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Research Paper

Effects of *Globularia alypum* L. on the gastrointestinal tract

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ABSTRACT

The effect of *Globularia alypum* L. leaf aqueous extract was studied on the guinea pig isolated ileum. It has been shown that *Globularia alypum* L. aqueous extract induces on basal tone guinea pig isolated ileum a dose dependent contraction with of a maximal effect of 96.57 ± 1.39% of the response to histamine 10^{-6}M. *Globularia alypum* L.-induced contraction was demonstrated to be induced by acetylcholine, histamine and arachidonic derivatives. The order of potency of the recorded antagonism was: acetylcholine > histamine > arachidonic acid derivatives. It has also been shown by charcoal meal test in the rat that *Globularia alypum* L. induces an increment of intestinal peristalsis that was significant at the dose of 400 mg/kg with a proportion of intestine traversed of 71.68 ± 11.76% (n=10) *versus* 60.69 ± 8.55% (n=10) for the control group ($P<0.05$). Finally, *Globularia alypum* L. was shown to exert an anti-ulcer activity against the gastric mucosal damages caused by indomethacin that the mechanism of action may result from an inhibition of intraepithelial lymphocytes migration.

Keywords: *Globularia alypum L.*; Guinea pig; Ileum; Intestinal peristalsis; Anti-ulcer activity.

INTRODUCTION

Globularia alypum L. (Globulariaceae) is a widely growing shrub in the Mediterranean area. Chemical investigations carried out on *Globularia alypum* L. have reported the isolation and characterization of the major constituents: globularin and cataptol (Bernard, et al., 1974). Wieffering (1966) also reported the existence of aucubin, catalposide, monotropein and catalpol. Four other iridoid glucosides: globularicisin, globularidin, globularimin and globularinin, as well as the lignan diglucoside liriodendrin, and syringin have been isolated from *Globularia alypum* L. (Chandhuri and Sticher, 1981). More recently, Es-Safi, et al. (2006) reported the isolation of a new chlorinated iridodoid glucoside, globularioside from a hydromethanolic extract of the aerial parts alypum L. growing in Morocco.

Globularia alypum L. is used in popular medicine for the treatment of rhumatism, gout, typhoid, intermittent fever and diabetes (Balansard and Delphaut, 1948). The

plant is also described to be an effective laxative (Balansard and Delphaut, 1948), to posse's antileukemic property (Calves, et al., 1975) and potent immunosuppressive activity (Fehri, et al., 1996). In North Africa the plant is also used to treat gastric ulcers (Ben Merabet and Abed, 1982). Hence, this work was undertaken to investigate the effects of *Globularia alypum* L. on the gastrointestinal tract.

MATERIALS AND METHODS

***Plant material*:** Leaves of *Gobulaira alypum* L. were collected on the hills of Testour (North West Tunisia). After drying in air, the leaves were used to prepare aqueous extract. Approximately 50 gm of leaf material was ground in a blender with 500 ml of distilled water for 3 minutes. The homogenate was centrifuged for 30 minutes at 2000 g and the supernatent filtered and lyophilized.

Quantification of globularin: Globularin, principal iridoid glucosid present in *Globularia alypum* L. leaves was quantified by a reversed phase high performed liquid chromatography method with gradient elution and multiwavelengh detection (Chandhuri and Sticher, 1981) using harpagoside (Extrasynthèse, France) as a standard, a commercially compound with very close structural similarity to globularin (Fehri, et al., 1996).

A Waters Series 510 pump was used as the solvent delivery system together with a Waters U6K injector. The system was equipped with a Spectra Physics Focus multiwavelength scanning detector and data manipulation was achieved with an IBM PS/2 computer. The column was a 250 mm x 4.6 mm I.D. Sherisorb 4um ODS column (Waters, Milford, MA). Separation was achieved using a mobile phase consisting of Acetonitrile: Water (30:70) to pH 2.5 with concentrated orthophosphoric acid. The eluant was monitored at 280 nm with complete UV absorbance spectra of each eluting peak available between 200 and 360.

