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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF BARK AND LEAF EXTRACTS OF *KUNSTLERIA KERALENSIS*

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ABSTRACT

The hexane, chloroform and methanolic extracts of bark and leaf of the plant "*Kunstleria keralensis*" belonging to the family Fabaceae were screened for analgesic and anti-inflammatory activity. The analgesic activity was evaluated by tail flick and hot plate method. The anti-inflammatory activity was evaluated by Carrageenan induced paw edema method. The hexane extract (HB) and methanol extract (MB) of bark showed significant analgesic and anti-inflammatory activity at prefixed time. The chloroform extract (CB) of bark and hexane extract (HL) of leaf showed moderate analgesic and anti-inflammatory activity at prefixed time. The chloroform extract (CL) of leaf and methanol extract (ML) showed in significant analgesic and anti-inflammatory activity.

Keywords: Carrageenan, Analgesic, Anti-inflammatory, *Kunstleria keralensis*, Tail flick.

INTRODUCTION

Medicinal plants are used by tribal and rural population of India for their healthcare as well as for the health of their livestock. According to WHO still about 80% of the world population rely mainly on plant based drugs as they are cheap and no side effects [1]. Synthetic drugs that are currently used for the management of pain are opioids or nonopioids and for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and cortico steroids. All these drugs carry potential toxic effects and pose several health problems during their clinical use [2]. Hence, search for new and more effective drugs with fewer side effects is necessary [3]. Medicines of plant origin had been used since ages without any adverse effects. It is necessary to find new medicinal plants to develop more effective and cheaper drugs that might serve

as lead molecules in the development of plant based analgesic and anti-inflammatory drugs [4].

Kunstleria keralensis is a flowering plant belongs to the family Fabaceae, found in evergreen and semi evergreen forest in the Southern Western Ghats of India. It is mainly distributed in the districts of Kerala such as Kannur, Thiruvananthapuram, Thissur, Pallakad, Mallapuram, Kasaragod and certain parts of Karnataka [5]. It is reported that the bark of the plant *Kunstleria keralensis* used as a medicine by the tribal people of kerala to heal the body pain and also had antifertility activity [6-8]. In view of its medicinal properties, in the present study the solvent extracts of bark and leaf materials of *Kunstleria keralensis* were screened for analgesic and anti-inflammatory activity.

MATERIALS AND METHODS

Collection and authentication of plant

The bark and leaves of *Kunstleria keralensis* were collected in the month of January 2012 in Agumbe forest region. The materials were shade dried, powdered and stored in air tight containers. The plant was identified and authenticated by botanist Dr. K.G. Bhat, Professor in botany, Poornapragna first grade college, Udupi, Karnataka. The herbarium of the identified plant was prepared and submitted to the Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, India. The specimen number of the herbarium is NCP-14-2012-13 dated 22-11-12.

Preparation of plant extracts and evaluation of phytochemical tests

The bark and leaf extracts were made using the solvents hexane, chloroform and methanol by hot soxhlet and cold maceration methods [9-13]. The test samples were prepared and labeled the hexane extract as HB, chloroform extract as CB and methanol extract as MB. Similarly, the test samples of leaf extracts were also prepared and labeled the hexane extract as HL, chloroform extracts as CL and methanolic extract as ML respectively. The test samples of bark and leaf were analysed for various phytochemical constituents. The presence of various phytochemical constituents in these test samples has been reported earlier [14].

Experimental animals

Wistar albino rats weighing between 150-200 grams (g) and mice weighing between 25-30g of either sex were used. The animals were procured from the Central animal house, National college of Pharmacy, Balrajurs road, Shivamogga, Karnataka, India and the experimental protocol was approved by Institutional Animal Ethical Committee (Ref. No. NCP/IAEC/CL/02/ 12/2010-11) prior to the experiments. The animals were made to fast overnight for acute toxicity test. For analgesic and anti-inflammatory activity, the animals were made to fast for 3-4 hours prior to the experiment.

Acute toxicity Studies

Acute toxicity was evaluated on female Swiss albino mice weighing between 25-30g. The fixed dose method was adopted as per OECD Guideline No.423 of CPCSEA. The dose fixed was 250 and 500mg/kg body weight ie, $\frac{1}{20}$ th and $\frac{1}{10}$ th of the therapeutic dose [15-17].

