PHYTOCHEMICAL INVESTIGATION, ANALGESIC AND ANTIPYRETIC ACTIVITIES OF ETHANOLIC EXTRACT OF KARIYAT

Sachin Annasaheb Nitave 1,2, Nilesh B. Chougule 1,3 and Kailasam Koumaravelou 4

Centre for Research and Development 1, PRIST University, Vallam, Thanjavur - 613403, Tamil Nadu, India.
Dr. J. J. Magdum Trust’s 2, Anil Alias Pintu Magdum Memorial Pharmacy College Dharangutti, Shirol, Kolhapur - 416101, Maharashtra, India.
Shree Pushpasen Sawant College of D. Pharmacy 3, Kudal - 416820, Sindhudurg, Maharashtra.
Prist University 4, Pondicherry Campus - 605007, Pondicherry, Tamil Nadu, India.

ABSTRACT: An ethanolic extract of Kariyat (Andrographis paniculata) was prepared by using Soxhlet extractor. Kariyat ethanolic extract (KEE) was subjected for phytochemical investigations and screened for analgesic and antipyretic activities in experimental animals. High performance thin layer chromatography (HPTLC) and gas chromatography mass spectrometry (GC-MS) investigations indicated presence of steroids, phenols, terpenoids, alkaloids, saponins, flavonoids etc. The various concentrations ethanolic extract were tested for analgesic activity in mice by using acetic acid-induced writhing in mice and hot-plate method. The maximum protection of 56.23 % against writhing movement was observed at 300mg/kg when compared to standard drug which showed 79.43% protection. Analgesic activity of KEE using hot plate method indicates mean basal reaction time increased significantly in the treated group compared to control group from 30 min onwards. The various concentrations of KEE also tested for antipyretic activity in rats by using Brewer’s Yeast Induced Hyperpyrexia and results significant antipyretic activity 1 hr after drug administration. Mechanism of action was determined by studying prostaglandin inhibition studies. Results indicated that KEE produces action by inhibiting the synthesis of prostaglandin. The findings of present study indicate ethanolic extract of Kariyat shows significant analgesic and antipyretic activity.

INTRODUCTION: Andrographis paniculata commonly called as Kariyat and known as king of bitters or kalmegh used in ancient oriental and Ayurvedic medicine. Only a few are popular for their use in folk medicine for assorted health concerns.

This herb is found in different phytogeographical and edaphic zones of India, China, America, West Indies and Christmas Island. 1-5 Research work carried out on Kariyat has confirmed the broad range of pharmacological effects, some of them extremely beneficial. Kariyat is prominent in twenty six Ayurvedic formulations as evidenced from Indian Pharmacopoeia while in Traditional Chinese Medicine it is an important “cold property” herb used to release body heat in fever. 1 The species is well explored therapeutically and effectively used as immunostimulant and reduce fatigue 2 and for asthma, gonorrhea, pile 3, cancer 4, blood purification 5, influenza 6, gastric complaints,
diarrhea, pharyngitonsillitis, fever, loss of scalp hair, snake bite, myocardial ischemia, common cold, diabetes, respiratory tract infections, jaundice among others. The species also possesses antiulcerogenic, antityphoid, anti-snake venom, antiplatelet aggregation, anti HIV, antimalarial, antifertility, anti-inflammatory and antihyperglycemic properties. Ethanolic extract of the leaves of A. paniculata was reported to inhibit growth of Escherichia coli and Staphylococcus aureus while methanolic extract was effective against Proteus vulgaris. Komwatchara and Rassameemsmaung reported that A. paniculata has inhibitory effect against Porphyromonas gingivalis.

Prajjal et al. reported significant antimicrobial activity of aqueous extract containing andrographilide and arabinogalactan proteins. Roy et al. A. paniculata has been reported as having antifungal, antiviral, hepatoprotective, antibacterial activity. There is no much information is available on mechanism of action of ethanolic extract of Kariyat for analgesic and antipyretic activities hence, the present study was undertaken to investigate the analgesic and antipyretic activities of A. paniculata plant and its possible mechanism of action in experimental animals.

