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Antipyretic activity of leaf extracts of ethnomedicinal plant, *Kleinia Grandiflora* (Asteraceae)

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ABSTRACT

The methanolic and aqueous extracts of Kleinia grandiflora (Asteraceae) was investigated for antipyretic activity in rats using brewer's yeast-induced pyrexia model. Both the extract (200 and 400 mg/kg body weight p.o) produced a significant ($p<0.05$) inhibition of temperature elevation compared with the standard drug paracetamol (150mg/kg body weight). Peak antipyretic activity was observed by the methanolic extract at doses of 200 mg/kg b.w. These results indicate that Kleinia grandiflora leaves extracts possesses potent antipyretic effects and thus pharmacologically justifying its folkloric use in the management of fever.

Keywords: Acute toxicity, Antipyretic, Brewer's yeast, Prostaglandin, *Kleinia grandiflora*.

INTRODUCTION

Herbal medicines are assumed to be of great importance in the primary health care of individual and communities [1]. The World Health Organization has estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for the primary health care needs. The high degree of efficacy and safety with herbal medicines make them more acceptable compared to other therapeutic invention [2]. Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals [3, 4].

Pain, inflammation and fever are some of the most common manifestations of many diseases afflicting millions of people worldwide [5, 6]. Pyrexia or fever is caused as a secondary impact of infection, malignancy [7] or inflammatory disorders [8, 9]. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive [7]. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1β, α, β and TNF- α), which increase the synthesis of prostaglandin E2 (PGE2) in the region of the POAH (Preoptic area of the anterior hypothalamus) [10]. PGE2 is believed to be the proximal mediator of the febrile response. Preoptic neurons bearing E-prostanoid receptors alter their intrinsic firing rate in response to PGE2, evoking an elevation in the thermoregulatory set point. As the temperature regulatory system is governed by a nervous feedback mechanism, so when body temperature becomes very high, it dilate the blood vessels and increase sweating to reduce the temperature; but when the body temperature becomes very low hypothalamus protect the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration and existing complaints, as found in HIV [11].

Non-steroidal anti-inflammatory drugs (NSAIDs) and glucorticoids, opiods, etc. inhibit cycloxygenase 2 (COX-2) expressions to reduce the elevated body temperature by inhibiting PGE2 biosynthesis [12]. In contrast, inhibition of the COX-1 system produces prostaglandins critical to normal renal function, gastric mucosal integrity, vascular

hemostasis and the autocrine response to circulating hormones and explaining the side effects of gastrointestinal ulcer and renal dysfunction [13-19]. Medicinal plants with antipyretic properties are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents and antipyretic agents should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic, anti-inflammatory and antipyretic drugs with low toxicity profile [20].

Kleinia grandiflora (Wall. ex DC.) N.Rani (Family: Asteraceae), commonly known as "Common Fleshy Rag-weed," has been used for the treatment of ailments, such as aperient and used externally for pimples [21]. Traditionally, the plant is named as Muyal Kathilai (Tamil). The fresh leaf juice is used to treat earache [22-24]. However, no scientific data are available to validate the folklore claim. Therefore, this work was aimed at the scientific validation of the ethnopharmacological claim about the antipyretic activities of the leaf extracts of *Kleinia grandiflora*. In addition, the plant extracts were also subjected to preliminary phytochemical analysis and acute toxicity studies.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Kleinia grandiflora* was collected from their natural habitat at Grizzled Giant Squirrel Wildlife Sanctuary, Virudhunagar district, Tamil Nadu state, India in January 2011 and identified by referring the local flora [25]. The specimen (No: VHNSN 407/2012/TN) was deposited in the Department of Botany, V.H.N.Senthikumara Nadar College (Autonomous), Virdhunagar for future reference. The plant leaves were washed and rinsed with tap water to remove all the dirt and unwanted particles prior to the drying process. Then the plant parts were kept under a shad dried for 3-4 weeks at room temperature (27 ± 1° C). The leaves then pulverized into a coarse dry powder (<1 mm from our observation) with a mechanical grinder and passed through 60# sieve and stored in airtight container.

