RESEARCH ARTICLE ISSN: 2349-2678

Contents lists available at www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

NLM (National Library of Medicine ID: 101738825)

Index Copernicus Value 2019: 71.05

Volume 10 Issue 1; January-February; 2022; Page No. 01-08

Phytochemical Screening and Antipyretic activity of hydroalcoholic leaves extract of *Kalanchoe Crenata*

[1]Mr. Deena Nath Jaiswal,[*1]Dr. C.K. Tyagi, [2]Dr. Prabhakar Budholiya

[1]College of Pharmacy, SSSUTMS Sehore (M.P.)

[2]Government Medical College Ratlam (M.P.)

Conflicts of Interest: Nil

Corresponding author: Mr. Deena Nath Jaiswal

ABSTRACT

Pyrexia occurs due to infection, malignancy and other diseases. Majority of the antipyretic drugs are synthetic in nature which exerts side effects such as gastric ulcer, hepatic necrosis and renal damage. The antipyretic potential of the hydro-alcoholic extracts of Kalanchoe Crenata was investigated on the yeast-induced pyrexia in albino rats. Paracetamol was used as a positive control. Rectal temperature of albino rats was verified immediately before the administration of the extracts or vehicle or paracetamol and yet again at 1-hour gap for 4 hours using a digital thermometer. The extracts were also phytochemically screened for alkaloids, tannins, saponins, flavonoids, cardiac glycosides and phenols. The hydro-alcoholic extracts of plants with the dose of 500mg/kg showed significant ($p<0.0001$) decrease in yeast-induced pyrexia, as compared with that of set drug paracetamol (50mg/kg) where the extract dose 250mg/kg was less effective than that of standard drug ($p<0.05$). Phytochemical screening showed the presence of alkaloids, tannins, flavonoids, saponins and phenols. This study showed that hydro-alcoholic extracts of all plants under study at a dose of 500mg/kg have significant antipyretic potential in yeast-induced elevated temperature.

Keywords: *Kalanchoe Crenata.,*

1. Introduction

1.1 Pyrexia, also known as fever, is an increase in the body temperature of an individual beyond the normal range. This increase in temperature is usually considered dangerous, but it is a natural defensive mechanism of the body to fight against infections. Pyrexia is usually associated with other symptoms such as lethargy, headache, cough, and cold. A mild increase in body temperature can be relieved by over the counter medications such as Paracetomol and Ibuprofen. A sudden and higher increase in the body temperature beyond the normal range should be treated medically as it could be due to some major illnesses such as a brain hemorrhage or cancer. A fever can be caused by many medical conditions ranging from non-serious to life-threatening. This includes viral, bacterial, and parasitic infections—such as influenza, the common cold, meningitis, urinary tract infections, appendicitis, COVID-19, and malaria. Non-infectious causes include vasculitis, deep vein thrombosis, connective tissue disease, side effects of medication, and cancer. It differs from hyperthermia, in that hyperthermia is an increase in body temperature over the temperature set point, due to either too much heat production or not enough heat loss.

1.2 Causes of Pyrexia:

Causes of pyrexia may be infectious or non-infectious. Some of the common reasons of pyrexia are listed here:

Infectious Causes:

- Lower respiratory tract infections like bronchitis (inflammation of the air tubules that carry blood in and out of the lungs)

- A bacterial lung infection called tuberculosis
- Complicated urinary tract infections
- Bone infections like Osteomyelitis
- A bacterial infection of the cardiac tissue called endocarditis
- Viral infections like HIV (Human-Immunodeficiency syndrome) and Cytomegalovirus

Non-infectious Causes:

- Neurological conditions like brain fever or hemorrhages
- Malignant conditions like Leukaemia and renal cell carcinoma
- Reactions to drugs
- Bowel or bladder related problems
- Reactions to blood transfusion

1.3 Signs and Symptoms Associated with Fever:

Fever is clinically manifested as additional signs and symptoms such as:

- Shivering or chills
- Headache
- Generalised body pains and weakness
- Irritability
- Dehydration
- Loss of appetite
- Joint pains
- Sweating

Children in the age of 6 months to 5 years may get febrile seizures (which is marked by the loss of consciousness, stiffening, jerking and fainting) when the temperature reaches >103^{o}F.

