytochemical screening of the plant revealed the presence of saponins, cumarins, flavonoids, tannins, sterols, triterpenes, and absence of alkaloids, anthraquinones glycosides, and cyanogenic glycosides (Table1).

The highest EI%s were achieved in all groups four hours post oral administration of indomethacin or *Euphorbia aegyptiaca* extracts. The mean of EI% of rats treated with indomethacin at a dose of 5 mg/kg over different time intervals (64.0%) was significantly lower compared to those treated with *Euphorbia aegyptiaca* at a dose of 800 mg/kg (75.0%, $P < 0.001$), but higher when compared to rats treated at a dose of 400 mg/kg (57.4%, $P < 0.001$) (table 2). In contrast, MPT of rats treated with indomethacin at a dose of 5 mg/kg (6.5±1.1 mm) was significantly higher compared to those treated with *Euphorbia aegyptiaca* at a dose of 800 mg/kg (6.1±.7 mm, $P< 0.001$) as well as 400 mg/kg (5.9±.5, $P< 0.001$) (figure 1 and table 1).

Table 1. Phytochemical screening results of *Euphorbia aegyptiaca*

Phytoconistituent	Result
Saponins	+
Cumarins	+
Flavonoids	+++
Taninns	+++
Sterols	++
Triterpenes	+++
Alkaloids	_
Anthraquinon glycosides	_
Cyanogenic glycosides	_

Table 2. Effects of ethanolic extracts of *Euphorbia aegyptiaca* and indomethacin on rat's EI% and MPT at different time intervals

Extract/Drug	Parameter	Time interval					Mean
		1 hour	2hours	3hours	4hours	24hours	
Euphorbia aegyptiaca 400 mg/kg	EI%	62.8	53.3	45.9	90.4*	63.5	57.4
	MPT (mm)	6. 6±0.3	6.2±0.2	6.0±0.5	5.9±0 .3	5.7±0.2	5.9±0.5
Euphorbia aegyptiaca 800 mg/kg	EI%	62	65.2	69.7	97.1*	79.8	75.0
	MPT (mm)	6.9±0.2	6.6±0.6	6.4±.2	5.4±0.2	5.8±0.3	6.1±0.7
Indomethacin 5 mg/kg	EI%	24.2	32.2	50.9	97.0*	71.4	64.0
	MPT (mm)	8.3±0.6	7.6±0.4	6. 7±0.5	5.6±0.3	6.0±0.2	6.5±1.1
Normal saline	MPT (mm)	9.0±0.7	8. 5±0.4	7.7±1.4	8.2±0.4	7.2±0.2	7.7±1.2

*The highest edema-inhibition percent, EI% = edema Inhibition percentage, MPT = mean paw thickness in mm. All values are means of 5 observations.

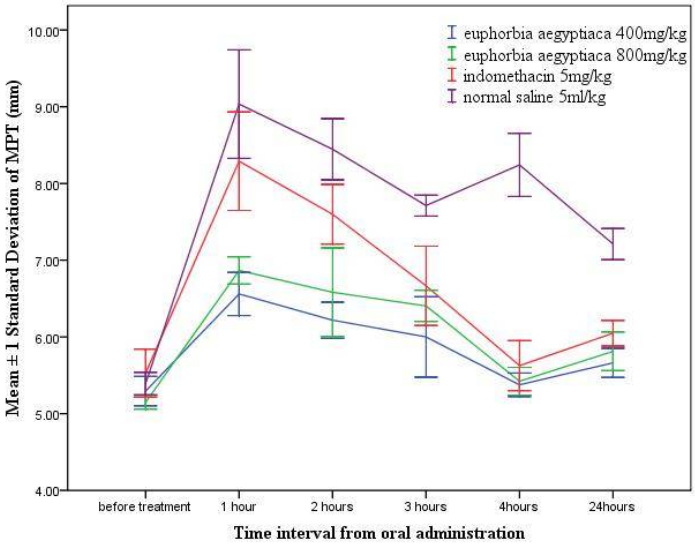

Figure 1. Effects of ethanolic extracts of *Euphorbia eagyptiaca* seeds and indomethacin on rat's MPT at different time intervals

