

Anti-inflammatory effect of some cucurbitaceous plants

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Summary

The anti-inflammatory effect of three plants of *Cucurbitaceae* family: *Ecballium elaterium*, *Cucumis prophetarum* and *Luffa cylindrica* were studied using their petroleum ether and alcohol extracts. The recorded LD₅₀ due to i.p. administration of petroleum ether of *E. elaterium* and *L. cylindrica* and alcohol of *C. prophetarum*, as representative extracts, were 3.4, 0.45 and 1.5 g/kg b.wt., respectively. The carrageenan induced edema in rats was significantly reduced by pre-treatment with petroleum ether extract of *E. elaterium*, *C. prophetarum* herbs and *Luffa cylindrica* fruits after 2h, while alcohol extract of *E. elaterium* herb caused a maximum inhibition of edema which amounted to 76.9% in comparison with the other extracts.

Key words: anti-inflammatory, *Cucurbitaceae*, carrageenan induced edema rats

INTRODUCTION

Cucurbitaceae is one of the most important plant families supplying humans with edible products and useful fibers. They are also well-recognized source of secondary metabolites. Cucurbitacins, a bitter tasting and highly oxygenated group of compounds are predominantly found in this family. However, they are also present in several other families, e.g. *Brassicaceae* [1]. The cucurbitacins are of great interest due to their wide range of biological activity. A number of compounds of this group has been investigated for their cytotoxic, hepatoprotective, anti-inflammatory, cardiovascular effects and as diabetics. Previous reports on the anti-inflammatory activities of some cucurbitacins were linked with the inhibition of the cyclooxygenase enzymes, although, no research was conducted to support this hypothesis [2].

Three plants of *Cucurbitaceae* family were included in this investigation: *Ecballium elaterium* is known as a squirting cucumber, common in Mediterranean region. Its juice has been used in traditional medicine for various therapeutic treatments including inflammation, sinusitis and rheumatic conditions [3]. Elaterium and cucurbitacin B (isolated from *Ecballium elaterium*) had preventive and curative effects against CCl_4 -induced hepatotoxicity [4]. Fruit juice of *E. elaterium* is used for the treatment of jaundice in folk medicine. It was reported that cucurbitacins D, E and B decreased the levels of direct bilirubin and total bilirubin in human plasma, while cucurbitacin I, glycosyl derivatives and proteins of the juice did not modify the bilirubin levels [5].

Cucumis prophetarum, a plant locally known as khiyar dakar, endemic to Egypt, is grown at Saint Katherine Mountains. It is a perennial, trailing herb with ellipsoidal echinate fruits. The plant grows wild in various regions of Pakistan, India and tropical Africa. The fruit is used in indigenous medicine as an emetic and purgative. It is known to contain cucurbitacins B and D as well as traces of cucurbitacins G and H [6]. Cucurbitacin B, isocucurbitacin B, dihydrocucurbitacin B, cucurbitacin E, dihydrocucurbitacin E, isocucurbitacin D, dihydroisocucurbitacin D, cucurbitacin I, dihydrocucurbitacin I, cucurbitacin Q1, and dihydrocucurbitacin Q1 were identified for the first time as a constituents of *C. prophetarum* L [7].

Luffa cylindrica (*Cucurbitaceae*) or loofa sponge is a tropical running vine with rounded leaves and yellow flowers. The fruits are smooth, of cylindrical shape. Young fruits are used as cooked vegetable while mature fruits are bitter and not edible but the fibrous skeleton can be used as a sponge [8]. The alcohol extract of *L. cylindrica* seeds significantly reduced the blood glucose levels in streptozotocin diabetic rats during first three hours of treatment [9]. Two constituents (L-6a and L10) from leaves of *L. cylindrica* showed tendency to enhance memory keeping activity [10]. Luffin from the seeds of *L. cylindrica* is cytotoxic to human metastatic melanoma cells and in murine *Ehrlich ascites* tumor cells [11]. Sapogenins isolated from *L. cylindrica* showed immunomodulatory effect on *in vivo* immune functions in mice [12].

The objective of present investigation was to determine the acute toxicity and study the anti-inflammatory effects of different extracts of the three cucurbitaceous plants.

