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Research Article

Evaluation of Analgesic and Anti-Inflammatory Activity of *Kalanchoe spanthulata* Leaves Extract

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ABSTRACT

With many of kalanchoe's traditional uses verified by animal research, it is not unusual that it continues to be a popular natural remedy throughout the tropics where it grows. In the present investigation anti-inflammatory and analgesic profile of *Kalanchoe spanthulata* has been explored using carrageenan induced paw oedema and tail flick method respectively. The obtained results indicate that Aqueous extracts of K. spanthulata in dose range of 100 and 200 mg/kg showed promising anti-inflammatory and analgesic activity in experimental animals.

Keywords: Kalanchoe spanthulata, aqueous extract, anti-inflammatory, analgesic.

1. INTRODUCTION

Kalanchoe spanthulata Dc. (syn - *Kalanchoe integra*) Sanskrit Syn –Asthibhaksa, Parnvija or leaf seed [1], is an indigenous plant and belongs to the family crassulaceae. It is a medicinal plant that grows at the altitude from 1000' to 3000' in Bhutan, India, Burma, Warm China and Java [2]. In india it grows in the hilly tracts of the Himalayan region and is locally known as "Slundhru". *Kalanchoe spanthulata* is a succulent perennial plant that grows 3-5 feet tall. Commonly known as 'air plant,' it has tall hollow stems, fleshy dark green leaves that are distinctively scalloped and trimmed in red, and bell-like pendulous flowers. Kalanchoe is rich in alkaloids, triterpenes, glycosides, flavonoids [3], steroids and lipids. The leaves contain a group of chemicals called bufadienolides which are very active and have sparked the interest of scientists. They are very similar in structure and activity as two other cardiac glycosides, digoxin and digitoxin (drugs used for the clinical treatment of congestive heart failure and related conditions). Kalanchoe's bufadienolides have demonstrated in clinical research to possess antibacterial, antitumorous, cancer preventative, and insecticidal actions. In the Himalayan region cases of acute poisoning in sheep were reported with the plant [4]. Preliminary investigations regarding its toxicity and pharmacology were carried out by Singh, Uppal and Ahmad [5]. Its Leaves are applied to abscess and reckoned as a specific medicine in Cholera. Its bitter variety is used in enlarge spleen and healing activity of inflammatory wounds [6]. In the present study preliminary clinical aspect with the inflammatory responses has been observed in aqueous extract of *Kalanchoe spanthulata* leaves.

2. MATERIAL AND METHODS

2.1. Collection of plant

The leaves of plant were procured from Himachal Pradesh in the month of September 2010. The leaves were authenticated as *K. spanthulata Dc.* A voucher specimen of plant has been deposited in museum of department of pharmacognosy, College of pharmacy, Teerthanker Mahaveer University, Moradabad, U.P., India for future reference.

2.1.1. Preparation of aqueous extract of K. spanthulata leaves

The powdered dried leaves of the plant were extracted in various polar and non-polar solvents. The cold aqueous extract (10% W/V) was prepared by macerating finely powdered material in distilled water for 24h during which occasional vigorous shaking. At the end of 24 h, it was filtered through muslin cloth; the sediment free supernatant was collected and made to original volume by adding distilled water [7]. The acute toxicity study was conducted in the laboratory animals.

2.2. Experimental animals

Male wistar strain rats, weighing about 200-250 g were used for the study. All animals were kept and maintained under laboratory conditions of temperature (22±2°C), humidity (45±5°C) and 12 hr day: 12 hr night cycle as per CPCSEA guidelines [8]. Animals were allowed free access to food (standard pellet diet) and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee of Teerthanker Mahaveer College of Pharmacy, Moradabad, India.

2.3. Analgesic activity

Control group received 0.5% sodium CMC (1mg/kg) orally whereas the standard reference group received Nimesulide (50mg/kg) orally. The rest two groups were treated as test groups and received *K. spanthulata* aqueous extract in dose level of 100mg/kg and 200mg/kg respectively. The animals which showed reaction time of 2-3s, were selected for the experiment and analgesic activity of the compound was studied by tail flick method [9]. After the administration of the solvent, nimesulide and leaf extract in different dose level was over, the basal reaction time was noted at 0, 1, 2, 3 and 4 hours by immersing the tail tips of the rats (last 1-2cm) in hot water heated at 55˚C±0.5˚C. The actual flick response of rats i.e. time taken (in sec) to withdraw tail from hot water source was calculated and compared with control group.