1.4 Risk factors for Fever:

People with the following conditions are at a higher risk for developing fever:

- Bronchitis
- Sinusitis
- Rheumatoid arthritis
- Allergic rhinitis (hay fever)

Common Complications of Fever:

High-grade fever (>104^{o}F) for a prolonged period may give rise to complications such as:

- Seizures
- Brain damage
- Coma
- Death

People with high-grade fever and those with fever since prolonged times require immediate medical treatment to prevent the development of complications due to a weakened immune system.

1.5 Treatment of Pyrexia:

The following methods can treat pyrexia:

- **Medications:** Use of drugs like Ibuprofen (Advil) and acetaminophen (Tylenol) helps to control fever and any discomfort associated with it. These medications should be used at the exact doses as recommended by the physician as higher doses may damage the liver or kidney.
- **Antibiotics:** These drugs are recommended if the doctor suspects that the fever is caused by some bacterial infections in the bladder or bowel.
- **Antiviral drugs:** These medicines are used if the doctors diagnose that the fever is caused by viral infections.
- **Rest:** The patient should take adequate rest.
- **Fluids:** Adequate fluids along with regular supplements should be taken to prevent dehydration.

Patients admitted with very high fever and weakness are immediately put on intravenous vitamin supplements or medications to prevent excessive loss of salts and minerals from the body.Fever itself is not a disease but is a sign that alerts you about some underlying infection or health condition. The following first-aid measures are helpful while treating fever:

- Drink plenty of fluids.
- Use blankets to control shivering.
- Rub the palms and soles (the peripheral parts of the body) to increase the internal temperature of the body.
- Use over-the-counter medications like Paracetomol to reduce the body temperatures, but only to a limited dose and seek doctors advice before using them for a prolonged time.

Infants lesser than 6 months are to be properly checked for the associated symptoms of fever such as stiff neck, continuous crying, difficulty in breathing and rash on the body; on incidence of any of these signs seek medical help.

1.6 Prevention of Pyrexia

The following measures can prevent pyrexia:

- Maintaining a proper self-hygiene

- Washing hands regularly before eating
- Using hand sanitizers where there is no access to water
- Covering the nose and mouth when travelling in public transport to prevent the entry of disease-causing organisms into the body
- Sharing plates, glasses or cups along with other people must be avoided.

2. Material and Method

2.1 Plant material collection

Leaves of *Kalanchoe Crenata* Linn. was collected from Vindhya herbals Bhopal (M.P.) in the month of December 2020.

2.2 Extraction of plant material

Dried powdered leaves of *Kalanchoe Crenata* has been extracted with hydro-alcoholic using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

2.3 Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

2.4 Phytochemical Screening

The *Kalanchoe Crenata* extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of alkaloids, glycosides, phenols, flavonoids, Amino acid, Cabohydrates, Terpenoids, Saponins, Steroids.

2.5 Estimation of total Phenolic, flavonoid and alkaloid Content

2.5.1 Total Phenolic content estimation

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

2.5.2 Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method.

2.6 *In vivo* antipyretic activity of *Kalanchoe Crenata* of plant extract

2.6.1 Yeast-induced hyperpyrexia in rats

Yeast induced pyrexia was used to evaluate the antipyretic activity of the extract. The rats were divided into four groups of six animals and the body temperature of each rat was recorded by measuring rectal temperature at predetermined time intervals. Fever was induced by injecting 15% suspension of Brewer's yeast (*Saccharomyces cerevisiae*) in the back below the nape of the rat. In brief, the rats were allowed to remain quiet in the cage for sometimes. A thermistor probe was inserted 3-4 cm deep into the rectum, after fastened the tail, to record the basal rectal temperature. The animals were then given a subcutaneous (s.c.) injection of 10 ml/kg of 15% *w/v* Brewer's yeast suspended in 0.5% w/v methyl cellulose solution and the animals were returned to their housing cages. Twenty four hour after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the hydroalcoholic extract of *Kalanchoe Crenata* leaves were administered orally at doses of 250 and 500 mg/kg to the treatment control groups animals, the normal control group received distilled water and standard control groups animals received 45 mg/kg of paracetamol. Pre-drug control temperatures of all the rats was recorded at 24h immediately before the extract or paracetamol administration and again at 1h interval up to 4h after yeast injection.

