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ACUTE AND CHRONIC ANTIINFLAMATORY PROPERTIES OF *Kalanchoe crenata Andr.* (Crassulaceae) EXTRACTS

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Abstract

Kalanchoe crenata is used in folk medicine for the treatment of several inflammatory disorders. Methylene chloride/methanol extract of *K. crenata* was successively exhausted in hexane, methylene chloride, ethyl acetate and n-butanol. The anti-inflammatory profile of the obtained extracts was investigated on carrageenan-induced paw oedema. The oral administration of n-butanol extract (600 mg/kg) produced a maximum inhibition of about 45% of carrageenan-induced paw oedema. The n-butanol extract also exhibited acute anti-inflammatory activity on histamine (47.51%), serotonin (54.51%) and formalin (35.97%) induced paw oedema. In the chronic inflammation, this extract showed significant maximum inhibition of 58.33% on the ninth day of treatment. The ulcerogenic assay shows that ulcer indices after *p. o.* treatment with n-butanol extract were 0.0 ± 0.0 at 300 mg/kg and 0.4 ± 0.2 at 600 mg/kg. On the basis of these findings, it may be inferred that *K. crenata* is an anti-inflammatory and anti-arthritic agent which blocks the cyclooxygenase and histamine pathways. The results are in agreement with the traditional use of the plant in inflammatory conditions.

Key words: carrageenan, histamine, 5-HT, formalin, anti-inflammatory activity, *Kalanchoe crenata*

1-Introduction

Kalanchoe crenata Andr. (Crassulaceae) commonly known as "never die" or "Dog's liver" has been traditionally used for the treatment of many ailments such as earache, small pox, headaches, inflammation, pain, asthma, palpitations, convulsion and general debility. Aqueous and alcoholic extracts of *K. crenata*'s leaves contain alkaloids and saponins (Sofowora, 1993).

In early studies, aqueous and ethanolic extracts of *K. crenata* was found to inhibit pain induced by acetic acid, formalin, hot plate and pressure (Nguelefack *et al.*, 2004). This work was

aimed at providing scientific validation of the claimed ethnopharmacological properties, by investigating anti-inflammatory properties of the leaves extracts.

2-Materials and methods

2-1-Plant materials

K. crenata's leaves were collected in Dschang City (West Cameroon) in October 2002, and authentified by comparison with a voucher specimen number 50103/YA in the National Herbarium, Yaounde, Cameroon. 2 kg of the air dried leaves were ground to a fine powder and extracted with methylene chloride/methanol (CH_2Cl_2/CH_3OH) for 3 days (72 h). The resulting extract was concentrated in vacuum over rotavapor to obtain 234.7g of CH_2Cl_2/ CH_3OH extract. 212.4 g of this extract were exhausted 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 g) and aqueous residue (23.5 g). CH_2Cl_2/MeOH, CH_2Cl_2 and n-butanol extracts were dissolved in 2.5 % DMSO and 2.5 % Tween 20; while hexane and ethyl acetate extracts were dissolved in 3% DMSO before giving orally at the doses of 300 and 600 mg/kg of the animal's body weight.

2-2. Phytochemical screening

The total extract as well as its fractions were submitted to the test of Liberman Buchard, the ferric chloride test, the copo of magnesium test and the Vanillin-sulphuric acid test in the goal to determine the presence of sterols, phenolic compounds, flavonoids and saponins respectively.

2-2 Chemicals

Indomethacin (Sigma), pyrilamine Maleate (Sigma), diclofenac, carrageenan (Sigma), Histamine (Fluka), 5-hydroxytryptomine (5-HT) (Fluka) and formaldehyde (Roth) were used.

0.1 ml of oedema-inducing agents was administered into the plantar surface of the right hind paw of the animals.

2-3 Animals

Male and female Wistar rats (140-190 g) were used for the investigations. They were obtained from the Animal House, Department of Animal Biology and Physiology, University of Yaoundé I Cameroon. The animals were divided into groups of five and fasted 24 h before the experiment.

2-4 Pharmacological studies

2-4-1 Carrageenan-induced paw oedema

The extracts (experimental groups) a control vehicle (solution contain 2.5% DMSO and 2.5% tween 20) (control group) and indomethacin 10mg/kg (reference group) were given orally one hour before subplantar administration of 1% carrageenan suspension in 0.9% NaCl. The volumes of the injected paws were measured 30, 60, 120, 180, 240, 300 and 360 min after

induction of inflammation using Ugo Basile Plethysmometer N° 7150 as described by winter *et al.* (1962). The oedema was expressed as an increase in paw volume due to carrageenan injection.

2-4-1Histamin and Serotonin-induced paw oedema

In another set of experiment serotonin and histamine were used as phlogogen agents. The n-butanol extract of *K. crenata* (experimental groups) and control vehicle (solution contain 2.5% DMSO and 2.5 % Tween 20) were administered one hour before injection of inflammatory mediators.