MATERIALS AND METHODS

Plant material

Ecballium elaterium and *Cucumis prophetarum* plants were collected from their places of growing: first from El-Arish, second from Sant-Katherine Mountains, while *Luffa cylindrica* fruits and herb were collected in Kalyobia and Giza governorates. The plants were authenticated by Prof. Loutfy Bolous (Botany Dept., Alexandria University), while a voucher specimen was deposited in the herbarium of the National Research Center.

Extraction of the plant materials

200 g of the dried herb of each plant and *L. cylindrica* fruits were extracted successively with petroleum ether 40–60°C and ethanol (95%) till exhaustion. The combined extracts were evaporated under reduced pressure till syrupy residue was obtained. The residues were kept in a desiccator over anhydrous calcium chloride until used for biological studies.

Determination of total cucurbitacins

Total cucurbitacins were determined in the alcohol extract using the method described by Attard and Scicluna-Spiteria [13].

Acute toxicity study

Female Sprague-Dawley albino rats of 20–25 g body weight were used to determine the acute toxicity after intraperitoneal administration (i.p.). The animals were kept in cages (6 mice per cage) at room temperature. Three plant extracts (petroleum ether of *E. elaterium* and *L. cylindrica* and alcohol extract of *C. prophetarum*) were used. LD₅₀ was calculated according to the method adopted by Karber [14].

Anti-inflammatory study

Animals

Female Sprague-Dawley Albino rats weighing 90–140 g were used for the examination of anti-inflammatory activities. All animals were conditioned in cages (6 rats per cage). They were fed and allowed free access to drinking water. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, National Research Centre, Cairo, Egypt, according to the guidance for care and use of laboratory animals.

Carrageenan-induced edema in rats

The anti-inflammatory properties of petroleum ether and alcohol extracts of the herb of all plants and fruits of *L. cylindrica* were investigated on rats paw, using the carrageenan-induced acute edema test inspired with the method described by Winter et al. [15]. The volume of rat's paw was measured before each treatment by a plethysmometer 7150, Ugo Basile. The average volume of the right back paw of each animal was determined (V_0). The rats were divided into groups as follows: group 1: control rats received i.p. injections of saline; groups 2, 3, 4 and 5: rats received i.p.

injections of petroleum ether extracts of *E. elaterium*, *C. prophetarum* and *L. cylindrica* herb and fruits; groups 6, 7, 8 and 9: rats received i.p. injections of alcohol extracts of *E. elaterium*, *C. prophetarum* and *L. cylindrica* herb and fruits. The petroleum ether and alcohol extracts of *E. elaterium* and *C. prophetarum* were administered intraperitoneally at 334 and 150 mg/kg b.wt., respectively, while petroleum ether and alcohol of *L. cylindrica* herb and fruits were administered at 45 mg/kg b.wt. Sixty min. after these administrations each rat received in its right back paw a sub-planter injection of 1% carrageenan suspension (0.05 ml per animal). Then, the average volume of the back paw of each rat was calculated at 1, 2, 3 and 4 hours after injection of the inflammatory agent. The edema rate and inhibition rate were calculated.

Statistical analysis

The obtained results were expressed as mean \pm SE (n=6). Student's-test was used for comparison between each treatment and the control animal group. The limit for significant difference was $p < 0.05$.

RESULTS AND DISCUSSION

Estimation of total cucurbitacins

The total cucurbitacins content in the herbs of *E. elaterium*, *C. prophetarum* and both *L. cylindrica* herb and fruits were determined and compiled in table 1. The highest content of total cucurbitacins (4.45%) was found in *E. elaterium* followed by *C. prophetarum* herb (4.23%) while *L. cylindrica* herb and fruits were 3.91% and 1.34%, respectively.

Table 1.

Total cucurbitacins content in the three plants under study

plant	total cucurbitacins (% w/v)
<i>E. elaterium</i> (herb)	4.45 \pm 0.2
<i>C. prophetarum</i> (herb)	4.23 \pm 0.3
<i>L. cylindrica</i> (herb)	3.91 \pm 0.2
<i>L. cylindrica</i> (fruits)	1.34 \pm 0.1

Determination of LD50 dose

The LD₅₀ of i.p. administration of petroleum ether extract of *E. elaterium* and *L. cylindrica* and alcoholic extract of *C. prophetarum* as a representative extracts were 3.34, 0.45 and 1.5 g/kg b.wt., respectively.