Analgesic activity

Tail flick method

The test samples prepared from bark extract (HB, CB and MB) and leaf extract (HL, CL and ML) were tested for their analgesic activity by tail flick method. The evaluation parameter was tail flick by the mice on dipping the tail into hot water kept at $55\pm 1^\circ\text{C}$ in an analgesiometer.

Wistar albino mice weighing between 25-30g of either sex were used for the study. The animals were divided into 15 groups of 6 animals each. Group I was administered with 1ml of distilled water which served as control. Group II was administered with 1ml of 1% Tween-80 which served as vehicle. Group III was administered with diclofenac sodium (10mg/kg body weight) which served as standard. The test samples of bark HB, CB and MB were administered at 250mg/kg body weight to group IV, VI, VIII and at 500mg/kg body weight to group V, VII and IX respectively. Similarly, the test samples of leaf HL, CL and ML were administered at 250mg/kg body weight to group X, XII, XIV and at 500mg/kg body weight to group XI, XIII and XV respectively. All the test samples were prepared in sterile water and administered orally. The initial reading of tail flick was taken before administration of the test samples to the animals by exposing the tip of the tail (1-2 cm) to hot water. The reaction time of tail flick was measured after administration of test samples at every 30 minutes intervals upto 150 minutes [19-20].

Hot plate method

The test samples were also tested for their analgesic activity by hot plate method. The evaluation parameters were latency time for paw licking and jumping responses of mice on exposure to the hot plate surface, kept at $55\pm 1^\circ\text{C}$. Wistar albino mice weighing between 25-30g of either sex were used for the experiment. The animals were divided into 15 groups of 6 animals each. Group I was administered with 1ml of distilled water which served as control. Group II was administered with 1ml of 1% Tween-80 which served as vehicle. Group III was administered with diclofenac sodium (10mg/kg body weight) which served as standard. The test samples of bark HB, CB and MB were administered at 250mg/kg body weight to group IV, VI, VIII and at 500mg/kg body weight to group V, VII and IX respectively. Similarly, the test samples of leaf HL, CL and ML were administered at 250mg/kg body weight to group X, XII, XIV and at 500mg/kg body weight to group XI, XIII and XV respectively. All the test samples were prepared in sterile water and administered orally. The initial reading of Paw licking was taken before administration of the test samples to the animals by placing the rat on hot plate. The reaction time of paw licking was measured after administration of test samples at every 30 minutes intervals upto 150 minutes [21,22].

Anti-inflammatory activity by Carrageenan-induced rat paw edema method

The test samples prepared from bark extract (HB, CB and MB) and leaf extract (HL, CL and ML) were tested for their anti-inflammatory activity by carrageenan-induced rat paw edema method. The test was evaluated by measuring the paw volume of the rats plethysmographically. Wistar albino rats weighing between

150-200g of either sex were used for the experiment. The animals were divided into 15 groups of 6 animals each. Group I was administered with 1ml of distilled water which served as control. Group II was administered with 1ml of 1% Tween-80 which served as vehicle. Group III was administered with diclofenac sodium (10mg/kg body weight) which served as standard. The test samples of bark HB, CB and MB were administered at 250mg/kg body weight to group IV, VI, VIII and at 500mg/kg body weight was administered to group V, VII and IX respectively. Similarly, the test samples of leaf HL, CL and ML were administered at 250mg/kg body weight to group X, XII, XIV and at 500mg/kg body weight to group XI, XIII and XV respectively. All the test samples were prepared by sterile water and were administered orally. After 30 mins of administration of the test samples and standard, 0.1 ml of 1% suspension of carrageenan was administered at sub-plantar region of hind paws of all rats to induce edema. The initial reading of paw edema was taken soon after the administration of the carrageenan. The reduction in the Paw volume of control, standard and test group animals were measured using mercury displacement method (plethysmographically) at every 1hour upto 3 hours [23-24].

Statistical analysis

The statistical analysis was carried out by one way analysis of variance (ANOVA). All the data were presented as Mean \pm SEM.

RESULTS

Acute toxicity test

The test samples prepared from bark extract (HB, CB and MB) and leaf extract (HL, CL and ML) exhibited no death of test animals at maximum dose of 5000mg/kg body weight. Hence, the $1/20^{\text{th}}$ and $1/10^{\text{th}}$ concentration of 5000mg ie, 250mg and 500mg/kg body weight was considered as therapeutic dose for all pharmacological activities.