The respective strength of oedemogens, the volume injected and the time of determination of oedema volumes are indicated in parentheses ; serotonin (10^{-3} g/ml, 0.1 ml, 30 min) ; histamine (10^{-3} g/ml, 0,1 ml, 60 min) (Singh *et al.*, 1996). The reference groups were treated with indomethacin (10 mg/kg) or pyrilamine maleate (1 mg/kg) respectively.

The oedema volume was determined as mention previously.

2-4-4. Formalin-induced paw oedema

The inflammation was produced by subaponevrotic injection of 0.1 ml of 2% formaldehyde one hour after oral administration of n-butanol extract of *K. crenata*, diclofenac (5 mg/kg) or control vehicle (solution contain 2.5% DMSO and 2.5% tween 20). The oedema volume was determined 1, 2 and 4 hours after injection of formaldehyde.

The same animals were further treated with the extract or diclofenac for the following 9 days. A second injection of formaldehyde was done on the third day (Hosseinzadeh and Younesi, 2002). The daily changes in paw size were measured plethysmographically.

2-4-4.Ulcerogenic activity

Two hours after test drugs administration (n-butanol extract of *K. crenata*, indomethacin (10 mg/kg) or control vehicle), animals were sacrified by cervical dislocation, stomach isolated and opened along the greater curvature. Ulcerated surface was measured and scores were attributed according to the table described by Martin *et al.* (1993).

2-4-5. Statistical analysis

All values were presented as mean values ± S.E.M from five rats. The statistical significance between the treated groups and the control group was calculated through the analysis of variance (ANOVA) followed by the Student's "t" test. P values less than 0.05 ($P<0.05$) were considered significant.

3-Results

3-1. Phytochemical screening

The phytochemical analysis revealed the presence of sterols in the CH_3OH/CH_2Cl_2 and its hexane fraction. Sterols, flavonoids and saponins were found in the methylene chloride and

ethyl acetate fractions. Flavonoids and saponins have been detected in CH_3OH/CH_2Cl_2 extract and its n-butanol fraction.

3-2. Carrageenan induced paw oedema

The effect of the *K. crenata's* extracts on carrageenan induced-paw oedema is shown in table 1. The CH_2Cl_2/MeOH, ethyl acetate extracts and aqueous residue showed at 600 mg/kg a maximum anti-oedematous effect of about 41.75; 40.10 and 35.71% respectively one hour after carrageenan administration. The hexane, CH_2Cl_2 and n-butanol extracts showed a maximum anti-inflammatory effect of about 44.34 (30 min), 39.01 (1 h) and 45.80% (1 h) after carrageenan administration respectively. This effect was maintained up to 25.16 and 30% for the hexane, CH_2Cl_2 and n-butanol extracts respectively.

The anti-inflammatory effect induced by indomethacin progressively increased and reached a maximum (65.91%) at 3 h, and the effect was maintained up to 6 hours.

3-3. Serotonin and histamine induced paw oedema

Table 2 shows the effect of the n-butanol extract of *K. crenata* on serotonin and histamine induced paw oedema. At the dose of 300 mg/kg, the n-butanol extract of *K. Crenata* significantly reduced the paw oedema formation induced by histamine (41.98%). The percentage inhibition at the dose of 600 mg/kg was 54.51 and 47.51% on serotonin and histamine induced inflammation respectively. Pyrilamine maleate at the dose of 1mg/kg inhibited histamine-induced inflammation by 58.01% while indomethacin exhibited a percent inhibition of 48.49% on inflammation induced by serotonin.

3-4. Formalin induced paw oedema

The n-butanol extract of *K. crenata* (300 and 600 mg/kg) and diclofenac (5 mg/kg) produced significant inhibition of formalin induced inflammation by 32.57, 35.97 and 43.62% respectively 4 h after formalin administration (table 3).

In the chronic inflammation, the extract showed significant inhibition. On the seventh day of experiment, the inflammation of the paw of animals treated with extract (600 mg/kg) and diclofenac (5 mg/kg) was 31.40 and 31.07% respectively, showing an inhibition of 55.94%. An inflammation of 21.37% was observed in rats treated with extract (600 mg/kg) on the ninth day, presenting a maximum inhibition of 58.33%

3-5. Ulcerogenic activity

As shown in table 4, 300 mg/kg of the n-butanol extract of *K. crenata* fail to induce gastric ulceration. At the dose of 600 mg/kg, 2 over 5 rats treated show blood vessels dilatation, corresponding to general ulcer index of 0.4. All the animals that received indomethacin presented significant ulceration surface of 6.85 mm^2. Indomethacin as well as n-butanol extract (300 mg/kg) significant reduced mucus weight.

