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Analgesic activity of various Kariyat extracts in experimental animals

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Abstract
In the present study the various kariyat extracts were used to evaluate analgesic activity in various experimental animal models. Analgesic activity was carried out using acetic acid induced writhing as well as hot plate method in mice. Acute toxicity studies were conducted for the extracts 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 b.w, p.o, were used. Aspirin was used as standard for analgesic activity. Kariyat ethanolic extract and Kariyat aqueous extract and aspirin inhibited the writhing significantly ($P<0.001$) and extracts showed significant activity in hot plate methods by extending time of reaction.

Keywords: kiriyat, hot plate, analgesic, acetic acid

Introduction
Pain (Algesia) is an ill defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus [1]. The international association for the study of pain (1986) has defined it as, "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [2]. Here, analgesic activity was evaluated using Acetic acid induced writhing and hot plate methods. Literature search indicated not much work carryout on analgesic activity of alcoholic and aqueous extracts of Kariyat (*Andrographis paniculata)* on experimental animals. 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]

Fig 1

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. It is a valuable bitter tonic useful in general debility, in convalescence after fevers and in the advanced stages of dysentery. It acts as gentle aperients and is said to prove very useful in many forms of dyspepsia attended with torpidity of the bowels. It may be used in conjunction with other remedies required.

Materials and Methods
Drugs and Chemicals
Diclofenac, Aspirin and Formaldehyde were procured from Sigma Aldrich. Tragacanth and Tween 80 from Himedia and Diethyl ether, Glacial acetic acid Ethyl alcohol from S. D Fine chemicals.

Materials
Digital thermometer, Hot plate and Water bath were supplied by the department of Pharmacology SET'S College of pharmacy, Dharwad, Karnataka, India

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 continues 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 desiccators 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 light dark 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 observed 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.

***In-Vivo* Analgesic Activity**

A. Writhing test

Table. Acetic acid-induced Writhing test [4-5]

The swiss albino mice were divided into 6 groups, each containing 6 rats as follows

Table 1

Groups	Treatment
Group 1	0.6% v/v Acetic acid 10ml/kg i.p.
Group 2	0.6% v/v Acetic acid + aspirin 20mg/kg p.o.
Group 3	0.6% v/v Acetic acid + KEE (200 mg/kg)
Group 4	0.6% v/v Acetic acid + KEE (400 mg/kg)
Group 5	0.6% v/v Acetic acid + KAE (200 mg/kg)
Group 6	0.6% v/v Acetic acid + KAE (400 mg/kg)

The animals were administered orally with standard drug (aspirin 20mg/kg p.o), test extract (KEE and KAE) and vehicle (distilled water); 30 min later the animals were injected with 0.6% v/v acetic acid intraperitoneally (i.p.) in a dose of 10ml/kg to induce the characteristic writhings. Number of wriths occurring between 5 and 15 min was recorded. The response of treated animals was compared with that of control.

B. Hot plate method [6-7]

Swiss albino mice were divided into six groups of five in each. The mice were pretreated orally with standard drug with standard drug diclofenac (20 mg/kg), test drugs KEE (200 mg/kg and 400 mg/kg) and KAE (200 mg/kg and 400 mg/kg) respectively 30 min before experiment. The mice were dropped on the hot plate maintained at 55˚C and the latency to flick the hind paw or lick the hot plate was the reaction time noted at 0, 30, 120, and 240 minutes. The cut off time was considered as 15 seconds.

Statistical analysis

The data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post -test using Graph Pad Prism version 5.0, USA.

Results

Alcoholic and aqueous extracts of kariyat were prepared, chemically characterized and screened for the antipyretic and analgesic activities in animals. Results of percentage yield and physical characteristics of plant extracts of kariyat are presented in the Table 2

Table 2: 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 3 Extracts showed the presence of carbohydrates, tannins, flavonoids and alkaloids.

Table 3: Preliminary phytochemical analysis of ethanolic and aqueous extracts plant of kariyat

S. 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

Table 4 Acetic acid induced writhing in mice

KEE and KAE aspirin at all tested dose significantly ($P<0.001$) inhibited the writhing produced when compared with control (Table 4). The percentage inhibition of Aspirin was 89.37% and KEE and KAE was 71.91 and 75.85% respectively, (Table 4 and Figure 1)

Table 4

	No writhes in 5-15 min post inj of acetic acid			
Animal no	Control	KEE	KAE	STANDARD
1	41	12	10	1
2	42	13	10	9
3	38	12	4	3
4	20	2	5	2
5	15	1	8	2
6	22	10	6	2
Average (SD) % Inhibition	29.67 (11.98)	8.33 (5.39) 71.91%	7.17 (2.56) 75.85%	3.17 (2.93) 89.33%

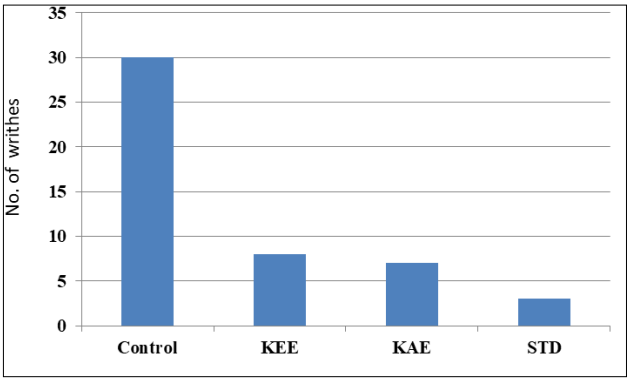

Fig 1: Analgesic activity by acetic acid induced writhing in mice.