Analgesic activity by Tail flick method

Bark extracts

The HB and MB test samples administered to the mice showed analgesic activity by exhibiting increase in time of 6.82 ± 0.04 and 6.80 ± 0.04 seconds respectively for tail flick response after 90 minutes of administration at 500mg/kg body weight when compared to control tail flick response time of 2.08 ± 0.04 seconds. The tail flick response time exhibited by test samples were found significant in comparison to standard drug diclofenac sodium response time 8.22 ± 0.02 seconds after 90 minutes at 500mg/kg body weight. The CB test sample showed comparatively less significant activity than HB and MB with a tail flick response time of 4.58 ± 0.04 seconds after 120 minutes of administration at 500mg/kg body weight. The analgesic activity was initiated after 1 hour of

administration for all the test samples. The activity of HB and MB test samples decreased after 90 minutes whereas for CB test sample, the decrease in activity was found after 120 minutes of administration. All the test samples showed less analgesic activity at a dose of 250mg/kg body weight when compare to 500mg/kg body weight. The results obtained for the test samples of bark are shown in table 1.

Leaf extracts

The HL test sample administered to the mice showed analgesic activity by exhibiting increase in time of 3.42 ± 0.04 seconds for tail flick response after 60 minutes of administration at 500mg/kg body weight when compared to control tail flick response time of 2.08 ± 0.04 seconds. The tail flick response time exhibited by test sample HL was found moderately significant in comparison to standard drug diclofenac sodium response time 6.74 ± 0.04 seconds after 60 minutes at 500mg/kg body weight. The activity of HL was initiated after 30 minutes of administration and decreased after 60 minutes. It showed less analgesic activity at a dose of 250mg/kg body weight when compare to 500mg/kg body weight. The test samples CL and ML did not exhibited the analgesic activity. The results obtained for the test samples of leaf are shown in table 2.

Analgesic activity by Hot plate methodBark extracts

The HB and MB test samples administered to the mice showed analgesic activity by exhibiting increase in time of 6.80 ± 0.04 and 6.77 ± 0.04 seconds respectively for paw licking response after 90 minutes of administration at 500mg/kg body weight when compared to control paw licking response time of 2.08 ± 0.04 seconds. The paw licking response time exhibited by test samples were found significant in comparison to standard drug diclofenac sodium response time 8.22 ± 0.02 seconds after 90 minutes at 500mg/kg body weight. The CB test sample showed comparatively less significant activity than HB and MB with paw licking response time of 4.56 ± 0.04 seconds after 120 minutes of administration at 500mg/kg body weight. The analgesic activity was initiated after 1 hour of administration for all the test samples. The activity of HB and MB test samples decreased after 90 minutes whereas for CB test sample, the decrease in activity was found after 120 minutes of administration. All the test samples showed less analgesic activity at a dose of 250mg/kg body weight when compare to 500mg/kg body weight. The results obtained for the test samples of bark are shown in table 3.

Leaf extracts

The HL test sample administered to the mice showed analgesic activity by exhibiting increase in time of 3.40 ± 0.04 seconds for paw licking response after 60 minutes of administration at 500mg/kg body weight when compared to control paw licking response time of 2.08 ± 0.04 seconds. The tail flick response time exhibited

by test sample HL was found moderately significant in comparison to standard drug diclofenac sodium response time 6.74 ± 0.04 seconds after 60 mins at 500mg/kg body weight. The activity of HL was initiated after 30 minutes of administration and decreased after 60 minutes. It showed less analgesic activity at a dose of 250mg/kg body weight when compare to 500mg/kg body weight. The test samples CL and ML did not exhibit the analgesic activity. The results obtained for the test samples of leaf are shown in table 4.

Anti-inflammatory activity by Carrageenan induced paw edema method

Bark extracts

The HB and MB test samples administered to the rat showed anti-inflammatory activity by exhibiting decrease in paw volume of 0.32 ± 0.02 and 0.35 ± 0.02 ml displacement of mercury respectively after 90 minutes of administration at 500mg/kg body weight when compared to control paw edema of 0.86 ± 0.04 ml. The decreased paw volume response time exhibited by test samples were found significant in comparison to standard drug diclofenac sodium of 0.22 ± 0.02 ml after 90 minutes at 500mg/kg body weight. The CB test sample showed comparatively less significant activity than HB and MB

with decreased paw volume of 0.44 ± 0.02 ml after 60 minutes of administration at 500mg/kg body weight. The anti-inflammatory activity was initiated after 1 hour of administration and decreased after 90 minutes for all the test samples. All the test samples showed less anti-inflammatory activity at a dose of 250mg/kg body weight when compare to 500mg/kg body weight. The results obtained for the test samples of bark are shown in table 5.

