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Analgesic And Anti-Inflammatory Activity of Kalanchoe Pinnata (Lam.) Pers

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The main objective of the present work is to find out good pharmacological activities in herbal source with their preliminary phytochemical study, and also it is aimed to investigate of ethanol and aqueous extracts of dried stem of plant 'Kalanchoe pinnata(lam.)Pers.' against with Anti-inflammatory and in rats and Analgesic activity in mice. Normally herbal products are free from side effects/adverse effects and they are low cost medicines, which will be beneficial for human being. The main objective of this work is to develop potent Anti-hyperglycemic, Anti-inflammatory, analgesic, having no or minimum side effects from indigenous plants for the therapeutic management.

Keyword : Analgesic and Anti-Inflammatory Activity, Kalanchoe Pinnata (Lam.) Pers

1. Introduction:

The World Health Organization (WHO) defined health as "a complete state of physical, mental, and social well-being and not merely the absence of disease or infirmity."

So during the past decade, traditional systems of medicine have become a topic of global importance. Current estimate suggest that, in many developing countries a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to

alternative or complementary therapies, including medicinal herbs.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material:

The specimen copy (Herbarium) of selected plant collected in month of July-2007 from ABS Botanical garden, Karpipatty, Distt. - Salem, Tamil Nadu Mr. A .Balsubramnian. (Consultant-central siddha research) Executive Director ABS botanical garden, Salem, authenticated the plant as **Kalanchoe Pinnata (Lam.) Pers.** (Family-Crassulaceae).

2.2 Preparation of extract:

The stem of **Kalanchoe Pinnata (LAM.) PERS.** Were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no. 30 and stored in an airtight container for further use.

2.3 Solvent For Extraction:

- Petroleum Ether (60-80° C)
- Alcohol (95% v/v)
- Distilled water with chloroform (0.25%)

2.4 Extraction Procedure:

The dried powders of stem of **kalanchoe pinnata** were defatted with petroleum ether (60-80°c) in a Soxhlet Apparatus by continuous hot- percolation. The defatted powder material (marc) thus obtained was further extracted with ethanol (95% v/v) with same method and fresh powder used for aqueous extraction by Cold maceration method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

2.5 Procurement of Experimental Animals:

Swiss albino mice (20-25 g) and albino Wister rats (150-200 g) of either sex and of approximate same age are used in the present studies were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols

were approved by **Institutional Animal Ethics Committee (IAEC No.-P.Col. / /2007)** after scrutinization. The animals received the drug treatments by oral gavage tube.

2.6 Acute Oral Toxicity Study:-

The lethal dose (LD 50) of the alcoholic and aqueous extract of dried stem of **kalanchoe pinnata (LAM.) PERS.** was determined by OECD guideline (423 guideline).

The LD50 of alcoholic extract and aqueous extract was found to be 3000 mg/kg therefore the ED50 value is 300mg/kg.

HYPOGLYCEMIC ACTIVITY:-

In present study, the hypoglycemic activity of plant extract of dried stem of **Kalanchoe pinnata (LAM.) PERS.** was studied for the decrease in blood glucose level (BGL) in normal fasted rats. 300 and 600 mg/kg were screened for hypoglycemic activity on normal rats up to 3 hrs. It produced significant hypoglycemic activity in a dose dependent manner.

Significant reduction in blood glucose level was seen at 2nd hrs and maximum reduction occurred at 3rd hrs by treatment with ethanolic and aqueous extracts which was compared with the control animal group and standard treated group.

The alcoholic and aqueous extract treated group shows the significant reduction in blood glucose level during the study hour.

2.7 Evaluation of Anti-Inflammatory Activity:-

Ethanolic and Aqueous extract of plant **kalanchoe pinnata (Lam.) Pers.** was tested for Anti-Inflammatory activity against carrageenan induced paw-edema in rats.

Both the extracts having anti-inflammatory activity against the carrageenan induce paw oedema in rats. The reductions of paw oedema of rats are compared with the standard drug i.e. indomethacin.