Results are shown in Table 5 and Figure 2, the standard drug significantly (P< 0.001) inhibited the pain threshold by extending reaction time to 9.83 and 8.0 sec at 3rd and 6th hr. respectively when compared with control. KEE extract had also extended up to 6.0 and 4.17 sec at 3rd and 6th hr. respectively and KAE showed 5.67 and 4.15 sec at 3rd and 6th hr respectively.

Table 5: Results of analgesic activity of various extracts of kariyat by hot plate methods.

Group	Rat	Reaction time in sec. at					
		0	30 min	1 hr	2 hr	3 hr	6 hr
Control	1	2	2	5	2	4	1
	2	2	5	4	4	2	2
	3	5	3	2	2	3	2
	4	3	4	2	2	4	4
	5	4	7	4	3	5	4
	6	4	2	2	2	1	2
	Average (SD)	3.33 (1.21)	3.83 (1.94)	3.17 (1.33)	2.5 (0.84)	3.17 (1.47)	2.5 (1.22)
KEE	1	1	5	5	2	9	6
	2	2	6	5	2	7	5
	3	5	5	6	6	3	7
	4	4	5	5	9	9	4
	5	4	7	2	9	5	2
	6	3	9	9	6	3	1
	Average (SD)	3.17 (1.47)	6.17 (1.60)	5.33 (2.25)	5.67 (3.14)	6 (2.76)	4.17 (2.32)
KAE	1	5	4	6	2	5	9
	2	2	6	2	3	7	5
	3	5	5	6	6	3	5
	4	4	2	2	4	5	1
	5	2	7	2	9	5	2
	6	3	2	9	7	9	3
	Average (SD)	3.50 (1.38)	4.33 (207)	4.5 (2.95)	5.17 (2.64)	5.67 (2.07)	4.15 (2.86)
STD	1	3	5	10	9	9	5
	2	5	7	9	11	5	9
	3	4	1	8	12	8	8
	4	2	2	7	10	12	9
	5	1	6	8	7	14	7
	6	2	9	6	9	11	10
	Average (SD)	2.83 (1.47)	5.00 (3.03)	8.00 (1.41)	9.67 (1.75)	9.83 (3.19)	8.0 (1.79)

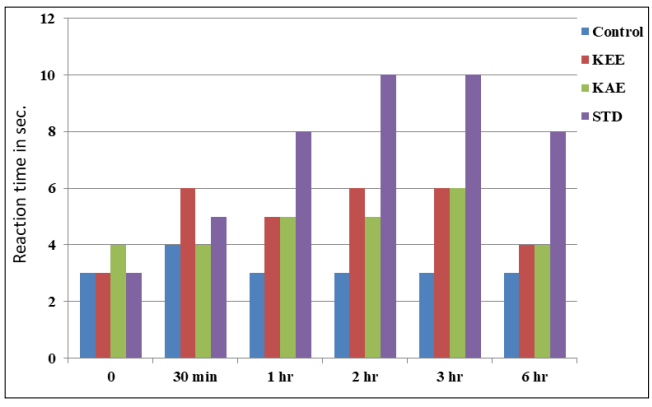

Fig 2: Results of analgesic activity of various extracts of kariyat.

Discussion

In the present research, alcoholic and aqueous extracts of kariyat were prepared and investigated for analgesic activities 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 analgesic activities. The methods for testing analgesic activity were selected peripherally mediated effects were investigated. The hot plate methods and acetic acid induced abdominal

constriction elucidated peripheral. This test is very useful not only for assessing analgesic drugs but also helping in the elucidation of mode of action In this study, aqueous and ethanolic extracts of plant of kariyat at tested dose significantly ($P<0.001$) inhibited the writhing produced when compared with control. In case of the analgesic activity ethanolic extracts of plant of in kariyat inhibited the pain threshold by extending reaction time to 9.83 and 8.0 sec at 3rd and 6th hr.

Conclusion

Alcoholic and aqueous extracts of plant of kiryat. were investigated for analgesics activities in experimental animals. Significant analgesic activity was studied using acetic acid induced writhings as well as hot plate method in mice and it can be concluded that the plant of kiryat. is endowed with peripheral acting analgesic properties. Further molecular mechanism studies are required to establish the mechanism of the analgesic effects.

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