Leaf extracts

The HL test sample administered to the rat showed anti-inflammatory activity by exhibiting decrease in paw volume of 0.46 ± 0.02 ml displacement of mercury after 60 minutes of administration at 500mg/kg body weight when compared to control paw volume response of 0.84 ± 0.03 ml. The decrease in paw volume response time exhibited by test sample HL found significant in comparison to standard drug diclofenac sodium of 0.30 ± 0.02 ml after 60 minutes at 500mg/kg body weight. The activity of HL test sample initiated after 30 minutes of administration and decreased after 60 minutes. It showed less anti-inflammatory activity at a dose of 250mg/kg body weight when compare to 500mg/kg body weight. The test samples CL and ML did not exhibit the anti-inflammatory activity. The results obtained for the test samples of leaf are shown in table 6.

Table 1. Analgesic activity of test samples of bark of *Kunstleria keralensis* by tail flick method

Drugs	Dose (mg/kg) body weight	Reaction time in seconds					
		0 min	30 mins	60 mins	90 mins	120 mins	150mins
Group-I Control) Water	—	2.08 ± 0.04	2.07 ± 0.02	2.08 ± 0.04	2.08 ± 0.04	2.07 ± 0.02	2.08 ± 0.04
Group-II (Vehicle) Tween-80	0.1%	2.07 ± 0.02	2.07 ± 0.02	2.08 ± 0.04	2.08 ± 0.04	2.07 ± 0.02	2.08 ± 0.02
Group-III (Standard) Diclofenac sodium	5	2.07 ± 0.02	5.62 ± 0.02	6.74 ± 0.04	8.22 ± 0.02	7.82 ± 0.02	6.78 ± 0.04
Group-IV HB	250	2.08 ± 0.03	2.14 ± 0.02	2.64 ± 0.02	3.59 ± 0.04	3.12 ± 0.03	2.32 ± 0.04
Group-V	500	2.07 ± 0.04	2.28 ± 0.02	4.00 ± 0.04	6.82 ± 0.04	4.02 ± 0.02	3.22 ± 0.03
Group-VI CB	250	2.08 ± 0.02	2.08 ± 0.02	2.20 ± 0.04	2.88 ± 0.03	2.98 ± 0.04	2.28 ± 0.03
Group-VII	500	2.08 ± 0.02	2.18 ± 0.02	2.68 ± 0.04	3.48 ± 0.04	4.58 ± 0.04	2.98 ± 0.03
Group-VIII MB	250	2.08 ± 0.03	2.14 ± 0.02	2.65 ± 0.02	3.58 ± 0.04	3.14 ± 0.03	2.34 ± 0.04
Group-IX	500	2.07 ± 0.04	2.27 ± 0.02	4.03 ± 0.04	6.80 ± 0.04	4.04 ± 0.02	3.20 ± 0.03

Values are mean \pm SEM, n = 6.

Note: HB (Hexane extract of bark), CB (Chloroform extract of bark), MB (Methanolic extract of bark).

Table 2. Analgesic activity of test samples of leaf of *Kunstleria keralensis* by tail flick method

Drugs	Dose (mg/kg) body weight	Reaction time in seconds					
		0 min	30 mins	60 mins	90 mins	120 mins	150mins
Group-I (Control) Water	—	2.08 ± 0.04	2.07 ± 0.02	2.08 ± 0.04	2.08 ± 0.04	2.07 ± 0.02	2.08 ± 0.04
Group-II (Vehicle) Tween-80	0.1%	2.07 ± 0.02	2.07 ± 0.02	2.08 ± 0.04	2.08 ± 0.04	2.07 ± 0.02	2.08 ± 0.02
Group-III (Standard)	5	2.07 ± 0.02	5.62 ± 0.02	6.74 ± 0.04	8.22 ± 0.02	7.82 ± 0.02	6.78 ± 0.04