Carrageenan-induced edema in rats

Figure 1 illustrates the average back paw volumes for control and tested extracts, while table 2 presents the edema and inhibition rate calculated for each group and at each recorded time. The pretreatments with petroleum ether extract of *Ecballium*, *Cucumis* and *Luffa* fruits significantly reduced the carrageenan induced edema after two hours to reach a maximum intensity from carrageenan injection, while treatment with petroleum ether extract of *Luffa* herb produced a local edema which increased progressively to reach a maximum intensity after 4 hours. Administration of alcohol extract of *E. elaterium* herb and *I. cylindrica* fruit significantly reduced the carrageenan induced edema.

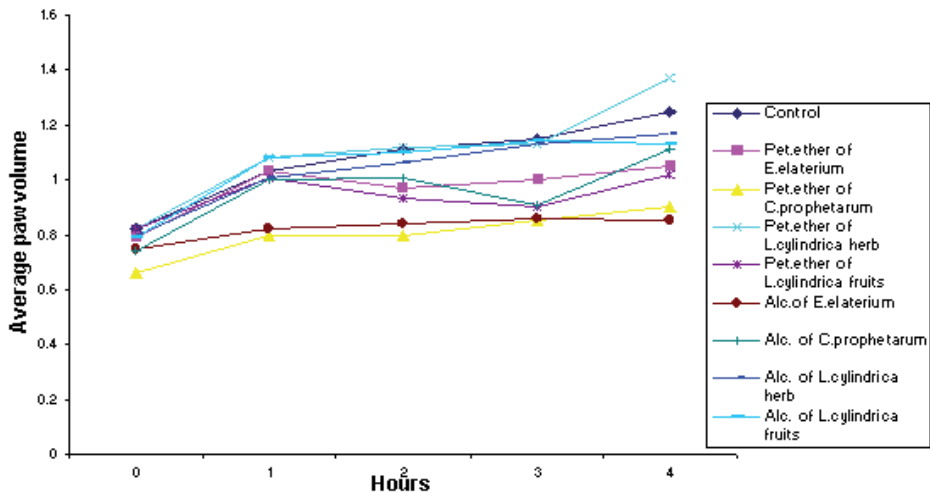


Figure 1. Influence of different extracts used on carrageenan-induced paw edema

Table 2 shows that alcohol extract of *Ecballium* herb caused a maximum inhibition of edema amounted to 76.9% in comparison with other extracts. This may be due to the high percentage of total cucurbitacins found in *Ecballium herb* (4.45%). It was reported that *Ecballium elaterium* has an anti-inflammatory activity induced by cucurbitacins [16], while the fruit juice of *Ecballium* and its cucurbitacin B dose dependently inhibited the serotonin and bradykinin-induced edema in mice [17]. Several authors reported that nitric oxide and prostaglandins participate in the inflammatory and nociceptive events. Prostaglandins, mostly prostaglandin E_2 and prostacyclin are important mediators of inflammation and fever. They are synthesized in tissues by the constitutive enzyme cyclooxygenase-1 and by its isoform cyclooxygenase-2, which can be induced with cytokine, growth factors, or other inflammatory stimuli [18, 19]. Prostaglandins are hyperalgesic and enhance nociception produced by other mediators such as bradykinin [20].

Table 2.