2.6.2 Treatment protocol:

Group	No. of Animals in each group	Treatment/Dose
Group -1 Normal Control	5	Brewer's yeast suspension (10 mL/kg b.w., s.c.)
Group -2 Standard Control	5	Brewer's yeast suspension (10 mL/kg b.w., s.c.) + Paracetamol (150 mg/kg *p.o.)*

Group -3 Treatment Group	5	Brewer's yeast suspension (10 mL/kg b.w., s.c.) + *Kalanchoe Crenata* hydroalcoholic extract at a dose of 250 mg/kg *p.o.*
Group -4 Treatment Group	5	Brewer's yeast suspension (10 mL/kg b.w., s.c.) + *Kalanchoe Crenata* hydroalcoholic extract at a dose of 500 mg/kg *p.o.*

3. Results and Discussion

3.1 Result of Percentage Yield

Table 3.1: % Yield of plant material

S. No.	Solvent	Leaves of *kalanchoe crenata*
1.	Hydroalcoholic	4.2

3.2 Phytochemical screening of extracts

Table 3.2: Phytochemical screening of extracts *of kalanchoe crenata*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's test Dragendroff's test	 -ve -ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenolics** Ferric Chloride Test	 +ve
5.	**Proteins** Xanthoproteic test	 +ve
6.	**Carbohydrates** Fehling's test	 -ve
7.	**Saponins** Froth Test	 +ve
8.	**Diterpins** Copper acetate test	 +ve

3.3 Results of estimation of total phenolic contents

Table 3.3: Total phenolic and total flavonoid content of *kalanchoe crenata* extract

S. No.	Extract	Total Phenol content (mg/100mg)	Total flavonoid content (mg/100mg)
1.	Hydroalcoholic *extract*	0.952	0.640

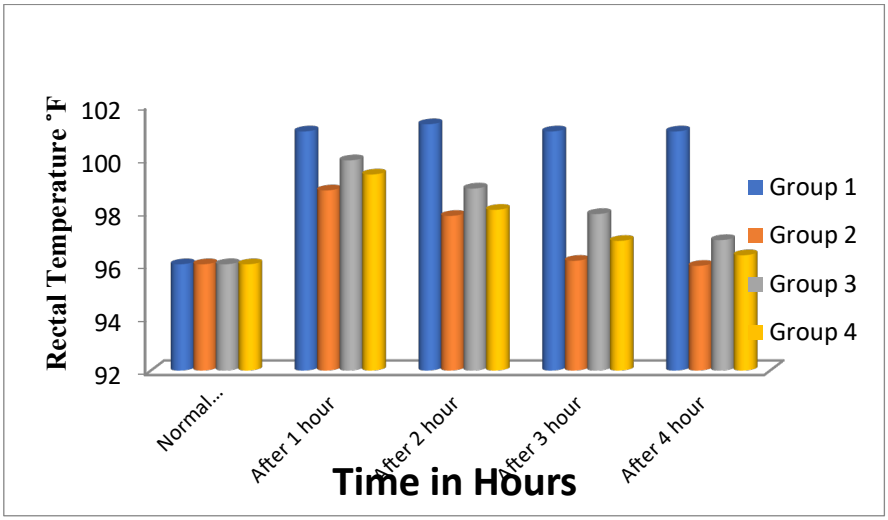
3.4 Results of Antipyretic activity of plant extract

3.4.1 Yeast-induced hyperpyrexia in rats

It is well known that pharmaceutical companies around the world are interested in developing safer and more effective drugs to treat pain, inflammation and fever. Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration. Treatment with the hydroalcoholic extract of kalanchoe crenata leaves at the doses of 250 and 500 mg/kg significantly decreased the rectal temperature of the rats. The antipyretic effect started as from the first hour and the effect was maintained for 4 h, after administration of the extract. The result obtained from both the standard paracetamol (50 mg/kg, p.o.) and hydroalcoholic extract of *kalanchoe crenata* (250 and 500 mg/kg) treated rats were compared with that of control and a significant reduction (*P<0.05; **P<0.01; ***P<0.001) against yeast induced pyrexia was observed. Hydroalcoholic extract at a dose of 500mg/kg, after 4 h showed more effect as compared to standard drug.