Diclofenac sodium							
Group-X HL	250	2.08 ±0.04	2.08 ±0.02	2.48 ±0.03	2.67 ±0.04	2.38 ±0.04	2.10 ±0.03
Group-XI	500	2.08 ±0.04	2.18 ±0.02	3.42 ±0.04	3.12 ±0.04	2.64 ±0.04	2.14 ±0.03
Group-XII CL	250	2.07 ±0.04	2.07 ±0.03	2.16 ±0.02	2.12 ±0.03	2.10 ±0.09	2.08 ±0.09
Group-XIII	500	2.07 ±0.03	2.07 ±0.03	2.21 ±0.04	2.20 ±0.02	2.09 ±0.04	2.08 ±0.04
Group-XIV ML	250	2.08 ±0.04	2.07 ±0.02	2.10 ±0.04	2.11 ±0.03	2.08 ±0.03	2.08 ±0.09
Group-XV	500	2.08 ±0.04	2.08 ±0.02	2.20 ±0.04	2.18 ±0.02	2.18 ±0.04	2.10 ±0.04

Values are mean ± SEM, n = 6.

Note: HL (Hexane extract of Leaf), CL (Chloroform extract of Leaf), ML (Methanolic extract of Leaf).

Table 3. Analgesic activity of test samples of bark of *Kunstleria keralensis* by hot plate method

Drugs	Dose (mg/kg) body weight	Reaction time in seconds					
		0 min	30 mins	60 mins	90 mins	120 mins	150mins
Group-I (Control) Water	—	2.08 ±0.04	2.07 ±0.02	2.08 ±0.04	2.08 ±0.04	2.07 ±0.02	2.08 ±0.04
Group-II (Vehicle) Tween-80	0.1%	2.07 ±0.02	2.07 ±0.02	2.08 ±0.04	2.08 ±0.04	2.07 ±0.02	2.08 ±0.02
Group-III (Standard) Diclofenac sodium	5	2.07 ±0.02	5.62 ±0.02	6.74 ±0.04	8.22 ±0.02	7.82 ±0.02	6.78 ±0.04
Group-IV HB	250	2.08 ±0.03	2.12 ±0.02	2.62 ±0.02	3.59 ±0.04	3.14 ±0.03	2.32 ±0.04
Group-V	500	2.07 ±0.04	2.28 ±0.02	3.98 ±0.04	6.80 ±0.04	4.02 ±0.02	3.23 ±0.03
Group-VI CB	250	2.08 ±0.02	2.08 ±0.02	2.22 ±0.04	2.85 ±0.03	2.95 ±0.04	2.26 ±0.03
Group-VII	500	2.08 ±0.02	2.16 ±0.02	2.68 ±0.04	3.46 ±0.04	4.56 ±0.04	2.98 ±0.03
Group-VIII MB	250	2.08 ±0.03	2.13 ±0.02	2.63 ±0.02	3.60 ±0.04	3.12 ±0.03	2.35 ±0.04
Group-IX	500	2.07 ±0.04	2.27 ±0.02	4.02 ±0.04	6.77 ±0.04	4.06 ±0.02	3.18 ±0.03

Values are mean ± SEM, n = 6.

Note: HB (Hexane extract of bark), CB (Chloroform extract of bark), MB (Methanolic extract of bark).

Table 4. Analgesic activity of test samples of leaf of *Kunstleria keralensis* by hot plate method

Drugs	Dose (mg/kg) body weight	Reaction time in seconds					
		0 min	30 mins	60 mins	90 mins	120 mins	150mins
Group-I (Control) Water	—	2.08 ±0.04	2.07 ±0.02	2.08 ±0.04	2.08 ±0.04	2.07 ±0.02	2.08 ±0.04
Group-II (Vehicle) Tween-80	0.1%	2.07 ±0.02	2.07 ±0.02	2.08 ±0.04	2.08 ±0.04	2.07 ±0.02	2.08 ±0.02
Group-III (Standard) Diclofenac sodium	5	2.07 ±0.02	5.62 ±0.02	6.74 ±0.04	8.22 ±0.02	7.82 ±0.02	6.78 ±0.04
Group-X HL	250	2.08 ±0.04	2.08 ±0.02	2.45 ±0.03	2.65 ±0.04	2.38 ±0.04	2.09 ±0.03
Group-XI	500	2.08 ±0.04	2.16 ±0.02	3.42 ±0.04	3.12 ±0.04	2.65 ±0.04	2.14 ±0.03
Group-XII CL	250	2.07 ±0.04	2.08 ±0.03	2.15 ±0.02	2.10 ±0.03	2.08 ±0.09	2.08 ±0.09
Group-XIII	500	2.07 ±0.03	2.08 ±0.03	2.23 ±0.04	2.20 ±0.02	2.09 ±0.04	2.08 ±0.04
Group-XIV ML	250	2.08 ±0.04	2.07 ±0.02	2.09 ±0.04	2.11 ±0.03	2.07 ±0.03	2.07 ±0.09
Group-XV	500	2.08 ±0.04	2.08 ±0.02	2.18 ±0.04	2.18 ±0.02	2.18 ±0.04	2.10 ±0.04

Values are mean ± SEM, n = 6.

