Research Paper

Antiinflammatory activity of leaf extracts of *Kalanchoe crenata* Andr.

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Received: 19.1.2005
Revised: 31.10.2005
Accepted: 3.11.2005

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ABSTRACT

Objective: To evaluate the acute and chronic antiinflammatory properties of leaf extracts of *Kalanchoe crenata* in rats.

Material and methods: The methylene chloride/methanol extract of *K. crenata* was extracted by using hexane, methylene chloride, ethyl acetate, and n-butanol. The antiinflammatory profile of these extracts was investigated on the basis of paw edema induced by carrageenan. The n-butanol fraction (most potent) was further assessed through acute inflammatory models induced by histamine, serotonin, and formalin. The chronic antiinflammatory and the ulcerogenic activities of the n-butanol fraction were also examined.

Results: The oral administration of n-butanol fraction (600 mg/kg) caused a maximum inhibition of about 45% in paw edema induced by carrageenan. The n-butanol fraction also exhibited acute antiinflammatory activity on paw edema induced by histamine (47.51%), serotonin (54.71%), and formalin-(40.00%) . In the chronic inflammation model, this extract showed maximum inhibition of 61.26% on the ninth day of treatment. The ulcerogenic assessment showed that ulcer indices after oral treatment with n-butanol fraction were zero and 0.4±0.2, for the 300 and 600 mg/kg doses, respectively.

Conclusion: On the basis of these findings, it may be inferred that *K. crenata* is an antiinflammatory and antiarthritic agent that blocks histamine and serotonin pathways. The results are in agreement with the traditional use of the plant in inflammatory conditions.

KEY WORDS: Antiarthritic, paw edema, ulcer index.

Introduction

Kalanchoe crenata Andr. (Crassulaceae), commonly known as "never die" or "Dog's liver," has been traditionally used for the treatment of ailments, such as, earache, smallpox, headache, inflammation, pain, asthma, palpitations, convulsion, and general debility. Aqueous and alcoholic extracts of *K. crenata* leaves contain alkaloids and saponins.[1]

In an earlier study, the aqueous and the ethanolic extracts of *K. crenata* were found to possess antinociceptive activity against acetic acid, formalin, and hot plate, as well as pain models induced by pressure.[2] This work was aimed at the scientific validation of the ethnopharmacological claim about the antiinflammatory property of the leaf extracts.

Materials and methods

Plant material

K. crenata leaves were collected from Dschang (West province, Cameroon) in October 2002 and authenticated by comparison with a voucher specimen number 50103/YA in the National Herbarium, Yaounde, Cameroon. Two kg of the air-dried leaves were blended to a fine powder and extracted with methylene chloride/methanol (CH_2Cl_2/CH_3OH) for 3 days (72 hours). The extract was concentrated using a rotavapor to obtain 234.7g of the CH_2Cl_2/ CH_3OH extract, which (212 g) was further extracted successively with hexane, methylene chloride, ethyl acetate and n-butanol. The following fractions were obtained: hexane (92.2 g), CH_2Cl_2 (4.7 g), ethyl acetate (7.1 g), n-butanol (37.0 g), and aqueous residue (23.5 g). The CH_2Cl_2/CH_3OH, CH_2Cl_2, and n-butanol fractions were dissolved in 2.5 % DMSO and 2.5 % Tween 20, while the hexane and the ethyl acetate fractions were dissolved in 3% DMSO before orally administrating 300 and 600 mg/kg of each fraction to the rats. The preliminary investigations for the antiinflammatory activity of the various fractions on paw edema induced by carrageenan, showed that the n-butanol fraction of the extract was the most potent. Therefore, it was chosen for further investigations.

Phytochemical screening

The extract and its fractions were tested by the Liberman Burchard, Ferric chloride, Magnesium tracings, and Vanillin-sulphuric acid tests to determine the presence of sterols, phenolic compounds, flavonoids, and saponins, respectively.