2.8 Evaluation Of Analgesic Activity:-

Alcoholic and aqueous extract of plant **Kalanchoe pinnata (Lam.) Pers.** Was evaluated for analgesic activity against acetic acid-induced writhing in mice. Both the extract (ethanolic and aqueous) having analgesic activity against the acetic acid-induced writhing in mice but the activity of higher concentration of extracts having greater activity than lower once. The reduction in writhing response are compared with the standard drug i.e. pentazocine.

2.9 Anti-Inflammatory Activity:

Everyone have has personal experience of inflammation and pain. The classic signs of inflammation have long been recognized; the tissues become red, swollen, tender, or painful, there is local heat and the patient may be febrile. Inflammation can be categorized mainly as Chronic and Acute inflammatory disease.(D.R.Laurence, et al., clinical pharmacology).

Acute and chronic inflammatory diseases are still one of the most important health problems in the world. Although several agent known to treat inflammatory disorders, their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt retention. For this reason there is a need to find and develop new anti-inflammatory drugs with low side effects (K.D.Tripathi, essential of med. Ph., 4th ed., 1998).

Formaldehyde, Dextran, Carrageenan, histamine, and other inflammagen induced inflammation model are frequently used in screening for the anti-inflammatory activity of new compounds, where implantation off foreign body under the skin is often used to investigate the effects of drugs on the proliferative phase of the chronic inflammation model.

In the present study the attempt has been focused to evaluate the **anti-inflammatory**

activity of extracts of stem of plant **kalanchoe pinnata (LAM.) PERS.** using carrageen induced paw-oedema in rats as a model. For comparison purpose, indomethacins were taken as a reference compound.

• Treatment Design

- **Group I:-** Normal control (Carrageenan 1%w/v)
- **Group II:-** Positive control (indomethacin 10mg/kg, i.p.)
- **Group III:-** Ethanolic extract (300mg / kg)
- **Group IV:-** Ethanolic extract(600mg / kg)
- **Group V:-** Aqueous extract (300mg / kg)
- **Group VI:-** Aqueous extract (600mg / kg)

Procedure:-

1. Male albino wistar rats weighing between 150-200 gm was purchased from Venkateshwara Enterprises, Bangalore
2. Animals are divided into 6 groups.
3. The different groups were treated as shown in design.
4. The paw-volume measured at 0, 30, 60, 120, 180 mins after carrageenan injection using the plathysmometer.
5. The animals of group III, IV, were pretreated with ethanolic extracts and V, VI with aqueous extracts, 60 minutes before the administration of Carrageenan.
6. Acute inflammation was produced by the sub plantar administration of 0.1% carrageenan in normal saline in the left paw or rats.
7. Inhibition of swelling is compared with that of control group.(Kulkarni S.K.-2005)

The % inhibition of paw-edema is calculated by :-

$$\% \text{ inhibition of Paw-edema} = \frac{C - T}{C} \times 100$$

Where,

C = increase in paw-volume of control group

T = increase in paw-volume after administration of extracts.

Table No. - 7.6 - Evaluations of Anti-Inflammatory Activity

Group	Treatment Design	Dose	Paw volume in ml as measured by mercury displacement at				
			0 min	30 min	60 min	120 min	180 min
I	Normal control+ (Carrageenan)	0.1ml	0.51 ± 0.018	0.562 ± 0.023	0.73 ± 0.023	0.86 ± 0.023	1.1 ± 0.084
II	Standard (indomethacin) +carrageenan	10mg/kg + 0.1 ml	0.45 ± 0.0244	0.48 ± 0.0337	0.55 ± 0.0244	0.55 ± 0.0244	0.48 ± 0.0183
III	Ethanollic extract	(300mg/kg)	0.45 ± 0.0244	0.5 ± 0.04	0.55* ± 0.0244	0.61* ± 0.033	0.71 ± 0.065
IV	Ethanollic extract	(600mg/kg)	0.38 ± 0.0337	0.48 ± 0.044	0.60* ± 0.04	0.7* ± 0.028	0.58* ± 0.052
V	Aqueous extract	(300mg/kg)	0.41 ± 0.0337	0.53 ± 0.0231	0.65 ± 0.024	0.76 ± 0.036	0.65 ± 0.046
VI	Aqueous extract	(300mg/kg)	0.36 ± 0.0366	0.45 ± 0.0244	0.566* ± 0.0228	0.55* ± 0.046	0.54* ± 0.0374

P values: ** P< 0.01; * P <0.05.