Note: HL (Hexane extract of Leaf), CL (Chloroform extract of Leaf), ML (Methanolic extract of Leaf).

Table 5. Anti-inflammatory activity of test samples of bark of *Kunstleria keralensis* by Carrageenan induced paw edema method

Drugs	Dose (mg/kg) body weight	Reaction time in seconds with % of inhibition				
		30 mins	60 mins	90 mins	120 mins	150 mins
Group-I (Control) Water	—	0.72 ±0.02	0.84 ±0.03	0.86 ±0.04	0.92 ±0.02	0.94 ±0.02

Group-II (Vehicle) Tween-80	0.1%	0.72 ±0.02	0.84 ±0.03	0.85 ±0.04	0.92 ±0.02	0.93 ±0.02
Group-III (Standard) Diclofenac sodium	5	0.56 ±0.02 (22.23%)	0.30 ±0.02 (64.28%)	0.22 ±0.02 (74.41%)	0.20 ±0.04 (78.26%)	0.26 ±0.02 (68.08%)
Group-IV HB Group-V	250	0.68 ±0.02 (5.5%)	0.66 ±0.01 (21.42%)	0.60 ±0.02 (30.23%)	0.64 ±0.04 (30.43%)	0.64 ±0.03 (31.91%)
	500	0.62 ±0.02 (13.88%)	0.40 ±0.02 (52.38%)	0.32 ±0.02 (62.79%)	0.45 ±0.03 (51.08%)	0.49 ±0.02 (47.87%)
Group-VI CB Group-VII	250	0.74 ±0.02 (-2.7%)	0.70 ±0.01 (16.64%)	0.68 ±0.02 (20.93%)	0.67 ±0.04 (31.08%)	0.70 ±0.03 (25.23%)
	500	0.70 ±0.02 (2.76%)	0.52 ±0.02 (38.09%)	0.44 ±0.02 (51.16%)	0.50 ±0.03 (41.88%)	0.51 ±0.02 (42.53%)
Group-VIII MB Group-IX	250	0.67 ±0.02 (6.94%)	0.66 ±0.01 (21.42%)	0.62 ±0.02 (27.90%)	0.64 ±0.04 (30.43%)	0.66 ±0.03 (31.25%)
	500	0.64 ±0.02 (11.12%)	0.44 ±0.02 (37.61%)	0.35 ±0.02 (59.30%)	0.47 ±0.03 (48.69%)	0.50 ±0.02 (46.80%)

Values are mean ± SEM, n = 6.

Note: HB (Hexane extract of bark), CB (Chloroform extract of bark), MB (Methanolic extract of bark).

Table 6. Anti-inflammatory activity of test samples of leaf of *Kunsteria keralensis* by Carrageenan paw edema method