Chemicals

Indomethacin (Sigma), pyrilamine maleate (Sigma), diclofenac, carrageenan (Sigma), histamine (Fluka), serotonin (5-HT) (Fluka), and formaldehyde (Roth) were used.

Animals

Wistar rats (140-190 g) of both sexes were used for the studies. These rats were obtained from the Department of Animal Biology and Physiology, University of Yaounde I, Cameroon. The animals were housed in cages under standard laboratory conditions (12:12 hour light/dark cycle at 25 ± 2°C). They had free access to standard commercial diet and water. The animals were divided into groups of five and fasted for 12 hours before the experiment. The ethical guidelines for the investigation of animals used in experiments were followed in all tests.

Paw edema induced by carrageenan

0.1 ml of 1% carrageenan in 0.9% NaCl was administered into the plantar surface of the right hind paw of the animals.[3] The experimental groups, negative control group (2.5% DMSO and 2.5% tween 20), and positive control group (10 mg/kg indomethacin) were given either the control drug or test compounds orally, an hour prior to the administration of the carrageenan. Before injection of carrageenan, the average volume (Vo) of the right hind paw of each rat was calculated from 3 readings that did not deviate more than 3%. After injection of the phlogistic agent, readings (Vt) were obtained for each rat at 30, 60, 120, 180, 240, 300 and 360 min, with the aid of a Ugo Basil Plethysmometer (7150). The edema was expressed as an increase in the volume of paw, and the percentage of inhibition for each rat and each group was obtained as follows:

$$\text{Percentage of inhibition} = \frac{(Vt - Vo)\ \text{control} - (Vt - Vo)\ \text{treated}}{(Vt - Vo)\ \text{control}} \times 100$$

Paw edema induced by histamine and serotonin

In another set of experiments serotonin and histamine were used as the phlogistic agents. The n-butanol fraction of *K. crenata* extract (experimental groups) and control vehicle (solution of 2.5% DMSO and 2.5 % Tween 20) were administered one hour before the injection of inflammatory mediators. The respective strength of the inflammatory mediators, the volume injected, and the time of determination of volume of edema are indicated in parentheses, serotonin (10^{-3} g/ml, 0.1 mL, 30 min.) and histamine (10^{-3} g/ml, 0.1 mL, 60 min).[4] Pyrilamine maleate (1 mg/kg) was used as the antagonist of histamine. The volume of paw edema was determined as mentioned previously.

Paw edema induced by formalin

Acute inflammation was induced by subaponeurotic injection of 0.1 ml of 2% formalin one hour after oral administration of n-butanol fraction, diclofenac (5 mg/kg), or vehicle (solution of 2.5% DMSO and 2.5% tween 20). The volume of paw was determined one, two, and four hours following the injection of formalin. For chronic inflammation study, the above animals were further treated with the n-butanol fraction, diclofenac or vehicle, once daily, for 9 consecutive days. A second injection of formalin was given on the third day.[5] The daily changes in the volume of paw were measured plethysmographically.

Ulcerogenic activity

The ulcerogenic potential of the n-butanol fraction (300 and 600 mg/kg), indomethacin (10 mg/kg), or control vehicle (solution of 2.5% DMSO and 2.5% tween 20) was tested on rats that had been fasted for 24 h. Two hours following oral administration of these drugs, the rats were sacrificed by cervical dislocation. The stomach was isolated and opened along the greater curvature. Ulcerated surfaces were measured and scored according to the table described by Martin *et al.* [6]

Statistical analysis

All values are presented as mean±SEM of five rats. Differences between means were assessed by one-way analysis of variance (ANOVA), followed by Dunnett's test using Stat-Direct-Software. $P<0.05$ was considered significant.