Table No. 3.4: Effect of hydroalcoholic extract of kalanchoe crenata leaves on yeast induced pyrexia in rats

Group	Normal temperature before yeast administration	Pre-drug control, 1 h before drug admin.	Rectal temperature after drug administration (% decrease)			
			1h	2h	3h	4h
Group I Normal control	96.48±0.68	101.67±0.78	101.36±0.42	101.27±0.38	101.17±0.48	101.09±0.56
Group II Standard Control	96.58±0.84	100.37±0.68	98.78±0.5*	97.82±0.56*	96.13±0.40*	95.94±0.42*
Group III Treatment Group	96.78±0.69	100.87±0.49	99.91±0.2*	98.85±0.29*	97.89±0.27*	96.91±0.24*
Group IV Treatment Group	96.88±0.42	100.64±0.51	99.38±0.3*	98.05±0.67*	96.88±0.46*	96.34±0.67*



Graph 3.1 Effect of hydroalcoholic extract of *kalanchoe crenata* leaves on yeast induced pyrexia in rats

Discussion

In present study the extract of hydroalcoholic extract of kalanchoe crenata significantly reduced the pyrexia induced by yeast in rats. The reference drug Paracetamol suppressed the yeast induced fever in rat by inhibiting the synthesis of prostaglandin E2. These result support the use of hydroalcoholic extract of kalanchoe crenata as an antipyretic for the treatment of fever. Plants have been used for centuries for medicinal purposes by various cultures and ethnicities around the world and continue to be important curative agents for various disorders even with the revolution in antibiotics and other synthetic agents in modern scientific world. Different mechanisms of action of phytoconstituents from medicinal plants have been proposed. They may different metabolic processes or may modify gene expression and signal transduction pathways or physiologic response elicited by aseptic stimuli or various infections. Rise in body temperature arises when the concentration of prostaglandin E2 (PGE2) upsurges inside the hypothalamic region of the brain. Such an elevation creates substantial alteration in the firing degree of neurons that regulate the thermoregulation process in the hypothalamus. It is now evident that most of the antipyretic drugs manifest their effect by impeding the enzymatic activity of cyclooxygenase and subsequently decrease the levels of PGE2 within the hypothalamic region of brain. A natural antipyretic agent with less or no toxicity is therefore, considered vital. Since antipyretic activity is normally cited as a characteristic of drugs or agents, which possess an inhibitory property on prostaglandins biosynthesis, the yeast induced hyperpyrexia in rodents model was employed to determine the antipyretic effect of hydroalcoholic extract of kalanchoe crenata. Yeast induced pyrexia is known as pathogenic fever which is elicited because of the formation of prostaglandins (PGE2) that regulate the thermoregulatory center at an elevated temperature.

Antipyretics have been reported to subdue fever by retarding the activity of prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or subduing the escalation of interleukin- 1α production subsequent to interferon production. This study also correlates with the study that suggested about flavonoids and saponins to act synergistically to exert the antipyretic effect. The results of present study indicate that extract possesses significant antipyretic activity compared to the activity on yeast induced hyperthermia in mice. This could be credited for the presence of flavonoids and saponins in leaves that might be responsible for the inhibition of prostaglandin synthesis. Moreover, there are several intermediaries accentuating the pathogenesis of fever. Reducing the activity of these intermediaries could further reduce fever significantly.

Conclusion

The plant based bio-active compounds have the effective dosage response with minimal side effects, when compared to the synthetic compounds. The presence of phytochemicals (secondary metabolites) is responsible for their therapeutic effects. It further reflects a hope for the development of many more novel therapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents. Plant contains various phytoconstituents including phenols and flavonoid which can be responsible for antipyretic activity.

