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ANTIPYRETIC ACTIVITY OF VARIOUS KARIYAT EXTRACTS IN EXPERIMENTAL ANIMALS

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ABSTRACT
The present study was undertaken to evaluate antipyretic activity of various kariyat extract in various experimental animals models. Antipyretic activity was carried out using Brewer's yeast inducing pyresia in rats. Acute toxicity study was conducted for the extract and the test drug doses for screening were selected depending upon LD_{50} values Aqueous and ethanolic extracts of kariyat in the dose of 200 & 400 mg/kg body weight per oral were used. Aspirin was used as standard for antipyretic activity. Aqueous (KAE) and ethanolic (KEA) extract at higher dose (400 mg/kg) showed significant ($P<0.01$) decrease in elevated body temperature when compared with the control group. The maximum percentage reduction of 81.25% was observed for aspirin whereas for KEE and KAE was 68.25 and 81.87% respectively after 3 hr.

KEYWORDS: Kiriyat, antipyretic, Brewer's yeast induced pyresia.

INTRODUCTION
Fever is a common medical sign characterized by an elevation of body temperature above the normal range.[1] It is a complex physiologic response to disease mediated by pyrogenic cytokines and characterized by a rise in core temperature, generation of acute phase reactants, and activation of immune system.[2] It serves as body's natural defense against bacteria and viruses which cannot live at a higher temperature. Here, anti-pyretic activity was carried out using Brewer's yeast as inducing agent in rats. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. The global search involves investigating single plant extracts or fractions thereof or mixtures of fractions extracts from different plants, which have been carefully standardized for their safety and efficacy.[3] "Literature survey shows no reported evidence of antipyretic activity hence the present study is taken up to evaluate the same."

Kariyat is an annual herbaceous plant and is extensively cultivated in Southern Asia, China and some parts of Europe and scientifically it is known as *Andrographis paniculata Nees.*, belongs to family Acanthaceae. In English it also called as King of Bitters. In traditional medicine is used to get rid of body heat, dispel toxins from the body; prevent common cold, upper respiratory tract infections including sinusitis and fever and as an antidote against poisons of snakes and insects.[4] The plant has been reported to exhibit various mode of biological activities *in vivo* as well as *in vitro* viz., antibacterial[5], antiviral[6], anti-inflammatory[7] extracts from different plants, which have been carefully standardized for their safety and efficacy.

MATERIALS AND METHODS

Drugs and Chemicals

Aspirin and Formaldehyde were procured from Sigma Aldrich. Brewers Yeast, Tragacanth and Tween 80 from Himedia and Diethyl ether, Glacial acetic acid Ethyl alcohol from S. D Fine chemicals.

Materials

Digital thermometer, Water bath were supplied by the department of Pharmacology, SET'S college of pharmacy Dharwad.

Collection and authentication of the plant material

The aerial parts of plant of kariyat were collected from surrounding areas of Dharwad Hubli in Karnataka. The plant were washed with distilled water, dried at room temperature under shade, It was ground to obtain coarse powder using an electric grinder.

Preparation of Extracts

A. Alcoholic extract

Powdered drug was extracted with ethanol (60°C-80°C), in a continous hot extraction method using Soxhlet extractor. The extracts were concentrated in a rotary flash evaporator (*Hahnvapor, Hahnshin Scifintic Korea)* and residue was dried in a vacuum desiccator over anhydrous calcium chloride to yield ethanolic extract [KEE] The percentage yield of ethanolic extract was calculated.

B. Aqueous extract

Powdered drug was macerated with chloroform water I.P. The mixture was filtered throw muslin cloth and concentrated in vacuum under reduced pressure using rotary flash evaporator (*Hahnvapor, Hahnshin Scifintic Korea)* and then the extract was kept on water bath to obtain crude extract and finally residue was dried in a vacuum desiccator over anhydrous calcium chloride to yield aqueous extract [KAE]. The percentage yield of aqueous extract was calculated.

Experimental Animals

Male wistar rats weighing 150–200 g were used for the study. For acute toxicity study female albino mice were used. The animals were maintained under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 5%) and 12-h lightdark cycles. They were fed commercial stock diet and water, *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET's College of Pharmacy, Dharwad, Karnataka (REG.No.112/1999/CPCSEA) according to the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute Toxicity Studies

Acute toxicity study was carried out using female Albino mice (20-30g) by up and down/staircase method. The kiryat plant extracts were orally administered to mice at the doses of 50, 300, 1000 and 2000 mg/ kg b. w respectively. The general behavior of animals, sign of discomfort and nervous manifestation were obsereved for 48h. The kiryat plant extracts were found to be devoid of mortality of animals at the dose of 2000 mg/kg body weight. Hence the 1/10th (200 mg/kg, p.o.) and 1/20th (100 mg/kg, p.o.) of the doses were selected.