Results

Phytochemical screening

The phytochemical analysis revealed the presence of sterols in the methylene chloride/methanol (CH_2Cl_2/CH_3OH) and its hexane fraction. Sterols, flavonoids, and saponins were found in the methylene chloride and ethyl acetate fractions. Flavonoids and saponins were detected in CH_2Cl_2/CH_3OH extract and its n-butanol fraction.

Paw edema induced by carrageenan

The effects of extracts *of K. crenata* on paw edema induced by carrageenan are shown in Table 1. The CH_2Cl_2/CH_3OH extract showed (600 mg/kg) a maximum antiinflammatory effect of about 43.47% (30 min), while the hexane, CH_2Cl_2, ethyl acetate, n-butanol, and aqueous fractions showed maximal antiinflammatory effects of 47.82% (30 min), 38.88% (1 h), 44.44% (1 h), 45.45% (2 h) and 39.13% (30 min), respectively. The antiinflammatory effect of the n-butanol fraction was more potent and significant during the three phases of inflammation, compared with other fractions. The antiinflammatory effect induced by indomethacin progressively increased and reached a maximum (65.82%) at three hours. It was maintained up to six hours.

Paw edema induced by serotonin and histamine

Table 2 shows the effect of n-butanol fraction on paw edema induced by serotonin and histamine. At 300 mg/kg dose, the n-butanol fraction significantly reduced the formation of paw edema induced by histamine (41.66%). The percentage of inhibition at the dose of 600 mg/kg was 47.51% and 54.71% for inflammation induced by histamine and serotonin, respectively. Inflammation induced by histamine was inhibited by 58.01% by pyrilamine maleate at the dose of 1 mg/kg.

Paw edema induced by formalin

The n-butanol fraction of extract *of K. crenata*

Table 1

Effect of extracts *of K. crenata* on paw oedema induced by carrageenan in rats

		Inflammation (Δ in ml)						
Treatments	***Doses (mg/kg)***	***30min***	***1h***	***2h***	***3h***	***4h***	***5h***	***6h***
Control	0	0.23±0.22	0.36±0.01	0.66±0.03	0.79±0.02	0.70±0.03	0.64±0.04	0.66 ± 0.04
CH_2Cl_2/CH_3OH extract	300	0.28±0.03	0.37±0.04	0.72±0.06	0.80±0.09	0.67±0.08	0.72±0.06	0.77 ± 0.06
	600	0.13±0.02	0.21±0.02*	0.50±0.08	0.75±0.10	0.66±0.10	0.70±0.013	0.61 ± 0.10
Hexane fraction	300	0.28±0.03	0.33±0.01	0.56±0.04	0.66±0.04	0.54±0.02	0.52±0.04	0.50 ± 0.05
	600	0.12±0.01	0.26±0.05	0.46±0.02	0.53±0.04*	0.51±0.02	0.48±0.03	0.41 ± 0.02*
CH_2Cl_2 fraction	300	0.21±0.04	0.19±0.04*	0.56±0.04	0.66±0.06	0.66±0.03	0.65±0.04	0.62 ± 0.02
	600	0.19±0.01	0.22±0.04*	0.49±0.05	0.65±0.07	0.58±0.07	0.54±0.05	0.42 ± 0.05*
Ethylacetate fraction	300	0.27±0.04	0.34±0.03	0.74±0.06	0.87±0.06	0.74±0.66	0.71±0.06	0.70 ± 0.06
	600	0.17±0.03	0.20±0.03*	0.41±0.07*	0.60±0.02	0.74±0.05	0.80±0.05	0.75 ± 0.04
n-butanol fraction	300	0.21±0.02	0.27±0.04	0.63±0.04	0.63±0.05	0.53±0.06	0.61±0.07	0.60 ± 0.06
	600	0.17±0.02	0.20±0.02*	0.36±0.04**	0.48±0.03**	0.48±0.04*	0.43±0.04	0.38 ± 0.06**
Aqueous fraction	300	0.25±0.02	0.39±0.03	0.69±0.05	0.70±0.03	0.63±0.05	0.61±0.04	0.60 ± 0.06
	600	0.14±0.01	0.23±0.01*	0.48±0.07	0.78±0.05	0.85±0.02	0.87±0.04	0.77 ± 0.02
Indomethacin	10	0.15±0.03	0.18±0.03***	0.28±0.06***	0.27±0.07***	0.29±0.07***	0.29±0.04**	0.30 ± 0.05***
One-way	F	3.20	4.25	5.72	6.57	5.27	5.92	6.76
ANOVA	df	13,66	13,66	13,66	13,66	13,66	13,66	13,66
	P	=0.001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001