4. Discussion

The experimental data of the present study indicates that *K. crenata* extracts possesses significant anti-inflammatory activities on various phlogistic agents.

Carrageenan induced inflammation involved three distinct phases of mediators release including serotonin and histamine in first phase, kinins in second phase and prostaglandin in third phase (Singh *et al.*, 1996). CH_2Cl_2/MeOH extract of *K. crenata* and aqueous residue have significantly inhibited carrageenan induced paw oedema only in the first phase, suggesting an inhibitory effect on the release of histamine and/or serotonin. Anti-inflammatory effect of the ethyl acetate extract was prolonged to the third hour, suggesting an inhibition of the release of kinins. Hexane, CH_2Cl_2 and n-butanol extracts showed a significant inhibition of the oedema on the three phases. This oedematous response was also significantly reduced in rats pre-treated with indomethacin, compound known to be cyclooxygenase inhibitors. According to Chawla *et al.* (1987) and Dongmo *et al.* (2001), 5-lipoxygenase inhibitors also possess anti-inflammatory activity on carrageenan-induced oedema. The inhibitory effect of the hexane and n-butanol extract on the third phase of the oedema could be due to an inhibition of 5-lipoxygenase and/or cyclooxygenase, both enzymes involved in the formation of leukotrienes and prostaglandins respectively. The n-butanol extract was more active than others extracts and was chosen for further studies. Results obtained with n-butanol extract on the carrageenan-induced oedema were confirmed on the acute inflammation induced by formalin. According to Yuh-Fung *et al.* (1995), acute inflammation induced by formalin results from cell damage which provoked the production of endogenous mediators such as histamine, serotonin, prostaglandins and bradykinin. Formalin-induced oedema was significantly inhibited by n-butanol extract of *K. crenata*.

In order to ascertain the effect of the extract on mediators' activities, the extract was tested against inflammation induced by histamine and serotonine. It has been observed that n-butanol extract of *K. crenata* inhibited histamine and serotonin-induced oedema. The inhibitory effect of *K. crenata* inferred that the extract may be acting like pyrilamine as an antagonist of these mediators which also significantly inhibited inflammation induced by histamine.

n-butanol extract of *K. crenata* was further essayed on chronic inflammation induced by formalin. It is well known that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis (Greenwald, 1991). Formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation (Banerjee *et al.*, 2000). Since n-butanol extract of *K. crenata* could significantly inhibit this model of inflammation, it can be though that it possesses

anti-proliferative and anti-arthritic activities which have been observed with diclofenac, a cyclooxygenase inhibitor.

Since results from the present study strongly indicate the non steroidal anti-inflammatory like activity of the extract, its ulcerogenic effect was tested on starved animals. Non steroidal anti-inflammatory drugs are thought to impair the mucosal defence properties of the stomach and intestine. They act by inhibition of cyclooxygenase, therefore inhibiting the production of gastric prostaglandins which leads to reduction of gastric mucus production and an increase in mucosal permeability (Jain *et al.*, 2002). According to Kryvola *et al.* (2003), anti-histaminic agents protect the gastric mucosa against ulceration by diminishing gastric acid production. The n-butanol extract of *K. crenata* which reduced the mucus weight but induced little ulceration of the gastric mucosa could act by dual inhibition of cyclo-oxygenase and histamine.

The presence of flavonoids in the n-butanol extract of *K. crenata* may account for its anti-inflammatory activity. Many compound from this class have been found to exhibit anti-inflammatory effects (Martini *et al.*, 2004; Toker *et al.*, 2004)

In conclusion, the results show the marked and dose-related anti-inflammatory and anti-arthritic activities of the *K. crenata* extracts. These results corroborated the traditional use of the plant in inflammatory conditions. Their active principles specifically inhibit certain chemicals-induced oedema in rats.

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Table 1: Effect of *K. crenata* extracts on carrageenan induced paw oedema in rats

Traitements	Doses (mg/kg)	Inflammations (ΔV en ml)							Pourcentages d'inhibition						
		30min	1h	2h	3h	4h	5h	6h	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	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH_2Cl_2/CH_3OH	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	-25.21	-3.29	-8.08	-1.00	4.28	-12.03	-16.16
extract	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	40.00	41.75	23.95	6.01	5.14	-8.33	8.68
Hexane extract	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**	-25.21	7.69	14.97	17.04	21.71	19.75	24.55
	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	44.34	27.47	29.94	32.83	26.00	25.92	38.32
CH_2Cl_2 extract	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	8.69	46.70	15.56	16.79	5.14	-1.54	6.28
	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*	17.39	39.01	26.04	17.54	16.28	16.35	36.22
Ethylacetate	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	-21.73	4.39	-11.97	-9.77	-6.57	-11.11	-7.48
extract	600	0.17± 0.03	0.2± 10.03**	0.41± 0.07*	0.60± 0.02**	0.74± 0.05	0.80± 0.05	0.75± 0.04	22.60	40.10	38.62	24.81	0.00	-23.76	-12.27
n-butanol	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	6.95	25.27	5.38	20.05	23.42	5.86	9.28
extract	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**	24.39	42.85	45.80	39.34	30.85	32.71	42.21
Aqueous	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	-9.56	-7.69	-3.59	12.28	10.10	5.86	9.28
residue	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	39.13	35.71	26.94	1.25	-22.28	-34.25	-16.16
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**	31.30	48.90	57.78	65.91	57.42	54.01	54.49