The dried powder material was defatted with petroleum ether (60-80^{o}c) then successively extracted with 95% methanol in the ratio of 1:10 (w/v) and double distilled water using Soxhlet extractor. The methanolic and aqueous extracts were dried under reduced pressure using a rotary vacuum evaporator. The aqueous extract was spray dried further to remove trace of solvent. The extracts were kept in refrigerator (4°C) for future use [26]. Immediately before use, the extract was dissolved in normal saline at concentrations required to produce doses of 200 and 400 mg/kg and administered before subjecting animals to the respective assays.

Phytochemical screening

Freshly prepared *Kleinia grandiflora* extracts were subjected to preliminary phytochemical screening tests for the detection of various phytoconstituents using conventional protocol [27, 28].

Pharmacological screening

Drugs

Paracetamol was obtained from Ranbaxy Chemicals, Bangalore. All other analytical grade chemicals used in the experiments were purchased from Sigma-Aldrich, U.S.A. All preparations were freshly made in distilled water prior to the experiments.

Experimental animals

Healthy adult cross-bred *Wistar* albino rats (weighing 110-170 g) were used throughout the experiment. Animals were procured from the animal house of Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi. Albino rats of either sex was kept under standard environmental conditions (12:12 hour light/dark cycle at 25 ± 2^{o}C and relative humidity of 45-55%) in sanitized polypropylene cages. Standard animal feed and drinking water were provided *ad libitum* throughout the experimental period. The animals were acclimatized to laboratory conditions one week prior to the initiation of experimental work to minimize if any of non-specific stress. The animals were divided into six groups of four animals each. Institutional Animal Ethical Committee IAEC (SBCP/ 2012-2013/CPCSEA/IAEC-III/07) has approved the experimental protocol and care of animals was taken as per the guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Acute toxicity study

Acute oral toxicity study was performed as per revised OECD [29] (Organization for Economic Cooperation and Development) guidelines No. 425. *Wistar* rats (n = 3) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which a single high dose, 2000 mg/kg of methanolic and aqueous extracts of leaves of *Kleinia grandiflora* suspended in normal saline was administered orally. After a single administration, a sign of toxicity and behaviour was observed each

hour up to the 24 h. If this higher dose caused mortality, then one lesser dose of that higher dose (1000, 800, 400 200, 100 or 50 mg/kg of b.w. consecutively) was administered to the next group of animals and observed for sign of toxicity and behaviour. The same procedure was followed until to find out the highest non-lethal dose in which no mortality was observed.

Study of antipyretic activity by brewer's yeast induced pyrexia model

The antipyretic activity of the methanolic and aqueous extract of leaves of *Kleinia grandiflora* were evaluated using brewer's yeast-induced pyrexia in rats as described by Loux *et al* [30]. Rats were fasted overnight with water *ad libitum* before the experiments. Twenty-four rats were randomly divided into six groups (n=4). Group 1 was treated as positive control and received 10 ml/kg/b.w of normal saline. Group 2 served as negative control which received paracetamol (A NSAID drug) 150 mg/kg/b.w suspended in 1% DMSO which served as standard anti-pyretic agent. Group 3 and 4 were treated with 200 mg/kg/ml (sub maximal dose) and 400 mg/kg/ml (maximal dose) of methanolic extract of *Kleinia grandiflora* leaves suspended in DMSO respectively. The 5 and 6 groups were treated with 200 mg/kg/ml (submaximal dose) and 400 mg/kg/ml (maximal dose) of aqueous extract of *Kleinia grandiflora* leaves respectively. The normal body temperature (pre-treatment temperature) of each rat was recorded before starting of the experiments. The fever was induced by administering 10 ml/kg of 20% w/v aqueous suspension of brewer's yeast in normal saline subcutaneously into the animal's dorsum region. All groups were fasted overnight but allowed free accesses to drinking water and after 24 h rectal temperature of each rat was recorded. The induction of pyrexia was confirmed by rise in temperature more than 0.5°C, while animals showed rise in temperature less than 0.5°C were excluded from experiment [31]. All the drugs were administered by orally. Rectal temperature was determined by digital thermometer (Model No: 461 R) at 1, 2, 3 and 4 hrs after test extract/reference drug administration.