Values expressed as mean ± SEM, n= 5 in each group. *P<0.05, **P< 0.01, *** P<0.001 compared with control.

Table 2

Effect of the n-butanol fraction of extract *of K. crenata* on paw oedema induced by histamine and serotonin

		Mean oedema volume (ml)		*Percentage inhibition*	
Treatments	***Dose (p.o.)***	***Histamine***	***Serotonin***	***Histamine***	***Serotonin***
Control	10 ml/kg	0.36 ± 0.02	0.53±0.04	-	-
n-butanol fraction	300 mg/kg	0.21 ± 0.04*	0.41±0.02*	41.66	22.64
n-butanol fraction	600 mg/kg	0.19 ± 0.01**	0.24±0.01***	47.51	54.71
Pyrilamine	1 mg/kg	0.15 ± 0.03***	-	58.01	-
One-way	F	8.37	21.05		
ANOVA	df	3,16	2,12		
	P	=0.001	=0.0001		

Values represent means ± SEM; n=5 in each group. *P<0.05, **P< 0.01, *** P<0.001 compared with control .

(300 and 600 mg/kg) and diclofenac (5 mg/kg) significantly inhibited inflammation induced by formalin by 32.85,% 40.00%, and 44.28%, respectively, four hours after administration of formalin. [Table 3]

The n-butanol fraction showed a significant inhibition of chronic inflammation. An inflammation of 21.37% was observed in rats treated with the extract (600 mg/kg) on the ninth day, presenting a maximum inhibition of 61.26%. [Figure 1]

Ulcerogenic activity

As shown in Table 4, 300 mg/kg of the n-butanol fraction of extract of *K. crenata* failed to induce gastric ulceration. At the dose of 600 mg/kg, two rats showed blood vessel dilatation, corresponding to an average ulcer index of 0.4. All the animals that received indomethacin presented a significant ulceration of the stomach mucosa (ulcerated area = 6.85 mm^2). Indomethacin as well as n-butanol fraction (300 mg/kg) significantly reduced the mucus weight.

Discussion

The results of this study indicate that the leaf extracts *of K. crenata* possess acute and chronic antiinflammatory activity against various phlogistic agents. Inflammation induced by carrageenan involves three distinct phases of the release of the mediator, including serotonin and histamine in the first phase (0 – 2 h), kinins in the second phase (3 h), and prostaglandin in the third phase[4] (>4 h). The CH_2Cl_2/CH_3OH extract of *K. crenata* and the aqueous fraction significantly inhibited paw edema induced by carrageenan in the first phase,

Table 3

Effect of the n-butanol fraction of extract *of K. crenata* on acute inflammation of paw oedema induced by formalin

		Mean oedema volume (ml)			*% inhibition*		
Treatments	***Dose (p.o)***	***1h***	***2h***	***4h***	***1h***	***2h***	***4h***
Control	10 ml/kg	0.52±0.04	0.63±0.06	0.70±0.06	-	-	-
n-butanol fraction	300 mg/kg	0.41±0.04	0.49±0.04	0.47±0.06*	21.15	22.22	32.85
n-butanol fraction	600 mg/kg	0.35±0.03*	0.38±0.04**	0.42±0.05**	32.69	39.68	40.00
Diclofenac	5 mg/kg	0.32±0.02**	0.43±0.01*	0.39±0.02**	38.46	31.74	44.28
One-way	F	5.36	5.01	6.57			
ANOVA	df	3,16	3,16	3,16			
	P	=0.009	=0.01	=0.004			

Values are mean±SEM. n=5 in each group. *P<0.05, **P<0.01 compared with control.