Antipyretic Activity

Preparation of standard[8-9]

Aspirin was used as standard drug (positive control). aspirin (100 mg/kg body wt) was dissolved in normal saline.

Induction of yeast-induced pyrexia

Rats were divided into six groups of six each for this experiment. The normal body temperature of each rat was measured rectally at predetermined intervals and recorded. The rats were trained to remain quiet in a restraint cage. A digital thermometer was inserted 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. The temperature was measured on a thermometer. After measuring the basal rectal temperature, animals were given a subcutaneous injection of 10 ml/kg body wt. of 15% w/v yeast suspended in 0.5% w/v methyl cellulose solution. Rats were then returned to their housing cages. The Animals were restrained after 19 h of yeast injection, in individual cages for another recording of their rectal temperature as described above. By oral administration the animals were given the test compound, standard drug and vehicle. Rectal temperatures were recorded at 1, 2, 3, 4 and 5 h post treatment.

Statistical analysis

All the results were expressed as mean ±SEM. ANOVA by Dunnett Compare all Vs Control.

RESULTS

Alcoholic and aqueous extracts of kariyat were prepared, chemically characterized and screened for the antipyretic activity in animals. Results of percentage yield and physical characteristics of plant extracts of kariyat are presented in the Table 1.

Table 1: Percentage yield and physical characteristics of plant extracts of kariyat.

Extracts	%Yield (gm)	Color	Odour	Consistency
Ethanol Extract	05.53 %	Greenish brown	Characteristic	Salary
Aqueous Extract	9.12 %	Dark Brown	Characteristic	Salary

Results of preliminary photochemical analysis of ethanolic and aqueous extracts of kariyat are presented in Table 2 Extracts showed the presence of carbohydrates, tannins, flavonoids and alkaloids.

Table 2: Preliminary phytochemical analysis of ethanolic and aqueous extracts Plant of kariyat.

Sl. No	Extracts	Phytoconstituents
1	**Ethanol Extract**	Tannins, Carbohydrates, Flavonoids, phenolic compounds, Alkaloids, Terpinoids and Steroids
2	**Aqueous extract**	Saponin glycosides, Carbohydrates, Tannins and phenolic compounds and Alkaloids

The KEE & KAE extracts were subjected to evaluate the antipyretic activity by yeast induced pyrexia in rats. Aspirin was taken as standard drug. Aqueous and ethanolic extract at higher dose (400 mg/kg) showed significant ($P<0.01$) decrease in elevated body temperature when compared with the control group. Results are shown in Table 3 and Figure 1.

Table 3: Antipyretic activity of various extracts of kariyat in rats.

Treatment (Dose/BW)	Initial Rectal Temperature (°C) before yeast injection		Rectal Temperature (°C) after 19th hour of yeast injection			
	Rat	Temperature (°C)	0th hr	1st hr	2nd hr	4th hr
Group 1 (Baker's yeast of 10 ml/kg of 15% w/v yeast suspended in a 0.5% w/v CMC solution	1	38.9	39	39.8	39.1	38.9
	2	40.1	39.9	38.7	40	40.1
	3	39.5	39.4	38.4	39.1	39.5
	4	39.1	39.1	39.1	39.1	39.1
	5	39.6	39	39	38.7	39.6
	6	39.9	39.4	39.4	38.7	39.9
	Average (SD)	**37.52 (0.66)**	**39.52 (0.46)**	**39.3 (0.35)**	**39.07 (0.50)**	**39.12 (0.48)**
Group 2 (Aspirin100mg/kg BW) in a 0.5% w/v CMC	1	37	39.5	37.1	37.3	37.7
	2	37.2	39.7	38	37.2	37
	3	37.5	38.1	38.1	36.4	37.9
	4	37.5	39.9	37.9	37.3	38.2
	5	37.2	39.2	37.8	38.2	37.8
	6	37.5	38.1	38.2	37.7	37.1
	Average (SD)	**37.32 (0.21)**	**39.08 (0.80)**	**37.85 (0.39)**	**37.35 (0.60)**	**37.62 (0.47)**
Group 3 (Kiryat ethanol extract, 400 mg/KG BW) in a 0.5% w/v CMC	1	37.2	39.7	38.1	37.8	37.1
	2	37.2	39.9	38.5	38.5	37.8
	3	37.6	38.5	38.1	37.6	38.6
	4	37.1	38.8	39.3	37.8	38.3
	5	37.1	39.9	38.1	37.1	37.8
	6	37.3	39.7	38.3	37.1	36.9
	Average (SD)	**37.25 (0.19)**	**39.42 (0.61)**	**38.40 (0.47)**	**37.65 (0.52)**	**37.75 (0.66)**
Group 4 (Kiryat aqueous extract, 400 mg/KG BW) in a 0.5% w/v CMC	1	37.2	39.7	38.1	37.8	37.1
	2	37.5	39.3	38.9	37.5	37.6
	3	37	40.1	39.2	37.5	37.5
	4	37.5	39.2	38.3	37	37.6
	5	37.1	39	39.1	37.8	38.3
	6	37.9	39.1	38.5	37.1	37.1
	Average (SD)	**37.33 (0.36)**	**39.37 (0.40)**	**38.82 (0.35)**	**37. 50 (0.41)**	**37.62 (0.39)**