Edema rate (%) of different extracts used on carrageenan-induced edema

edema rate (%after carrageenan administration)				groups
+4h	+3h	+2h	+1h	
58.9±6.7	41.5±5.6	37.4±5.8	27±4.92	control
36.8±2.93* (37.5)	24.8±1.86* (40.2)	22.4±2.54* (40.1)	26.7±2.87 (1.1)	pet. ether of <i>E. elaterium</i> herb
38.1±4.49* (35.3)	27±2.1* (34.9)	27±2.78* (27.8)	22.5±2.69 (16.6)	pet. ether of <i>C. prophetarum</i> herb
69±6.0 (-17.1)	46.1±3.1 (-11.1)	40.5±4.8 (-8.2)	33.5±5.2 (-24)	pet. ether of <i>L. cylindrica</i> herb
23.6±3.7* (59.5)	15.3±3.90* (63.1)	14.2±2.3* (62)	23.3±1.65 (13.7)	pet. ether of <i>L. cylindrica</i> fruits
13.6±1.08* (76.9)	18.1±1.77* (56.3)	10.35±1.59* (72.3)	10.6±1.36* (60.7)	alc. of <i>E. elaterium</i> herb
51.1±2.2 (13.2)	30±2.56 (27.7)	35.2±2.12 (5.8)	33.6±3.8 (-24.4)	alc. of <i>C. prophetarum</i> herb
47.1±4.26 (20)	33.5±4.43 (19.2)	32.1±2.79 (14.1)	29±3.0 (-7.4)	alc. of <i>L. cylindrica</i> herb
39.1±6.7* (33.6)	40.1±6.2 (3.3)	40.3±4.4 (-7.7)	37±4.9 (-37)	alc. of <i>L. cylindrica</i> fruits

Values represent the mean ± SE.

p<0.05, significantly different from control group

Values with parentheses indicate percent inhibition rate.

Non-steroidal drugs and plants used in folk medicine to treat inflammatory conditions have been found to contain triterpenoids displaying potentially important anti-inflammatory actions. They are used as analgesics by suppression of prostaglandins and cytokine production [21, 22]. Several species containing cucurbitacins are commonly used as anti-inflammatories: *Ecballium elaterium*, *Bryonia alba* and *Cayaponia tayuya* are applied topically in popular medicine to cure problems of sinusitis or rheumatism [17]. Furthermore, cucurbitacins B and E isolated from *Wilbrandia ebracteata* (*Cucurbitaceae*) showed anti-inflammatory activity in carrageenan-induced paw edema in rats [23]. The anti-inflammatory action of cucurbitacin B could be related to inhibition of eicosanoid synthesis since this substance modifies the synthesis of leukotriene B4 [24].

In conclusion, *Ecballium elaterium* extracts have the highest anti-inflammatory activity in comparison to other plant extracts. This may be due to the high percentage of total cucurbitacins found in *Ecballium* herb.