MATERIALS AND METHODS:
Collection and Authentication of the Plant Material: The aerial parts of plant of Kariyat were collected from surrounding areas of Taluka Shirol, District Kolhapur and Maharashtra. The plant was washed with distilled water, dried at room temperature under shade. The sample was identified and authenticated by “Nikhil analytical & research laboratory (ISO 9001-2008 certified, central govt. Approved for Agmark), Sangli, Maharashtra, India as well as by Head, Department of Botany Dattajirao Kadam Arts, Science & Commerce College, Ichalkaranji, Taluka Hatkanagale, Dist. Kolhapur, Maharashtra, India.

Preparation of Kariyat Ethanolic Extract (KEE): Dried arial part of the Kariyat was grinded to obtain coarse powder using an electric grinder. Powdered drug was extracted with ethanol in 25% concentration (25 gm of weighed Kariyat powder and 100 ml of solvent at 60°C - 70°C), in a continuous hot extraction method using Soxhlet extractor. The extract was concentrated in a rotary flash evaporator (Hahnvapor, Hahnshin Scifintic Korea) and residue was dried in vacuum desiccators over anhydrous calcium chloride to yield ethanolic extract (KEE). The extract was stored in refrigerator at 4°C for further use.

Phytochemical Screening: Preliminary qualitative phytochemical analysis of the extract was carried out using various procedures to detect the presence of several phytoconstituents.

HPTLC Analysis: HPTLC analysis was carried out using CAMAG Linomat HPTLC instrument. Sample preparation was done by taking weighed quantity of extract residue was dissolved in 1ml of chromatographic grade methanol which was used for sample application on pre-coated silica gel 60 F 254 aluminium sheets.

Developing Solvent System: A number of solvent systems were tried, for each extract for better resolution and maximum number of spots, but the satisfactory resolution was obtained in the solvent of tolnaftate: ethyl alcohol: formic acid, 10:3:1.

Sample Application: Application of bands of each extract was carried out (15mm in length and 3μl in concentration) using spray technique. Samples were applied in duplicate on pre-coated silica gel 60 F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of Chromatogram: After the application of sample, the chromatogram was developed in Twin trough glass chamber 10 x 10 cm saturated with and solvent system in and tolnaftate: ethyl alcohol: formic acid: 10:3:1 for aqueous extract.

Detection of Spots: 3mg/ml concentration of extracts were prepared in methanol of chromatographic grade and then filtered by whatman filter paper No. 1. Prepared samples of different extracts were applied on TLC aluminium sheets silica gel 60 F 254 (Merck) 07 μl each with band length of 8 mm, sample applicator set at a speed of 150 nl/sec.
GC-MS Analysis: GC-MS analysis was carried out on a Turbomass 2017. The column used was capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. Sample injection volume of 1 micro litter was utilized and inlet temperature was maintained as 250 °C. The oven temperature was programmed initially at 110 °C for 4 min, then an increase to 240 °C. And then programmed to increase to 280 °C at a rate of 20 °C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200 °C. The source temperature was maintained at 180 °C. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library.

Animals Care and Handling: The experiment was approved by the institutional animal ethics committee of S. E. T. College of Pharmacy, S. R. Nagar Dharwad (REG. NO. 112/1999/CPCSEA, Proposal No. 2 Dated 02/02/2017). The experiment was carried out on Albino rats of wistar strain and Swiss albino mice of both sexes weigh around 200 grams and 25 grams respectively. Animals were procured from Venkateshwara Enterprises, Bangalore and were housed in the animal house. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25± 2°C relative humidity 44 to 56 % and light and dark cycles of 12:12 hours, fed with standard pallet diet and water ad libitum during experiment. The animals were kept in polypropylene cages in groups. The research was conducted in accordance with the ethical rules on animal experimentation.

Pharmacological Screening: Assessment of Analgesic Activity:
Eddy’s Hot Plate Method: The mice were divided into 5 groups of six mice in each group. Group 1 was control received only vehicle. Mice in group 2nd, 3rd and 4th were given KEE orally with doses of 100, 200 and 300 mg / kg respectively. Before administration of drugs animals were fasted for 12 hours. Animals in 5th group received aspirin 150 mg/kg dose orally. The hot plate was maintained at 55±0.5°C and the animals were placed into the perspex cylinder on the heated surface and time to discomfort reaction like licking paws or jumping was recorded as response latency by using a stop watch. Each of the mice was placed on the hot plate and the latency was recorded after 0 min, 30 min, 60 min, 90 min and 120 min after the administration of test and standard drug. A cutoff time of 20 seconds was considered. This short lasting stimulus elicited from the hot plate surface causes little or no damage at all to paw tissues.