The percent reduction in pyrexia was calculated by the following formula.

$$\text{Percent reduction} = \frac{B - C_n}{B - A} \times 100$$

Where, B represents temperature after pyrexia induction; Cn temperature after 1, 2, 3 and 4 h and A, normal body temperature.

Statistical analysis

The data are expressed as the means ± S.E.M. and statistical significance was determined using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's t-test for multiple comparisons. A probability level of less than 5% ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of methanolic and aqueous extract of *Kleinia grandiflora* demonstrated the presence of flavonoids, saponins, tannins, coumarin, xanthoprotein and alkaloids (Table 1), which are suggested to act synergistically to exert the observed pharmacological activity [32-34]. The fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound, is always overlooked. Moreover, this may help to nullify the toxic effects (if any) of individual constituents.

Acute toxicity study

The methanolic and aqueous extracts of *Kleinia grandiflora* plant did not produce any significant toxic symptoms or mortality at single dose (2000 mg/kg b.w.) and hence the drug was considered broad nontoxic and safe for further pharmacological screening. So $1/10^{th}$ and $1/5^{th}$ (200mg and 400mg) of extracts were selected for experiments as sub maximal and maximal dose respectively.

Antipyretic study

The effects of methanolic and aqueous extract of leaves of plant *Kleinia grandiflora* on brewer's yeast induced pyrexia in rats are depicted (Table 2 and Figure 1). Both the extract of *Kleinia grandiflora* leaves produced significant ($P<0.05$) antipyretic effect comparable with standard drug paracetamol. Methanolic extract produced dose-dependent activity but aqueous extract not dependent on selected dosage. Peak antipyretic effect was observed by the methanolic extract at 200 mg/kg/b.w at all the time intervals and showed comparable percentage of inhibition as standard drug paracetamol (Figure 1).

Table 1. Result of chemical group tests of the aqueous and methanol extract of *Kleinia grandiflora.*

Extract	Flavonoids	Coumarin	Saponin	Alkaloids	Tannin	Xantho protein
AE	+	+	+	-	+	+
ME	+	+	+	+	-	+

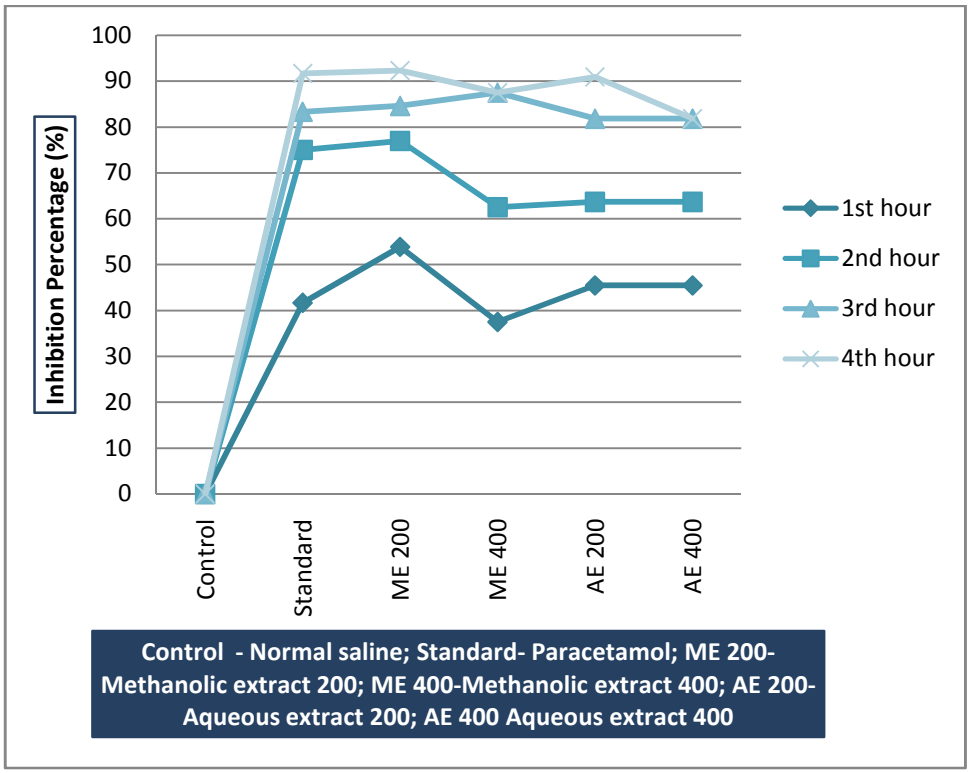
AE: Aqueous extract; ME: Methanol extract