2.4. Anti-Inflammatory Study

Carrageenan induced hind paw oedema

The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control; Group 2 received indomethacin 10mg/kg/ml, suspended in 1% sodium carboxymethyl cellulose. Group 3 and 4 were treated with 100 and 200 mg/kg of Aqueous extracts of *K. spanthulata* leaves suspended in Tween 80/ ethanol / saline (1:1:10). Oedema was induced by injecting 0.1 ml of a 1% solution of carrageenan in saline into the subplantar region of the right hind paw of the rats. The vehicle, extracts and the standard drugs were administered 60 min. prior to the injection of the phlogestic agent. The volumes of oedema of the injected and the contralateral paws were measured at 1, 2, 3, 4h after the induction of inflammation using a plethysomgraph to calculate the percentage of paw oedema inhibition [10].

2.5. Statistical Analysis

The results were presented as mean ± SEM. One way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons were used for statistical evaluation. *p* values less than 0.05 were considered as significance.

3. RESULTS AND DISCUSSION

Acute toxicity studies revealed the non-toxic nature of the aqueous extract of *K. spanthulata* leaves. After the administration of aqueous extract of *K. spanthulata* leaves rats were immediately observed for 2 h for behavioral, neurological and autonomic profiles for any changes or lethality for the next 48 h. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

3.1. Analgesic activity

The extract produced a significant analgesia even in the first hour in the dose of 100 and 200 mg/Kg body weight (data are given in Table-1). These effects were well comparable with the standard drug used in this present study. It will be worth to mentioning that although different constituents were extracted in different solvents as per polarity but aqueous fraction is more effective as compared to other solvent extracts and analgesic activity was found to be more in dose of 200 mg/Kg body weight. The activity showed by this extract is of considerable importance. By employing one way ANOVA, all data were found to statistical significant at 5 % level of significance ($p<0.05$). It could be concluded that the plant *K. spanthulata* leaves is having analgesic activity and better result are obtaining from aqueous extract. Further study needed to identify the chemical constituents present in extract of this plant that may elicit analgesic activity.

Table 1: Analgesic activity of K. spanthulata leaf extract on tail flick method

S.No.	Treatment	Tail flick response (sec)				
		0 hr	1 hr	2hr	3 hr	4 hr
1	Control (0.5% Sodium CMC)	0.064±0.173	0.386±0.311	0.334±0.292	0.50±0.217	0.366±0.275
2	K.spanthulata (100 mg/Kg)	1.02±0.064	2.19±0.200	2.79±0.230	2.89±0.290	3.48±0.140
3	K.spanthulata (200 mg/Kg)	1.06±0.201	2.31±0.380	2.86±0.300	3.62±0.185	3.84±0.201
4	Standard Nimesulide(50 mg/Kg)	0.90±0.152	2.22±0.195	2.31±0.142	3.16±0.302	4.02±0.165

Results are expressed as mean ±SEM from five observations.
P<0.05

3.2. *Anti-inflammatory activity*

Interplanetary injection of carrageenan in the hind paw induced gradual increase in the edema paw volume in the control group. Aqueous extracts of *K. spanthulata* at doses of 100 and 200 mg/kg significantly ($p<0.05$) inhibited the edema formation of rat paw at 3 h after carrageenan challenge. The results are expressed in Table 2. The extract of *K. spanthulata* possessed varying degree of anti-inflammatory activity when tested at various doses. This study revealed that total aqueous extract possesses good anti inflammatory property which may be attributed to the individual or combined actions of phytoconstituents present in it.

Table 2: Effect of K. spanthulata leaf extract on carrageenan induced rat paw oedema

S.No.	Treatment	Initial Paw Thickness in mm	Mean Paw thickness			
			1h	2h	3h	4h
1.	Control (0.5% Sodium CMC)	0.18 ± 0.08	0.23 ± 0.08	0.29 ± 0.06	0.32 ± 0.05	0.23 ± 0.04
2.	K.spanthulata (100 mg/Kg)	0.21 ± 0.01	0.28 ± 0.01	0.31 ± 0.01	0.37 ± 0.02	0.26 ± 0.01*
3.	K.spanthulata (200 mg/Kg)	0.15 ± 0.03	0.23 ±0.04	0.29 ± 0.01	0.31 ± 0.12	0.27 ± 0.08*
4.	Standard Indomethacin (10 mg/Kg)	0.23 ± 0.01	0.43 ± 0.06	0.53 ± 0.03	0.64 ± 0.02	0.67 ± 0.01

**P<0.001*

4. CONCLUSION

On the basis of the parameters investigated in the present study and the results attained, it can be concluded that aqueous extract of *K. spanthulata* expressed remarkable analgesic and anti-inflammatory activities when given in different dose level in experimental rodents. Though at this stage it is not possible to identify the exact phytochemical constituent(s) responsible for anti-inflammatory activities of *K. spanthulata*, it may still be suggested to explore out the exact phytoconstituent(s) present in the aqueous extract by qualitative tests and chromatographic techniques. It seems safe, however to conclude that this part of the plant do possess biological activities following oral administration. The above results need to be verified in other experimental models to be totally authentic.

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