Acetic Acid Induced Writhing: The analgesic activity of KEE was also investigated by the acetic acid-induced writhing test in mice. The mice were divided into 5 groups of 6 mice in each group. A freshly prepared solution 0.6% acetic acid in the volume of 10 ml/kg was administered intra peritoneally to each animal of all groups. Administration of KEE and Aspirin 150 mg/kg dose is done by oral route 1 hr prior to acetic acid injection. The mice in group 1 received only vehicle treated as control group. KEE in doses of 100, 200 and 300 mg / kg were administered for animals in group 2nd, 3rd and 4th respectively. Aspirin 150 mg/kg dose orally administered in 5th group and treated as standard. The mice were placed singly in glass jars and numbers of writhing movements were counted for following acetic acid injection. Percentage of reduction in writhing syndrome was calculated and compared with control group. Percent reduction indicates the percentage protection against abdominal constriction which was taken as an index of analgesia.

\[
\text{% Inhibition} = \frac{(Wc-Wt) \times 100}{Wc}
\]

Where \(Wc\) = No. of writhing of the control group
\(Wt\) = No. of writhing of the treated group

Screening of Antipyretic Activity: Antipyretic study was carryout using Brewer’s Yeast Induced Hyperpyrexia as per method described by Adam et al., (1968). The animals used for this study were fasted over night before the experiment but water was made available ad libitum. Rats of either sex were randomly divided into five groups (1 to 5) containing six rats per groups. Pyrexia was induced by subcutaneous injection of 20 % (w/v) brewer’s yeast suspension (10 ml / kg) in the dorsum region
of the rats. 17 hrs after injection, the rectal temperature of each rat was measured, using a
digital thermometer. Only rats that showed an increase in temperature of at least 0.7°C were used
for this study. The rats in group 2nd to 4th were given (orally) 100, 200, and 300 mg / kg of the
KEE respectively while those in group 5 animals
were administered with Aspirin 150 mg/kg. The
temperatures were subsequently measured at 0, 1, 2
and 3 hours post extract administration and the
mean temperature of each group was recorded 45,46.

Screening of Prostaglandins Inhibition: Screening
of Prostaglandins inhibition was determined by
inhibition of Castor oil induced diarrhea in rats. In
the present study animals were divided into three
groups of six rats each. Group 1 was administered
vehicle orally and served as control. Group 2
served as standard and received aspirin (150
mg/kg) orally. Group 3 was administered with KEE
300 mg/ kg by orally. Before administration of test
and standard drugs animals were fasted overnight
but allowed free access to water. After 30 min of
administration of above dose all the rats were given
with 1 ml of castor oil orally. The numbers of wet
fecal dropping were measured for four hours 49.

Statistical Analysis: All data was expressed as
mean ± standard error of the mean (S.E.M.) of 6
rats/mice per experimental group. Statistical
analysis was performed using Graph pad prism 5.0
statistical software. Parametric one way analysis of
variance (ANOVA) followed by Tukey’s post test.
The minimal level of significance was identified at
P < 0

RESULTS AND DISCUSSION: Extraction of
Kariyat was carried out by continuous hot
extraction method by Soxhlet apparatus using ethyl
alcohol. Temperature of apparatus was kept around
60 - 70°C to avoid degradation of chemical
constituents. Thus obtained ethanolic extract was
further concentrated by rotary evaporator to get
greenish brown colored slurry and around 8.13 %
yield was obtained (Table 1). Extracts was
subjected for preliminary qualitative analysis to
detect the presence of several phytochemicals like
alkaloids, flavonoids, steroids, saponins, cardiac
glycoside, tannins, terpenoids and free amino acid
etc. and confirmed the presence of aleurone grains,
aldehyds, amino acids, carbohydrate, fat & fixed
oils, flavonoids, glycoside, inulin, mucilage,
tannin, proteins, steroids and triterpenoids. These
results were further confirmed by spectral and
chromatographic studies.