Reference

1. World Health Organization: Quality control methods for medicinal plant materials. Published by WHO, Geneva, 1998.
2. El SN and Karakava S. Radical scavenging and ironchelating activities of some greens used as traditional dishes in Mediterranean diet. Int J Food Sci Nutr, 2004, 55: 67.
3. Samy PR, Iushparaj PN, Gopalakrishnakone PA. Compilation of bioactive compounds from Ayurveda Bioinformation, 2008.
4. Subhose V, Narian A. Basic principles of pharmaceutical science in Ayurvĕda. Bull Indian Inst Hist Med Hyderbad, 2005, 35: 83.
5. Ballabh B and Chaurasia OP. Traditional medicinal plants of cold desert Ladakh--used in treatment of cold, cough and fever. J Ethnopharmacol, 2007, 112: 341.

6. Dev S Ethnotherapeutic and modern drug development: The potential of Ayurveda. Current Sci, 1997, 73: 909.
7. Perumal Samy R and Ignacimuthu S. Screening of 34 Indian medicinal plants for antibacterial properties. J Ethnopharmacol, 1998, 62: 173.
8. Perumal Samy R and Gnacimuthu SI. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharmacol, 2000, 69: 63.
9. Kamboj V P. Herbal medicine – Some comments. Current Sci, 2000, 78: 35.
10. Rabe and Staden J V. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol, 1997, 56: 81.
11. Nayar M P. The *ecological biogeography* of the lowland endemic tree flora. Bull Bot Surv Ind, 1987, 29: 319.
12. Cox PA, Ethnopharmacology and the search for new drugs Bioactive Compounds from PlantsCiba Foundation Symposium 154, Chichester, John Wiley & Sons, 1990, 40.
13. Cox P, Balick M. The ethnobotanical approach to drug discovery. Sci American, 1994, 82.
14. Tiwari S, Singh A. Toxic and sub-lethal effects of oleadrin on biochemical parameters of freshwater air breathing murrel, Chant punctatus (Bloch). Indian J Exp Biolo, 2004, 42: 413-18.
15. Tiwari S. Plants: A Rich Source of Herbal Medicine. Journal of Natural Products, Vol 1, 2008, 27-35.
16. Ved DK, Mudappa A, Shankar D. Regulating export of endangered medicinal plant species-need for scientific vigour. Curr Sci, 1998, 75: 341-4.
17. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. NISCIR, CSIR, Delhi 2002.
18. Singh J, Singh AK, Pravesh R. Product ion and trade potential of some important medicinal plants: an overview. In: Proceeding of first national interactive meet on medicinal and aromatic plants, CIMAP, Lucknow, India, 2003, 50-8.
19. Nadkarni AK. Indian Materia Medica.Popular Press Bldg. 2000.
20. Cardinali PD and Esquifino IA. Circardian disorganization in experimental arthritis.Neuro Signals. 2003;12:267-282.
21. Pervical M. Understanding the natural management of pain and inflammation, Clinical Nutrition insights. 1999:4:1-5
22. S. Kumar, BS. Bajwa, Singh Kuldeep and AN. Kalia 'Anti-Inflammatory Activity of Herbal Plants: A Review' IJAPBC – Vol. 2(2), Apr-Jun, 2013 ISSN: 2277 – 4688
23. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. Annu Rev Pathol. 2008;3:279-312.
24. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol. 2001 Jul;2(7):612-9.
25. Serhan CN, Chiang N, Van Dyke TE.Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008 May;8(5):349-61.
26. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med. 1999 Jun;5(6):698-701
27. Rajakariar R, Hilliard M, Lawrence T, Trivedi S, Colville-Nash P, Bellingan G, Fitzgerald D, Yaqoob MM, Gilroy DW. Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyDelta12 14 PGJ2. Proc Natl Acad Sci U S A. 2007 Dec 26;104(52):20979-84. Epub 2007 Dec 5.
28. Dai C, Stafford RS, Alexander GC. National trends in cyclooxygenase-2 inhibitor use since market release: nonselective diffusion of a selectively cost-effective innovation. Arch Intern Med 2005;165:171-7.
29. Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell AA. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey.JAMA 2002;287:337-44.
30. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al.

Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 2005;352:1092-102.

31. Juni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. Lancet 2004;364:2021-9. [PubMed]
32. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. JAMA 2006;296:1633-44