Discussion

The present results indicate that *Euphorbia aegyptiaca* possesses significant anti-inflammatory activity, which exceeds indomethacin when the first was administered at a dose rate of 800 mg/kg. In all groups, the peak edema inhibition was achieved within four hours after oral dosing regardless of type of drug/extracts or the dose. However, the effect of *Euphorbia aegyptiaca* extract at a dose rate of 800 mg/kg is more than that of indomethacin at the time intervals of 1, 2, 3, 4 and 24 hours post-treatment. Although *Euphorbia aegyptiaca* ethanolic extracts at dose rates of 400 and 800 mg/kg achieved comparable EI% and MPT in the first hour following oral administration, these parameters were significantly less thereafter in rats offered the extract at a dose rate of 400 mg/kg. The higher degree of edema inhibition offered by the dose 800 mg/kg suggests a dose-dependent anti-inflammatory effect of *Euphorbia aegyptiaca*.

To our best of knowledge the present study is the first to confirm the anti-inflammatory activity of *Euphorbia aegyptiaca*, and to screen the plant for possible phytoconistituents. The potential anti-inflammatory effects of other species of *Euphorbia* were documented in the literature especially for *Euphorbia hirta.* [1, 9] Ahmed and his group investigated the potential anti-arthritic effects of *Euphorbia hirta* in mouse models of adjuvant induced arthritis. [10] Mice treated by *Euphorbia hirta* in Ahmed *et al* study were proved to have substantially lower levels of pro-inflammatory cytokines (interferon-γ (IFN-γ), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and higher concentrations of anti-inflammatory cytokines (IL-4 and IL-5), pointing to the therapeutic anti-inflammatory potential of this herb. In a separate study, *Euphorbia hirta* was proved to inhibit the inflammatory effects of prostaglandin E2 (PGE2) on rabbit synovial fibroblasts. [1] Alternatively, the diterpenoids isolated from the roots of *Euphorbia fischeriana* were able to inhibit production of inflammatory mediators such as PGE2, nitric oxide (NO), IL-6 and TNF-α. [11] Likewise, local application of tirucallol isolated from *Euphorbia lacteal* latex was proved to suppress ear edema in a mouse model in a dose-dependent manner, probably by suppressing synthesis of NO in macrophages stimulated by lipopolysaccharide. [12] Llanes-Coronel *et al* evaluated the activity of fourteen extracts derived from seven *Euphorbiaceae* herbs on immune cell cultures from healthy individuals.[4] Results showed that 14 Euphorbiaceae's extracts could at least minimally modulate one of the immunological parameters investigated in that study. Nonetheless, only the latex extracts of *Euphorbia cotinifolia* and *Euphorbia tirucalli* strongly enhanced both production and death in mononuclear cells of the peripheral blood.

A Polyamide column chromatography followed by preparative TLC of *Euphorbia aegyptiaca* resulted in the isolation of three flavonoid compounds, [16] which may explain anti-inflammatory activity of this herb. [14, 15] This is because previous reports demonstrated that flavonoids can suppress both pathways of arachidonic metabolism, namely lioxygenase and cyclooxygenase pathways. [17, 18] In a separate study, Luyen and his colleagues (19) isolated twenty six compounds from *Euphorbia humifusa.* [19] Most of these compounds demonstrated anti-inflammatory activities as indicated by their inhibitory effects on soluble epoxide hydrolase, lipopolysaccharide-induced NO and TNF-α production. In a comparable study, thirteen diterpenoids were isolated from the roots of *Euphorbia ebracteolata*. At least three of the isolated compounds exhibited significant inhibition of NO production in lipopolysaccharide-induced macrophages. [3] Fan et al (20) attributed the anti-inflammatory activity of *Terminali acatappa* to triterpenic acids. [20] The presence of flavonoids and triterpenes in *Euphorbia aegyptiaca* may account for the observed anti-inflammatory activity. [15] In a recent study, Abodola et al attributed anti-inflammatory effect of *Blumea aurita* to presence of triterpenes, flavonoids, saponin, cumarins, and tannins. [14] The present study documents the presence of flavonoids, sterols, triterpenes, saponins, tannins and cumarins in *Euphorbia aegyptiaca,* which explains anti-inflammatory activity of this herb based on results of previous studies on these phytoconistituents.

Conclusion

The results of the present study demonstrate that *Euphorbia aegyptiaca* possesses sustained, dose-dependent anti-inflammatory activity. These findings explain the traditional use of this herb in the treatment

of rheumatoid arthritis and other inflammatory conditions in Sudan. Further studies are needed to determine the potential toxic effects and the ideal dose of *Euphorbia aegyptiaca*.

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