Values are expressed in mean ±SEM, n=6 animals in each group.

One way ANOVA followed by DUNNETT'S, multiple comparison tests

2.9 Analgesic Activity

Analgesia is defined as a state of reduced awareness to pain, and analgesics are substances, which decrease pain sensation (pain - killers) by increasing by increasing threshold of painful stimuli. The commonly used analgesics are Aspirin, Paracetamol (non - narcotic type) and Morphine (narcotic type).

Painful reaction in experimental animals can be produced by applying noxious (unpleasant) stimuli such as (i) thermal

(radiant heat as a source of pain), (ii) chemical (irritants such as acetic acid and bradykinin) and (iii) physical pressure (tail compression).(Kulkarni S.K.-2005).

In the present study the attempt has been focused to evaluate the **Analgesic activity** of extracts of stem of plant **kalanchoe pinnata (LAM.)PERS.** against Acetic acid-induced writhing response in mice.

Treatment design

- **Group I :-** Normal control (Acetic acid 3%v/v)
- **Group II:-** Positive control (Pentazocine 5mg / kg)
- **Group III:-** Ethanolic extract (300mg / kg)
- **Group IV:-** Ethanolic extract(600mg / kg)
- **Group V:-** Aqueous extract (300mg / kg)
- **Group VI:-** Aqueous extract (600mg / kg)

Procedure:-

1. Albino mice weighing between 150-200 gm was purchased from Venkateshwara Enterprises, Bangalore
2. Animals are divided into 6 groups.

3. Acetic acid is administered in the dose of 30mg/kg or 0.3 ml to the first group (normal control) and number of writhing responses (constriction of abdomen, twisting of trunk and extension of hind limbs) are recorded for a period of 10 mins.
4. The animals of group III, IV, were pretreated with ethanolic extracts and V, VI with aqueous extracts, 15 minutes before the administration of Acetic acid.
5. Reduction in number of writhes is taken as analgesic activity and compared with that of control group.(Kulkarni S.K.-2005)

Evaluate the Analgesic activity of extracts of stem of plant **Kalanchoe Pinnata (Lam.)Pers.** Against Acetic acid-induced writhing response in mice.

Table 7.7: Evaluation of Analgesic Activity

Group	Treatment design	Dose	Mean No. of wriths (In 10 mins.)
I	Normal control (Acetic acid)	1%v/v	23.75±0.75
II	Positive control (Pentazocine)+ acetic acid	5mg/kg +1%v/v	4.25 ± 0.25* *
III	Ethanolic extract + Acetic acid	300 mg/kg + 1%v/v	11.5 ± 0.86* *
IV	Ethanolic extract + Acetic acid	600 mg/kg + 1%v/v	8.5 ± 0.64* *
V	Aqueous extract + Acetic acid	300 mg/kg + 1%v/v	12.25 ± 1.10* *
VI	Aqueous extract + Acetic acid	600 mg/kg + 1%v/v	7.75 ± 0.62* *

* P values:
* P< 0.01, * P <0.05

Values are expressed in mean ±SEM, n=6 animals in each group.
One way ANOVA followed by DUNNETT'S, multiple comparison tests

3. Conclusion

Both the extract of dried stem of *Kalanchoe pinnata* (LAM.) PERS. Having analgesic activity against the acetic acid-induced writhing in mice. Both the extract of dried stem of *Kalanchoe pinnata* (LAM.) PERS. having anti-inflammatory activity against the carragenan -induce paw edema in rats.

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