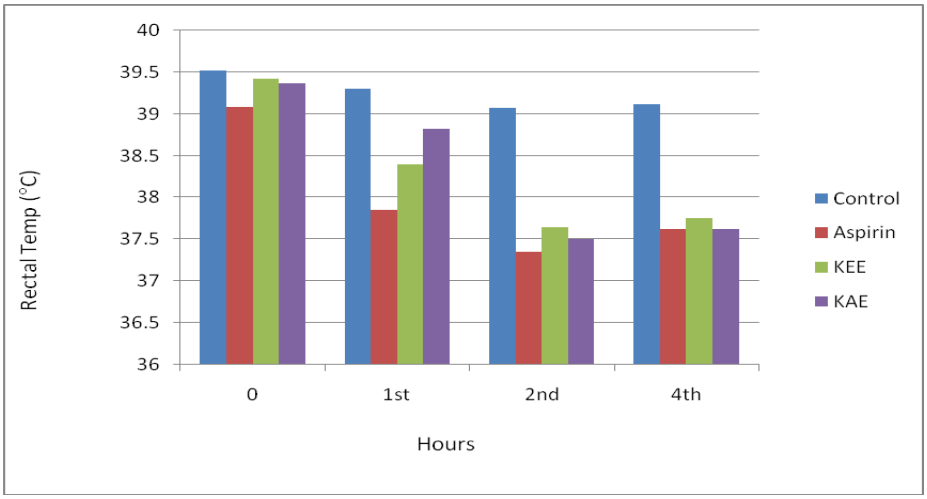

Fig 1: Antipyretic Activity of various extracts of kariyat in rats.

DISCUSSION

In the present research, alcoholic and aqueous extracts of kiryat were prepared and investigated for antipyretic activity in experimental models. Extracts were subjected for acute toxicity study by up and down/staircase method to fix the dose. Since, the main purpose of the preliminary acute toxicity study in animals is to get some idea on conspicuous behavioral changes and death, if any. Since no toxicity or lethality was found with the extract even at higher dose of 4000 mg/kg b.w, two scalar doses (200 & 400 mg/kg b.w) were selected for antipyretic activity.

The present study reveals the aqueous and ethanolic extract of kiriyat possesses a significant antipyretic effect in yeast provoked elevated body temperature. The ethanol and aqueous extract at higher dose caused a significant reduction in body temperature, with the effect being comparable to that of aspirin. Oral administrations of aqueous and ethanolic extracts of plant of kiriyat have suppressed the fever and pain. Aqueous and ethanolic extract at higher dose (400 mg/kg) showed significant ($P<0.01$) decrease in elevated body temperature when compared with the control group. The maximum percentage reduction of 81.25% was observed for Aspirin whereas for KEE and KAE was 68.25 and 81.87% respectively after 3 h.

CONCLUSION

Alcoholic and aqueous extracts of plant of kiryat were investigated for anti-pyretic activity in experimental animals. Significant anti-pyretic activities by reducing elevated temperature in Brewer's yeast induced pyresia in rats and it can be concluded that the plant of kiryat. is endowed with antipyretic properties. Further molecular mechanism studies are required to establish the mechanism of the antipyretic effects.

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