Drugs	Dose (mg/kg) body weight	Reaction time in seconds with % of inhibition				
		30 mins	60 mins	90 mins	120 mins	150 mins
Group-I (Control) Water	—	0.72 ±0.02	0.84 ±0.03	0.86 ±0.04	0.92 ±0.02	0.94 ±0.02
Group-II (Vehicle) Tween-80	0.1%	0.72 ±0.02	0.84 ±0.03	0.85 ±0.04	0.92 ±0.02	0.93 ±0.02
Group-III (Standard) Diclofenac sodium	5	0.56 ±0.02 (22.23%)	0.30 ±0.02 (64.28%)	0.22 ±0.02 (74.41%)	0.20 ±0.04 (78.26%)	0.26 ±0.02 (68.08%)
Group-X HL Group-XI	250	0.76 ±0.02 (-5.5%)	0.66 ±0.01 (21.42%)	0.64 ±0.02 (25.58%)	0.71 ±0.04 (26.08%)	0.74 ±0.03 (13.51%)
	500	0.72 ±0.02 (0.00%)	0.46 ±0.02 (38.09%)	0.48 ±0.02 (46.51%)	0.66 ±0.03 (28.06%)	0.70 ±0.02 (25.53%)
Group-XII CL Group-XIII	250	0.78 ±0.02 (-8.32%)	0.80 ±0.01 (4.76%)	0.78 ±0.02 (9.30%)	0.88 ±0.04 (7.02%)	0.86 ±0.03 (6.94%)
	500	0.77 ±0.02 (-6.32%)	0.78 ±0.02 (7.14%)	0.76 ±0.02 (11.62%)	0.82 ±0.03 (10.86%)	0.82 ±0.02 (10.62%)
Group-XIV ML Group-XV	250	0.79 ±0.02 (-8.12%)	0.82 ±0.01 (2.38%)	0.75 ±0.02 (12.62%)	0.82 ±0.04 (10.86%)	0.82 ±0.03 (10.97%)
	500	0.78 ±0.02 (-6.15%)	0.80 ±0.02 (5.32%)	0.72 ±0.02 (16.32%)	0.80 ±0.03 (13.04%)	0.81 ±0.02 (13.36%)

Values are mean ± SEM, n = 6.

Note: HL (Hexane extract of Leaf), CL (Chloroform extract of Leaf), ML (Methanolic extract of Leaf).

DISCUSSION

In the present study the test samples of bark and leaf extracts of plant *Kunsteria keralensis* belongs to the family Fabaceae were tested for analgesic and anti-inflammatory activities. Several reports are available on many plant species belonging to the presently studied family Fabaceae with analgesic and anti-inflammatory activities. The anti-inflammatory activity has also been reported for bark and leaf extracts of many plants like, *Desmodium triflorum*, *Pterocae puserinaceus* and *Crotalaria burhia* of the same family [25-27]. Apart from

anti-inflammatory activity, many plants of Fabaceae like *Pongamia pinnata*, *Dalber giasisoo*, *Acacia suma*, *Pterocarpus santalinoides*, *Cajanuscajan* and *Brachystegianeurycoma* of Fabaceae have been reported with significant analgesic and anti-inflammatory activity [28-33]. However, analgesic and anti-inflammatory activity has not been reported for the metabolites of the plant *Kunsteria keralensis*. Hence, in the present study the extracts of *Kunsteria keralensis* has been evaluated for these activities.

In the present study analgesic activity was evaluated by tail flick and hot plate method. The test samples HB and MB of bark extract exhibited significant analgesic activity with 82.96% in comparison with standard at 500mg/kg body weight. The test sample CB exhibited moderately significant analgesic activity with 58.56% at 500mg/kg body weight. The test sample HL of leaf extract also exhibited moderately significant analgesic activity with 50.74% at 500mg/kg body weight. Whereas, the test samples CL and ML exhibited insignificant analgesic activity.

In the present study anti-inflammatory activity was evaluated by paw edema method. The percentage of inhibition of paw edema of standard and test samples were compared with control. The test sample HB of bark extract exhibited anti-inflammatory activity with 62.79%. This was found to be significant in comparison with standard which exhibited 78.26% of paw edema. The test sample MB exhibited significant anti-inflammatory activity with 59.30% followed by other test samples CB and HL which exhibited 51.16% and 46.51% respectively at 500mg/kg body weight. The test samples CL and ML exhibited insignificant anti-inflammatory activity.

Though both the bark and leaf extracts showed analgesic and anti-inflammatory activity, the bark extracts exhibited comparatively more analgesic and anti-

inflammatory activity than leaf extracts. There are several reports on alkaloids, flavonoids, steroids and triterpenoids exhibiting analgesic and anti-inflammatory activity [32-35]. The phytochemical investigation has showed the presence of alkaloids, flavonoids, steroids and triterpenoids in the presently tested sample. The analgesic and anti-inflammatory activity may be due the presence of these constituents.

CONCLUSION

The test samples of bark extract HB and MB exhibited significant analgesic and anti-inflammatory activity. The test sample CB and HL exhibited moderately significant activity. The test samples CL and ML did not exhibit analgesic or anti-inflammatory activity. The further isolation, purification and the spectral analysis of pure compounds may provide a potential analgesic and anti-inflammatory lead molecule. The test samples can be evaluated further for other pharmacological properties which may be useful in designing of new drugs.

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