<p>| TABLE 1: RESULTS OF % YIELD OF ETHANOLIC EXTRACT OF KARIYAT |
|-----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Batch</th>
<th>Initial weight of powder drug in grams</th>
<th>Weight of extract</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25.4</td>
<td>1.87</td>
<td>7.37</td>
</tr>
<tr>
<td>II</td>
<td>26.1</td>
<td>2.21</td>
<td>8.47</td>
</tr>
<tr>
<td>III</td>
<td>25.3</td>
<td>2.16</td>
<td>8.55</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>8.13</td>
</tr>
</tbody>
</table>

HPTLC profiling of ethanolic extract showed the
presence of 9 constituents at different Rf values at
254 nm. The developed TLC plates and HPTLC
cchromatograms are given in Fig. 1. Peak 1, 2, 3, 4, 5, 6, 7, 8 and 9 got Rf value of 0.36, 0.43, 0.47,
0.56, 0.61, 0.71, 0.76, 0.82 and 0.87 respectively.
KEE showed the presence of andrographolide and which was confirmed with retardation factor Rf 0.6.
The KEE showed the presence of andrographolide in the respective Rf region, which matched very
well with that of standard andrographolide (Fig. 1 and 2).
GC-MS Analysis: GC-MS analysis of KEE is presented in Fig. 3 and found 20 different fractions. Interpretation these fractions were done by confirming data bank of National Institute of Standards and Technique by comparing the molecular weight and molecular formula. The spectrum showed the presence of andrographolide and various analogs of andrographolide in KEE.

Pharmacological Screening: Results of analgesic activity of KEE using hot plate Method are presented in Table 2 and Fig. 4. Mean basal reaction time in animals treated with KEE have increased at various time. Reaction time of 3.4, 4.0, 4.2, and 3.0 sec were observed at 30, 60, 90 and 120 min respectively for 100 mg/kg dose of KEE. For 200 mg/ kg dose of KEE mean basal reaction time at 30 min was 4.0 sec, at 60 min 3.4 sec, at 90 min 3.9 sec & at 120 min it was 4.2 sec. For 300mg/ kg dose of KEE mean basal reaction time at 30 min was 3.1 sec, at 60 min 5.4 sec, at 90 min 5.7 sec while at 120 min it was 6.2 sec. which was maximum. This indicates that KEE showed analgesic activity.

Table 2: Analgesic Activity of KEE by Hot Plate Method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Dose (per kg p.o)</th>
<th>Reaction time in second’s ± SEM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>2.9 ±0.16 2.7 ±0.40 2.5 ±0.35 2.3 ± 0.50 2.8 ± 0.37</td>
</tr>
<tr>
<td>2</td>
<td>KEE 100mg/kg</td>
<td>2.9 ±0.16 3.4 ± 0.10 4.0 ±0.35* 4.2 ± 0.31* 3.0 ± 0.38</td>
</tr>
<tr>
<td>3</td>
<td>KEE 200mg/kg</td>
<td>2.6 ±0.22 4.0 ±0.33 3.4 ±0.64 3.9 ± 0.40 4.2 ± 0.40*</td>
</tr>
<tr>
<td>4</td>
<td>KEE 300mg/kg</td>
<td>2.3 ±0.23 3.1 ± 0.28 5.4± 0.71** 5.7 ± 0.93** 6.2 ± 0.70**</td>
</tr>
<tr>
<td>5</td>
<td>Aspirin 150mg/kg</td>
<td>3.5 ±0.67 3.2 ±0.48 5.7 ±0.67** 5.5 ± 0.99** 5.7 ± 0.92**</td>
</tr>
</tbody>
</table>

Table represents significant reaction time in second values compare to control. Each value represents mean reaction time ± S.E.M. (n = 6). Significant in reaction time values according to one-way ANOVA followed by Tukey’s post-test, P values of *P<0.05, **P<0.01 were considered statistically significant.