Quantitative analysis was performed on an extract of 10 µg of dried plant material. The concentration of globularin in the aqueous extract of *Globularia alypum* L. as determined as from the calibration curve constructed over the range of 0.02-5µg/ml with each point taken as the mean of 3 determinations.

Guinea pig isolated ileum preparation and experimental procedure: Male guinea pigs weighting from 300 to 400gm were obtained in a homogeneous breeding center (Central Animal House, Tunisia Pharmaceutical Industries Society). They were killed by a blow and exsanguinated. The ileum was rapidly removed and cut into segments of 2 to 3 cm, which were equilibrated under a resting tension of 1g and suspended in 25 ml baths containing Tyrode's solution at 37°C and gassed continuously with 95% O_2 and 5% CO_2. The composition of the Tyrode's solution was (mM): NaCl 139.2; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 0.49; $NaHCO_3$ 11.3; Na_2HPO_4 0.4; glucose 5.5. Tension was measured isometrically with Ugo Basil strain gauges displayed on Geminy Ugo Basile recorders (Italy).

Ileum placed in baths were washed every 15 minutes for 1.25h, and then contracted with histamine 10^{-6}M until maximal contraction was achieved. At that stage, they were washed repeatedly and allowed to rest until they returned to baseline tension. When baseline was reached, concentration-response curves to *Globularia alypum* L. aqueous extract were established by cumulative addition. Pretreatment, if any, were performed 30 minutes before *Globularia alypum* L. aqueous extract addition.

The effect induced by *Globularia alypum* L. aqueous extract was expressed as a percentage of the contraction produced by histamine 10^{-6}M.

***Gastrointestinal effects**:* The gastrointestinal effects of *Globularia alypum* L. aqueous extract were evaluated by the charcoal meal test described by Green, 1959. Male Wistar rats weighing from 190 to 220 gm were given no solid food but water *ad libitum* the day before the test. The day of the test, the animals were divided in four groups of ten rats each. The first group (control group) received distilled water by oral route (1ml/100gm). The three other groups respectively received by oral route 100, 200 and 400 mg/kg of *Globularia alypum* L. aqueous extract. Thirty minutes later, the charcoal suspension (charcoal 10g, wheat floor 2.5 g and distilled qsp 100 ml) was administered to each rat at the rate of 2 ml per rat. Twenty minutes later, the animals were sacrified and the intestine was removed from pylorus to caecum. The intestine length over all (L) and the proportion of the intestine traversed by the charcoal (l) were determinate for each rat. The results were expressed as the percentage of intestine traversed (100.l/L).

***Determination of antiulcer activity**:* The method used is derived from those described by Mansoor et al., 1986. Male Wistar rats weighing between 200 and 300g were divided into five groups (groups 1 to 5) of 10 animals. Animals of each group were subjecting for three consecutive days to the following procedure:

- Group-1: served as control and received the vehicle (CMC: carboxymethylcellulose 1%)
- Group-2: received 100mg/kg/24h of an indomethacin suspension in CMC 1%
- Groups-3, 4 and 5: respectively received 100, 200 and 400 mg/kg/24h of *Globularia alypum* L. extract. One hour later, 100mg/kg/24h of indomethacin suspension in CMC was administered to all animals of each group.

All drugs were administered by oral route under a volume of 1mL/100g. During the treatment period, animals were only allowed to take water. On the fourth day (24h after the last treatment), all the animals were killed and the stomachs were immediately removed. The developed ulcers were evaluated according to the method of Robert et al., 1979:

- 0: no ulcer
- 1: punctiform ulcers
- 2: ulcers < 2mm
- 3: 2 mm <ulcers < 4 mm

Ulcer index in any animal was calculated as:

$$\frac{\textbf{Sum of total scores x percentage of stomachs presenting ulcers}}{\textbf{Number of animals}}$$

Afterwards, the stomachs were fixed in 10% of neutral formalin and processed for paraffin embedding. Then, the stomach tissues were sliced into 4-5µ pieces with a microtome and stained with haematoxylin and eosin for mucosa histological examination.