Table 2. Effect of the various extracts of *Kleinia grandiflora* leaves on brewer's yeast induced pyrexia in rats.

Group	Dose (mg/kg)	Pretemperature (°C)	Temp. after induced pyrexia (°C)	Temperature after drug administration (°C) (mean ± S. E. M)			
				1st hour	2nd hours	3rd hours	4th hours
I	Saline (10ml/kg)	37.5±0.359	38.0±0.327	38.2±0.179	38.2±0.249	38.3±0.212	38.3±0.212
II	Paracetamol (150mg/kg)	36.8±0.366	38.0±0.178	37.5±0.187**	37.1±0.188**	37.0±0.171**	36.9±0.212**
III	Methanol (200mg/kg)	37.4±0.168	38.7±0.075	38.0±0.085	37.7±0.063*	37.6±0.111*	37.5±0.155*
IV	Methanol (400mg/kg)	37.4±0.081	38.2±0.085	37.9±0.064	37.7±0.048*	37.5±0.047**	37.5±0.040*
V	Aqueous (200mg/kg)	37.1±0.170	38.2±0.232	37.7±0.108	37.5±0.091*	37.3±0.108**	37.2±0.147**
VI	Aqueous (400mg/kg)	37.1±0.217	38.2±0.193	37.7±0.149	37.5±0.143*	37.3±0.189**	37.3±0.201**
One-way ANOVA			F	03.13	05.86	08.081	07.56
			P	P<0.05	P<0.01	P<0.001	P<0.01

*n=6 in each group. Values mean ±S.E.M, *P<0.05, ** P<0.01*

Figure 2. Effect of the various extracts of *Kleinia grandiflora* leaves on brewer's yeast induced pyrexia in rats.



The yeast induced fever is a well-established model for assessing antipyretic effect and it has been used in a number of studies [35]. Yeast-induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins [36] in central nervous system. Baker's yeast binds to an immunological protein called Lipopolysaccharide-Binding Protein (LBP). The binding results in the synthesis and release of various endogenous cytokine factors such as interleukin 1 (IL-1), interleukin 6 (IL-6) and the tumor necrosis factor-alpha which in turn activate the arachidonic acid pathway and ultimately results in the synthesis and release of prostaglandin E2 (PGE2) in the surroundings of the hypothalamic thermoregulator centers [37]. PGE2 is the ultimate mediator of the febrile response induced by the baker's yeast [38-40]. It slows the rate of firing of warm sensitive neurons and results in increased body temperature. The set-point temperature of the body will remain elevated until PGE2 is no longer present [41, 42]. It may therefore be plausible to conclude that inhibition/reversion of the synthesis of prostaglandins

or COX is the possible mechanisms that contribute to antipyretic activities of the methanolic and aqueous extract of *Kleinia grandiflora*.