Analgesic activity of KEE was also performed by using acetic acid induced wriths in mice. Acetic acid produced significant abdominal constrictions in mice and treatment with KEE showed reduction of wriths as comparable to aspirin (See Table 3, Fig. 5). At 100, 200 & 300mg/kg dose of KEE protection against writhing movement are 27.78%, 33.91% and 56.23% respectively. The maximum protection against writhing movement was observed in A. paniculata at 300mg/kg (56.23%).
TABLE 3: ANALGESIC ACTIVITY OF KEE ACETIC ACID INDUCED WRITHING MODEL

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Dose (per kg p.o)</th>
<th>Writhing Test Number of writhing (per 20 minutes) ± SEM</th>
<th>% Change in abdominal wriths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>45.7 ± 5.33</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>KEE 100mg/kg</td>
<td>33.0 ± 3.71</td>
<td>27.78</td>
</tr>
<tr>
<td>3</td>
<td>KEE 200mg/kg</td>
<td>30.2 ± 3.43*</td>
<td>33.91</td>
</tr>
<tr>
<td>4</td>
<td>KEE 300mg/kg</td>
<td>20.0 ± 2.11**</td>
<td>56.23</td>
</tr>
<tr>
<td>5</td>
<td>Aspirin 150mg/kg</td>
<td>9.4 ± 1.93**</td>
<td>79.43</td>
</tr>
</tbody>
</table>

Table represents significant reduction in acetic acid induced writhing values compare to control. Each value represents mean writhing ± S.E.M. (n = 6). Significant reduction in acetic acid induced writhing values according to one-way ANOVA followed by Tukey’s post-test, P values of *P<0.05, **P<0.01 were considered statistically significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Dose (per kg p.o)</th>
<th>Rectal temperature in °C ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before yeast injection - 17 hrs</td>
<td>0 hrs</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>36.0 ± 0.58</td>
</tr>
<tr>
<td>2</td>
<td>KEE 100mg/kg</td>
<td>35.8 ± 0.31</td>
</tr>
<tr>
<td>3</td>
<td>KEE 200mg/kg</td>
<td>36.2 ± 0.31</td>
</tr>
<tr>
<td>4</td>
<td>KEE 300mg/kg</td>
<td>36.2 ± 0.48</td>
</tr>
<tr>
<td>5</td>
<td>Aspirin 150mg/kg</td>
<td>36.3 ± 0.33</td>
</tr>
</tbody>
</table>

Table represents significant reduction rectal temperature values compare to control. Each value represents mean rectal temperature ± S.E.M. (n = 6). Significant reduction rectal temperature values according to one-way ANOVA followed by Tukey’s post-test, P values of *P<0.05, **P<0.01 were considered statistically significant.

Antipyretic activity of KEE was carried out by using Brewer’s Yeast Induced Hyperpyrexia model. The experimental rats showed a mean increase of about 1.26 °C in rectal temperature, 17 hrs after Brewer’s yeast injection. KEE at 100, 200 and 300 mg/kg produced significant antipyretic activity 1 hr after drug administration. The highest dose 300mg/kg of KEE significantly reduces the rectal temperature of the rats from 37.7 ± 0.21 to 35.2 ± 0.31°C. (See Table 4 and Fig. 6).

Prostaglandins inhibition was studied by inhibition of Castor oil induced diarrhea in rats. Administration of castor oil induced diarrhea around 9 fecal drops with total weight of 5.5 grams in four hours. This was reduced in both test and standard groups. Indicating KEE produces action by inhibiting the synthesis of prostaglandins (Table 5).

TABLE 4: ANTIPYRETIC ACTIVITY OF KEE BREWER’S YEAST INDUCED HYPERPYREXIA

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Dose (per kg p.o)</th>
<th>Mean frequency of diarrhea ± SEM</th>
<th>Mean no. of fecal drops ± SEM</th>
<th>Mean wt. of feces after 4 hrs (gm) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>7.00 ± 0.52</td>
<td>9.50 ± 0.96</td>
<td>5.50 ± 0.43</td>
</tr>
<tr>
<td>2</td>
<td>KEE 150mg/kg</td>
<td>1.83 ± 0.31</td>
<td>3.83 ± 1.01</td>
<td>2.50 ** ± 0.56</td>
</tr>
<tr>
<td>3</td>
<td>Aspirin 150mg/kg</td>
<td>2.00 ± 0.26</td>
<td>4.67 ± 0.88</td>
<td>2.83 ** ± 0.31</td>
</tr>
</tbody>
</table>