***Drugs**:* Harpagoside (Extrasynthese), histamine dihydrochloride (Roussel), atropine sulphate, methysergide (Merch), mepyramine maleate (Fisons), phentolamine (Ciba), indomethacin and aspirin (Siphat), imidazole, propranolol, haematoxylin and eosin (Sigma).

Statistical analysis: All results in the text, tables and figures are expressed as means ± S.E.M. Statistical analysis of the results was performed by using Student's test.

RESULTS

***Quantification of globularin*:** Harpagoside and globularin were respectively eluted at 8.65 ± 0.02 min (n=12) and 6.53 ± 0.01 min (n=20). The calibration curve was linear with a correlation coefficient of 0.9987. Intra essay precision was determined as 2.1%. The concentration of globularin in the aqueous extract was of 23.30 ± 0.70% (n=10).

***Effect of Globularia alypum L. aqueous extract on the guinea pig isolated ileum*:** *Globularia alypum* L. aqueous extract exerted on basal tone guinea pig isolated ileum a dose dependent contraction with a maximal effect of 96.57 ± 1.39% of the response to histamine 10^{-6}M (Figure-1). This effect was neither modified by phentolamine, propranolol and methysergide 10^{-7}M nor by imidazole 5.10^{-5}M (Table-1). On the opposite, under the influence of aspirin 3.10^{-3}M, mepyramine 10^{-7}M, atropine 10^{-7}M, *Globularia alypum* L. aqueous extract-induced contraction was significantly antagonized (Table-1).

Table-1: Effect of pretreatment with atropine, propranolol, phentolamine, aspirin, imidazole, mepyramine and methysergide on *Globularia alypum* L. aqueous extract induced contraction on the guinea pig isolated ileum.

Pretreatment (M)	N	E_{max} (mg)	E_{max}/Histamine 10^{-6}M
Control	11	2305.69 ± 265.30	96.57 ± 1.36
Atropine 10^{-7}	6	206.25 ± 16.75***	10.68 ± 0.85***
Propranolol 10^{-7}	6	2099.76 ± 296.76	75.93 ± 21.02
Phentolamine 10^{-7}	5	1985.76 ± 431.03	74.23 ± 19.84
Aspirin 3.10^{-3}	5	1899.54 ± 467.11*	70.17 ± 29.72*
Imidazole 5.10^{-5}	5	2148.04 ± 471.20	76.03 ± 20.57
Methysergide 10^{-7}	6	2118.63 ± 328.91	80.64 ± 17.63
Mepyramine 10^{-7}	5	1310.56 ± 403.90*	58.17 ± 14.28**

- Values are means ± S.E.M.,
- E_{max}: maximal effect and N = number of experiments
- Significant differences from control are shown as: *P<0.05, **P<0.01,***P<0.001.

***Charcoal meal test*:** *Globularia alypum* L. aqueous extract administrated at the doses of 100 and 200mg/kg did not significantly increase the proportion of the intestine traversed (60.69 ± 8.55%, n=10 for the control group *versus* 63.63 ± 11.47% and 63.06 ± 11.34% for groups treated with 100 and 200 mg/kg, n=10 of the plant extract respectively) (P>0.05). However, when administered at 400 mg/kg, the drug significantly enhanced the passage of the charcoal to 71.68 ± 11.76% (n=10) in comparison to the control group (60.69 ± 8.55%, n=10) (P<0.05).

***Protection against indomethacin induced ulcers*:** Administration of indomethacin 100mg/kg/24 h to the rat resulted to the production of gastric mucosal damages with an ulcer index of 120 (Table-2). Pretreatment with *Globularia alypum* L. aqueous extract at the doses of 100, 200 and 400 mg/kg/24 h resulted in preventing the lesions induced by indomethacin decreasing by this way ulcer index to 70, 48 and 42 respectively (Table-2).