The flavonoids are known to inhibit prostaglandin synthetase [43, 44]: an enzyme which is involved in the pyrexia. As some flavonoids are act as a predominant inhibitors of cyclooxygenase or lipooxygenase [45, 46]. In many earlier studies, flavonoids have been reported to exhibit antipyretic effect [47, 48]. Therefore, it appears that the flavonoids content of the methanolic and aqueous extract of *Kleinia grandiflora* may also be responsible for its antipyretic activity.

CONCLUSION

Therefore, the plant extract of *Kleinia grandiflora* possesses a significant antipyretic effect in brewer's yeast induced elevation of body temperature in rats. These results support the traditional use of this plant in pain and related conditions. However, further studies are necessary to examine underlying mechanisms of antipyretic activities and to isolate the active compound (s) responsible for these pharmacological activities.

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REFERENCES

[1] JW Sheldon; MJ Balick; Laird SA. *Econ. Bot*., **1997**, 12, 1-104.
[2] A Chaturvedi; MM Kumar; G Bhawani; H Chaturvedi; M Kuma; Goel KR. *Indian J. Physiol. Pharmacol*., **2007**, 51, 131-140.
[3] Ghosh A. *Indian J. Trad. Knowledge*, **2003**, 2, 393-396.
[4] PP Sharma; Mujundar AM. *Indian J. Trad. Knowledge*, **2003**, 2, 292-296.
[5] SK Raghav; B Gupta; C Agrawal; K Goswami; Das, HR. *J. Ethnopharmacol*., **2006**, 104(1-2), 234-239.
[6] HP Rang; MM Dale; JM Ritter; RJ Flower; Henderson G. In Pharmacology, 7th ed., Elsevier Churchill Livingstone, Edinburgh, UK, **2011**.
[7] D Chattopadhyay; G Arunachalam; L Ghosh; K Rajendran; AB Mandal; Bhattacharya SK. *J. Pharm. Pharma. Sci*., **2005**, 8, 558-564.
[8] Zeisberger E. *J. Therm. Biol*. **1999**, 24, 287-326.
[9] J Roth; C Rummel; SW Barth; R Gerstberger; Hübschle T. *Neurol. Clin*., **2006**, 24, 421-439.
[10] L Cheng; L Ming-liang; Lars B. *Acta Pharmacol. Sin*., **2005**, 26, 926-933.
[11] PJ Veugelers; JM Kaldor; SA Strathdee; KA Page-Shafer; MT Schechter; RA Coutinho; IP Keet; Van Griensven GJ. *J. Infect. Dis*., **1997**, 176, 112-117.
[12] MM Coelho; G Luheshi; SJ Hopkins; IR Pela; Rothwell NJ. *Am. J. Physiol*. **1995**, 269, 527-535.
[13] S Revathi; AK Gupta; LK Soni; S Kavitha; R Wagh; Kaskhedikar SG. *J. Pharm. Biomed. Anal*., **2006**, 42, 283-289.
[14] WJ Tsai; YJ Shiao; SJ Lin; WF Chiou; LC Lin; TH Yang; CM Teng; TS Wu; Yang LM. *Bioorg. Med. Chem. Lett*., **2006**, 16, 4440-4443.
[15] L Navidpour; H Shafaroodi; K Abdi; M Amini; MH Ghahremani; AR Dehpour; Shafiee A. *Bioorg. Med. Chem*., **2006**, 14, 2507-2517.
[16] L Navidpour; M Amini; H Shafaroodi; K Abdi; MH Ghahremani; AR Dehpour; Shafiee A. *Bioorg. Med. Chem. Lett*. **2006**, 16, 4483-4487.
[17] A Moreau; PN Rao; Knaus EE. *Bioorg. Med. Chem*., **2006**, 14, 5340-5350.
[18] G Dannhardt; Kiefer W. *Eur. J. Med. Chem*., **2001**, 36, 109-126.
[19] SA Kraemer; EA Meade; DeWitt DL. *Arch. Biochem. Biophys*., **1992**, 293, 391-400.
[20] M Gupta; UK Mazumder; P Gomathi; Thamil Selvan V. *BMC Complement. Altern. Med*., **2006**, 6(36), 1-6.
[21] Khare CP. In Indian medicinal plants: An illustrated dictionary, Springer-Verlag Heidelberg Publication, New York, **2007**.
[22] B Sandhya; S Thomas; W Isabel; Shenbagarathai R. *Afr. J. Trad. CAM*, **2006**, 3(1), 101-114.
[23] S Ganesan; NR Pandi; Banumathy N. *eJ. Indian Med*., **2008**, 1, 1-18.
[24] Kottaimuthu R. *Ethnobot. Leaflets*, **2008**, 12, 195-203.
[25] Gamble JS. In; Flora of the Presidency of Madras, Botanical Survey of India, Calcutta, **1936**.
[26] R Ilavarasan; M Mallika; Venkataraman S. *Afr. J. Trad. CAM*, **2005**, 2(1), 70-85.
[27] GE Trease; Evans WC. In A Text Book of Pharmacognosy, Academic Press, London, **1989**.
[28] Harborne JB. In Phytochemical methods. A guide to modern techniques of plant analysis, Chapman and Hall Ltd., London, **1973**.