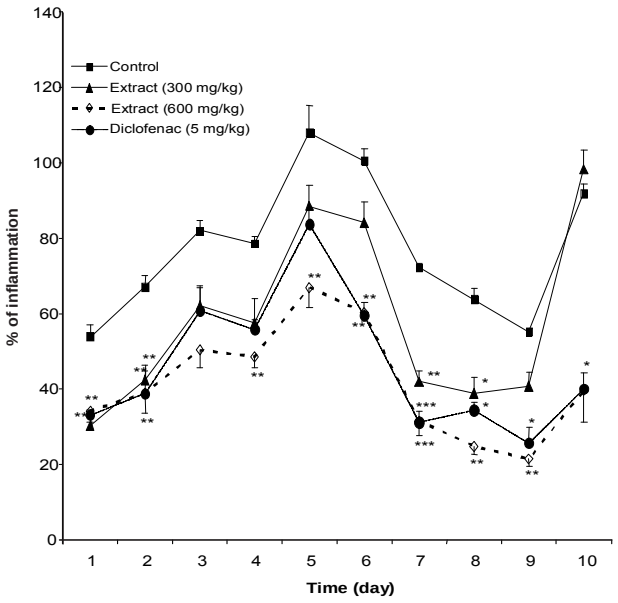
Table 4

Ulcerogenic activities of the n-butanol fraction of CH_2Cl_2/CH_3OH extract of *K. crenata*

Treatment	***Dose (mg/kg)***	***Mucus weight (mg)***	***Ulcer surface (mm²)***	***Ulcer index***	***Ulcer surface (%)***	***Animals with ulcer (%)***
Control		62.98±4.93	0.0±0.0	0.0± 0.0	0	0
n-butanol fraction	300	46.95±1.64*	0.0±0.0	0.0± 0.0	0	0
n-butanol fraction	600	45.32±6.09*	0.0±0.0@	0.4± 0.2	0	40
Indomethacin	10	40.86±3.59*	6.85±1.98*	2.66± 0.16*	0.39	100
One-way	F	4.34	11.34	74.88		
ANOVA	df	3,16	3,16	3,16		
	P	0.0203	0.0003	<0.0001		

Values are mean±SEM. n=5 in each group. *P<0.05 compared with control. @Blood vessels dilation.

Figure 1. Effect of n-butanol fraction of *K. crenata* extract on chronic inflammation induced by formalin. n= 5 in each group, *P<0.05, **P< 0.01, ***P<0.001 compared with control.



suggesting an inhibitory effect on the release of histamine and/or serotonin. The n-butanol fraction showed significant inhibition of the edema in all the three phases. This antiedematous response was also significantly reduced in rats pre-treated with indomethacin, a known cyclooxygenase inhibitor. The n-butanol fraction was chosen for further studies because it was more active, compared with the other fractions. To ascertain the effect of the n-butanol fraction on the activities of the mediator, it was tested on inflammation induced by histamine and serotonin, characterised by increased vascular permeability. It was observed that the n-butanol fraction was capable of inhibiting edema induced by histamine and serotonin. Furthermore, edema induced by formalin was also significantly inhibited by the n-butanol fraction of extract of *K. crenata*. According to Yuh-Fung *et al*,[7] acute inflammation induced by formalin results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin.