*p<0.05, **p<0.01 compared with control

Table 2. Effect of n-butanol extract of *K. crenata* on histamine and serotonin induced paw oedema

Group	Dose (*p.o.*)	Mean oedema volume (ml)		Percentage inhibition	
		Histamine	Serotonin	Histamine	Serotonin
Control	10 ml/kg	0.36 ± 0.02	0.53 ± 0.04		
n-butanol	300 mg/kg	0.21 ± 0.04*	0.41 ± 0.02	41.98	21.40
extract	600 mg/kg	0.19 ± 0.01**	0.24 ± 0.01**	47.51.	54.51
Pyrilamine	1 mg/kg	0.15 ± 0.03**		58.01	
Indomethacin	10 mg/kg		0.27 ± 0.02**		48.49

*p<0.05. **p<0.01 compared with control

Table 3.Effect of n-butanol of *K. crenata* on formalin induced paw oedema

Group	Dose	Mean oedema volume (ml)			Percent inhibition		
	(*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	300 mg/kg	0.41 ± 0.04	0.49 ± 0.04	0.47 ± 0.06**	19.92	21.58	32.57
extract	600 mg/kg	0.35 ± 0.03*	0.38 ± 0.04**	0.42 ± 0.05**	32.95	39.36	35.97
Diclofenac	5 mg/kg	0.32 ± 0.02**	0.43 ± 0.01*	0.39 ± 0.02**	37.93	30.47	43.62

*p<0.05. **p<0.01 compared with control

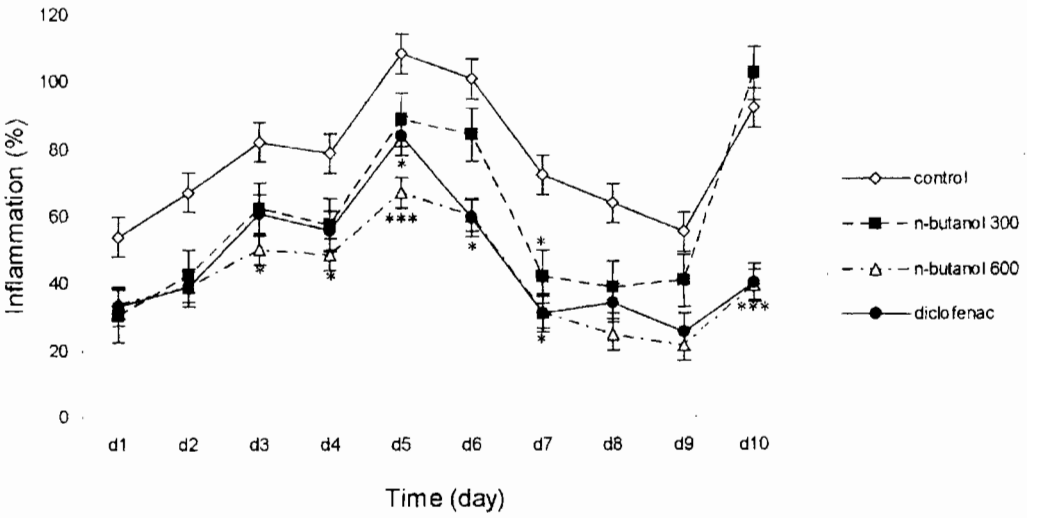

Figure1. Effect of n-butanol extract of *K. crenata* on chronic inflammation induced by formalin. *p<0.05, **p<0.01, ***p<0.001 compared with control

Table 4. Ulcerogenic activities of n-butanol extract of *K. crenata*

Drugs	Doses (mg/Kg)	Mucus weight (mg)	Ulcerated surface (mm^2)	Ulceration Indice	ulcerated surface (%)	ulcerated animals (%)
Control		62.98 ± 4.93	0.0 ± 0.0	0.0 ± 0.0	0	0
n-butanol extract	300	46.95 ± 1.64*	0.0 ± 0.0	0.0 ± 0.0	0	0
	600	45.32 ± 6.09	Blood vessels dilation	0.4 ± 0.2	0	40
indomethacin	10	40.86 ± 3.59**	6.85 ± 1.98**	2.66 ± 0.16***	0.39	100

*p<0.05, **p<0.01, ***p<0.001 compared with control