REFERENCES

1. Miro M. Cucurbitacins and their pharmacological effects. *Phytother Res* 1995; 9(3):159-68.
2. Jayaprakasam NP, Seeram M, Nair G. Anticancer and anti-inflammatory activities of cucurbitacins from *Cucurbita andreana*. *Cancer Lett* 2003; 189:11-16.
3. Uslu C, Karasen RM, Sahin F, Taysi S, Akcay F. Effect of aqueous extracts of *Ecballium elaterium* rich in the rabbit model of rhinosinusitis. *Int J Pediatr Otorhinolaryngol* 2006; 70(3):515-8.
4. Agil A, Miro M, Jimenez J, Aneiros J, Caracuel MD, Garcia-Granados A, Navarro MC. Isolation of anti-hepatotoxic principle from the juice of *Ecballium elaterium*. *Planta Med* 1999; 65(7):673-5.
5. Greige-Gerges A, Abou Khalil R, Abou Mansour E, Magdalon J, Chahine R, Quaini N. Cucurbitacins from *Ecballium elaterium* juice increase the binding of bilirubin and ibuprofen to albumin in human plasma. *Chem Biol Interac* 2007; 169:53-62.
6. Rahman AU, Ahmad VU, Khan MA, Zehra F. Isolation and structure of cucurbitacin Q-1. *Phytochem* 1973; 12: 2741 - 43.
7. Affi MS, Ross SA, El Sohly MA, Naeem ZE, Halaweish FT. Cucurbitacins of *Cucumis prophetarum*. *J Chem Ecology* 1999; 25(4):847-59.
8. Sastri BN. The wealth of India – raw materials series. CSIR Research 1962; 6:179.
9. El-Fiky FX, Abou-Karam MA, Afify EA. Effect of *Luffa egyptiaca* (seeds) and *Carissa eddulis* (leaves) extracts on blood glucose levels of normal and streptozotocin diabetic rat. *J Ethnopharmacol* 1996; 50(1):43-7.
10. Qi SB, Wang XY, Zhou YL, Zhang L, Ruan HZ, Wang J, Li XC. Effects of new constituents of L-6a and L-10 from *Luffa cylindrica* on learning, memory and hippocampal somatostatin in rats. *Zhongguo Yao Li Xue Bao Nov* 1997; 18(6):529-31.
11. Poma A, Miranda M, Spanol J. Differential response of human melanoma and Ehrlich ascites cells in vitro to the ribosome inactivating protein luffin. *Melanoma Res* 1998; 8(5):465-7.
12. Khajuria A, Gupta A, Garai S, Wakhloo BB. Immunomodulatory effects of two sapogenins 1 and 2 isolated from *Luffa cylindrica* in Balb/C mice. *Bioorg Med Chem Lett* 2007; 17(6):1608-12.
13. Attard E, Scicluna-Spiteria A. *Ecballium elaterium*: an *in vitro* source of cucurbitacins. *Fitoterapia* 2001; 72:46-53.
14. Karber G. Bietrag Zur Kollektiven behandlung pharmakologischem, Reihen versuche. *Arch Exp Pathol Pharmacol* 1931; 162:480-82.
15. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hand paw of rats an assay for anti-inflammatory drugs. *Proc Soc Exp Biol* 1962; 111:544.
16. Yesilada E, Tanaka S, Sezik E, Tabata M. Isolation of an anti-inflammatory principle from the fruit juice of *Ecballium elaterium*. *J Nat Prod* 1988; 51(3):504-8.
17. Yesilada E, Tanaka S, Tabata M, Sezik E. Anti-inflammatory effects of the fruit juice of *Ecballium elaterium* on edema in mice. *Phytother Res* 1989; 3(2):75-6.
18. Holthusen H, Arndt JO. Nitric oxide evokes pain in humans on intracutaneous injection. *Neurosci Lett* 1994; 165:171-74.
19. Vane JR, Bakhil YS, Botting RM. Cyclooxygenases 1 and 2. *Ann Rev Pharmacol Toxicol* 1998; 38:97-120.
20. Ferrira SH, Moncada S, Vane JR. Prostaglandins and the mechanism of analgesia produced by aspirin like drugs. *Br J Pharmacol* 1973; 49(1):86-97.
21. Safayhi H, Sailer ER. Anti-inflammatory actions of pentacyclic triterpenes. *Planta Med* 1997; 63:487-93.
22. Vane JR. Towards a better aspirin. *Nature* 1971; 367:215.
23. Peters RR, Saleh TF, Lora M, Patry C, Brum-Femandes AJ, Farias MR, Ribeiro-do-Valle RM. Anti-inflammatory effects of the products from *Wilbrandia ebracteata* carrageenan-induced pleurisy in mice. *Life Sci* 1999; 64(26):2429-37.
24. Stuppner H, Wagner H. New cucurbitacin glycosides from *Picrorhiza kuroa*. *Planta Med* 1989; 55:559-63.

DZIAŁANIE PRZECIWPALNE NIEKTÓRYCH ROŚLIN DYNIOWATYCH

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Streszczenie

Badano działanie przeciwzapalne trzech roślin z rodziny *Cucurbitaceae*: *Ecballium elaterium*, *Cucumis prophetarum* i *Luffa cylindrica*, stosując wyciąg alkoholowy i wyciąg uzyskany za pomocą eteru naftowego. Odnotowane wartości LD_{50} jako wynik podawania wyciągu otrzymanego z użyciem eteru naftowego z *E. elaterium* and *L. cylindrica* i wyciągu alkoholowego z *C. prophetarum* wynosiły odpowiednio 3,4, 0,45 i 1,5 g/kg m.c. Obrzęk łapy wywołany u szczurów przez karageninę wyraźnie zmniejszył się po 2 godzinach dzięki wcześniejszemu zastosowaniu wyciągów otrzymywanych przez ekstrakcję za pomocą eteru naftowego z ziela *E. elaterium*, ziela *C. prophetarum* i owoców *Luffa cylindrica*, natomiast wyciąg alkoholowy z ziela *E. elaterium* spowodował maksymalne zahamowanie obrzęku, nawet do 76,9% w porównaniu z innymi wyciągami.

Słowa kluczowe: działanie przeciwzapalne, *Cucurbitaceae*, test obrzęku łapy u szczura indukowany karageniną