Histological examination showed in the group of animals treated by only indomethacin the presence of mucosal ulcers represented by an exudation of fibrin and leukocytes [Figure-2 (photo-3)]. In this group, the loss of substance of the stomach mucosa was in 80% of animals (8/10). In the opposite, when animals were pretreated by the drug, histological examination showed in the quasi totality of the observed cases a gastric mucosa with normal fundus and antrum [Figure-2 (photos-1 and 2)]. The observed loss of substance of the stomach mucosa in animals receiving *Globularia alypum* L. aqueous extract were 1, 3 and 1 out of 10 at 100, 200 and 400

mg/kg/24 h respectively. On the other hand, one out of 10 cases in the group treated by only indomethacin 100 mg/kg/24 h revealed the presence of intraepithelial lymphocytes whereas, such observation was absent in all the other groups treated by *Globularia alypum* L. aqueous extract.

Table-2: Results of digestive tolerance study- Macroscopic observation.

Treatment	Number of rats	Number of graded stomachs 0 1 2 3	Mean scores	% of rats presenting ulcers	Ulcer index
Control (Group 1) Vehicle CMC 1%	10	0 0 0 0	0	0	0
Group 2 (Indomethacin) 100 mg/kg/24 h	10	2 3 3 2	1.5	80	120
100 mg/kg/24 h + *Globularia alypum* L. (Group 3) 100 mg/kg/24 h	10	3 4 3 0	1	70	70
(Group 4) 200 mg/kg/24 h	10	4 4 2 0	0.8	60	48
(Group 5) 400 mg/kg/24 h	10	4 5 1 0	0.7	60	42

DISCUSSION

Globularia alypum L. aqueous extract produced on the guinea pig isolated ileum a dose-dependent contraction with a maximal effect of 96.57 ± 1.39% of the response to histamine 10^{-6}M. This effect is independent of an interaction with serotoninergic, alpha or beta receptors since it is not modified by methysergide, phentolamine and propranolol 10^{-7}M (Table-1). Furthermore, *Globularia alypum* L.-induced contraction is not mediated by thromboxane A_2 since in the presence of imidazole 5.10^{-5}M, its effects were not modified (Table-1). On the opposite, under the influence of aspirin 3.10^{-3}M, mepyramine 10^{-7}M and atropine 10^{-7}M, *Globularia alypum* L.-induced contraction was significantly antagonized (Table-1). This may suggest that arachidonic acid derivatives, histamine and acetylcholine are involved in *Globularia alypum* L.-induced contraction. Taking into consideration E_{max} (maximal effect) values, it is possible to suggest the following order of potency of the recorded antagonism: Acetylcholine > histamine > arachidonic acid derivatives.

Globularia alypum L.-induced intestinal peristalsis increment was also demonstrated charcoal meal test in the rat. The studied drug induced a dose-dependent increase of charcoal passage that was significant at the dose of 400 mg/kg (71.68 ± 11.76% *versus* 60.69 ± 8.55% for the control group) ($P<0.05$).

These two observations demonstrating the enhancement of the intestinal peristalsis induced by the drug confirm the laxative and purgative properties conferred to the plant (Balansard and Delphant, 1948).

Administration of indomethacin 100 mg/kg/24h for three consecutive days induced the production of mucosal ulcers characterized by an exudation of fibrin and the presence of numerous leukocytes. The stomach mucosa loss of substance was observed in 80% of experimental animals. Pretreatment with *Globularia alypum* L. aqueous extract at the doses of 100, 200 and 400 mg/kg/24h resulted to the prevention of mucosal damages caused by indomethacin.

In groups of animals receiving the plant extract, histological examination showed a gastric mucosa with normal fundus as well as a significant decrease of

stomach mucosa loss of substance and a total absence of intraepithelial lymphocytes. The mechanism of action by which *Globularia alypum* L. aqueous extract exerts its anti-ulcer activity needs more elucidation. However, since *Globularia alypum* L. aqueous extract treatment resulted in the absence of intraepithelial lymphocytes and leukocytes, it is therefore possible to suggest that the studied drug induces its anti-ulcer activity by an inhibition of intraepithelial lymphocytes and polymorphonuclear leukocytes migration.

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