The n-butanol fraction was further tested on chronic inflammation induced by formalin. It is well known that inhibition of edema induced by formalin in rats is one of the most suitable test procedures to screen antiarthritic and antiinflammatory agents, as it closely resembles human arthritis.[8] Arthritis induced by formalin is a model used for

the evaluation of an agent with probable antiproliferative activity.[9] As the n-butanol fraction significantly inhibited this model of inflammation, it can be thought to possess antiproliferative and antiarthritic activities similar to diclofenac, a cyclooxygenase inhibitor.

As results from this study strongly indicate the non-steroidal, antiinflammatory-like activity of the extract, its ulcerogenic effect was tested on fasted animals. Non-steroidal, antiinflammatory drugs are thought to impair the mucosal defense of the stomach and the intestine. They act by inhibition of cyclooxygenase and, therefore, inhibit the production of gastric prostaglandins. This leads to a reduction in production of gastric mucus and an increase in mucosal permeability.[10] The reduced weight of mucus coupled with mild ulceration of the gastric mucosa caused by the n-butanol fraction could be due to inhibition of cyclo-oxygenase. The presence of flavonoids in the n-butanol fraction may account for its observed pharmacological activities. Many compounds from this class have been found to exhibit antiinflammatory effects.[11], [12]

To conclude, the results showed antiinflammatory and antiarthritic activities of the CH_2Cl_2/CH_3OH extract of *K. crenata* and its fractions. These activities were related to dose and these results corroborate the traditional use of the plant in inflammatory conditions.

References

1. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd ed. Ibadan. Polygraphic Ventures. Ltd.; 1993. p. 207-9.
2. Nguelefack TB, Fotio LA, Watcho P, Wansi S, Dimo T, Kamanyi A. Analgesic activities of aqueous and ethanolic extracts of the leaves of *Kalanchoe crenata* (Crassulaceae). Phytother Res 2004;18:385-8.
3. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of rat as an assay for anti-inflammatory drugs. Proc Soc Expt Biol Med 1962;111:544-47.
4. Singh S, Majumdar DK, Rehan HMS. Evaluation of anti-inflammatory potential of fixed oil of *Ocimum sanctum* (Holybasil) and its possible mechanism of action. J Ethnopharmacol 1996;54:19-26.
5. Hosseinzadeh H, Younesi H. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol 2002;2:1-8.
6. Martin MJ, Motilva V, Alarcon De La Lastra. Quercetin and Naringenin: Effect on ulcer formation and gastric secretion in the rat. Phytother Res 1993;7:150-3.
7. Yuh-Fung C, Huei-Yann T, and Tian-Shung W. Anti-inflammatory and analgesic activities from roots of *Angelica pubescens.* Planta Med 1995;61:2-8.
8. Greenwald RA. Animal models for evaluation of arthritic drugs. Method Find Clin Pharmacol 1991;13:75-83.
9. Banerjee S, Sur TK, Mandal S, Chandra Das P, Sikdar S. Assessment of the anti-inflammatory effects of *Swertia chirata* in acute and chronic experimental models in male albino rats. Indian J Pharmacol 2000;32:21-4.
10. Jain NK, Kulkarni SK, Singh A. Modulation of NSAID-induced antinociceptive and anti-inflammatory effects by a2 adrenoceptor agonists with gastro-protective effects. Life Sci 2002;70:2857-69.
11. Martini ND, Katerere DRP, Eloff JN. Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae). J Ethnopharmacol 2004;93:207-12.
12. Toker G, Küpeli E, Memisoðlu M and Yesilada E. Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of *Tilia argentea* (silver linden) J Ethnopharmacol 2004;95:393-97.

ERRATA

Indian Journal of Pharmacology, February 2006; Vol 38: Issue 1.

Title: Gatifloxacin-induced prolongation of QTC interval

Page 61: Col. 1, Para 3.

"An interesting finding of our study is QT_c interval prolongation in two female patients less than 40 years old without any known risk factor."

Should read as

"An interesting finding of our study is QTc interval prolongation in two female patients receiving chloroquine less than 40 years old without any known risk factor"

The error is regretted

-Chief Editor, IJP