[29] OECD, Guidelines for testing of chemicals, Revised Draft Guidelines 425: Acute Oral Toxicity-Up and Down Method, **2006**.
[30] JJ Loux; PD De Palma; Yenkell SL. *Toxicol. Appl. Pharmacol.* **1972**, 22 (4), 672-675.
[31] J Kang; M Khan; N Park; J Cho; M Lee; H Fujii; Hong Y. *J. Ethnopharmacol*., **2008**, 116(1); 187-190.
[32] J Maj; Rogoz Z. *Pol. J. Pharmacol*., **2000**, 52, 111-114.
[33] BA Chindo; S Amos; AA Odutola; HO Vongtau; J Abah; C Wambebe; Gamaniel KS. *J. Ethnopharmacol*., **2003**, 85, 131-137.
[34] IO Ishola; AJ Akindele; Adeyemi OO. *J. Ethnopharmacol*., **2011**, 135, 55-62.
[35] GK Chatterjee; TK Bunan; A Nagchandhum; Pal SP. *Planta Med*., **1983**, 48(2), 116-119.
[36] Moltz H. *Neurosci. Biobehav. Rev*., **1993**, 17(3), 237-269.
[37] GH Chan; Fiscus RR. *Exp. Gerontol*., **2004**, 39(3), 384-394.
[38] Al-Ghamdi MS. *J. Ethnopharmacol*., **2001**, 76, 45-48.
[39] B Uzcategui; D Avila; H Suarez-Roca; L Quintero; J Ortega; Gonzalez B. *Invest. Clin*., **2004**, 45, 317-322.
[40] R Botting; Ayoub SS. *Prostaglandins, Leukotrienes and Essent. Fatty Acids*, **2005**, 72(2), 85-87.
[41] FA Santos; Rao VS. *J. Pharm. Pharmacol*., **1998**, 50, 225-229.
[42] M Ryan; Levy MM. *Crit. Care*, **2003**, 7, 221-225.
[43] K Rajnarayana; MS Reddy; Chaluvadi MR. *Indian J. Pharmaceutical Sciences*, **2006**, 68(3), 380-384.
[44] A Chakraborty; BRK Devi; R Sanjebam; S Khumbong; Thokchom IS. *Indian J. Pharmacol*., **2010**, 42(5), 277-279.
[45] AG Mathew; Parpia, HAB. In; CO Chichester; EM Mark; Stewart GF. Food browning as a polyphenol reaction, Advances in food research, Academic Press, New York, **1971**, 75-145.
[46] K Rajnarayana; MS Reddy; MR Chaluvadi; Krishna DR. *Indian J. Pharmacol*., **2001**, 33, 2-16.
[47] J Baumann; SV Von Brucchau; Wurm G. *Prostaglandins*, **1980**, 20, 627-639.
[48] Brasseur T. *J. Pharm. Belg*., **1989**, 44, 235-241.