Table represents significant reduction weight of feces values compare to control. Each value represents mean weight of feces ± S.E.M. (n = 6). Significant reduction weight of feces values according to one-way ANOVA followed by Tukey’s post-test, P values of **P<0.01 were considered statistically significant.
FIG. 6: ANTIPYRETIC ACTIVITY OF KEE BREWER’S YEAST INDUCED HYPERPYREXIA

DISCUSSIONS: Analgesic activity of ethanolic extracts of Kariyat using hot plate method indicates mean basal reaction time increased significantly in the aspirin treated group compared to control group from 30 min onwards. There is increase in mean basal reaction time was observed at intervals 30 min, 60 min, 90 min & 120 min. at all three doses of KEE (Table 2). Pain is a subjective unpleasant experience at site due noxious stimuli and there are two types pain viz., peripheral and neurogenic pain. Peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by afferent inputs pain sensation. The hot plate method is considered to be selective for screening of the compound acting through the center receptor.

The intraperitoneal injection of acetic acid produces pain through the activation of chemosensitive nocireceptor or irritation of the visceral surface, thereby leading to the liberation of bradykinins, histamine, prostaglandins and serotonin. Thus, the extract has inhibited the pain induced by acetic acid which indicates that plants act through both mechanisms, i.e. central as well as peripheral analgesics.

Analgesic activity of KEE was performed by using acetic acid induced Writhing model. KEE produced significant reduction in number of abdominal constrictions in mice in dose dependent manner comparable to commercial drug. KEE significantly reduced the number of writhing in mice. KEE reduced writhing at 100, 200 & 300 mg/kg dose compared to control. At 100, 200 & 300 mg/kg dose of KEE protection against writhing movement are 27.78%, 33.91% and 56.23% respectively. The maximum protection against writhing movement was observed in KEE at 300 mg/kg dose (56.23%) (Table 3). Antipyretic activity of KEE was carried out by using Brewer’s Yeast Induced Hyperpyrexia model. The experimental rats showed a mean increase of about 1.26 ºC in rectal temperature, 17 hrs after Brewer’s yeast injection. KEE at 100, 200 and 300 mg/kg produced significant antipyretic activity 1 hr after drug administration. Non-steroidal anti-inflammatory drugs (NSAIDs), which are known to possess anti-inflammatory, antipyretic and analgesic activities. One of the major mechanisms involved in the anti-inflammatory activity of NSAIDs is inhibition of prostaglandins biosynthesis.

Furthermore, fever is known to be promoted by prostaglandin, mainly PGE2, synthesized upon induction of cytokines released from activated macrophages. Aspirin (NSAID) for example, is known to block the synthesis of prostaglandins. The results indicate that the KEE exerted both antipyretic and analgesic activities probably by interfering with the synthesis of prostaglandins. The highest dose 300 mg/kg of KEE significantly reduces the rectal temperature of the rats from 37.7 ± 0.21 to 35.2 ± 0.31 ºC (Table 4). Mechanism of action was determined by studying prostaglandin inhibition studies. Results indicated that KEE produces action by inhibiting the synthesis of prostaglandin (Table 5).

CONCLUSION: An ethanolic extract of Kariyat was investigated for analgesic and antipyretic activities in experimental animals and chemicals content in the extract was determined by high performance thin layer chromatography and gas chromatography mass spectrometry investigations indicated presence of steroids, phenols, terpenoids, alkaloids, saponins, flavonoids etc. The various concentrations ethanolic extract showed significant analgesic activity in hot-plate method and acetic acid-induced writhing in mice. Mechanism of action was determined by studying prostaglandin inhibition. Studies showed that KEE inhibits the synthesis of prostaglandin as evidenced by inhibition of diarrhea induced by Castor oil. The findings of present study indicate ethanolic extract Kariyat shows significant analgesic and antipyretic activity.
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CONFLICTS OF INTEREST: No conflicts of interest.

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