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Antinociceptive, antiinflammatory and antipyretic activities of *Rumex hastatus* D. don stem and roots

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ABSTRACT

The antinociceptive, antiinflammatory and antipyretic activities of Rumex hastatus D. Don stem and roots were evaluated using several experimental models. The antinociceptive activity of the ethanol and aqueous extract of stem and root was determined by using acetic acid induced writhing method, tail flick model and formalin-induced pain model in mice, using standard drugs. The antiinflammatory activity was evaluated by using carrageenan induced rat paw oedema and cotton pellet induced granuloma method and the antipyretic activity was evaluated by using the technique of yeast-induced pyrexia in Wistar rats. The ethanol and aqueous extract of stem and root at the doses of 200 and 400 mg/kg showed a significant ($P < 0.001$) inhibition of acetic acid induced abdominal constrictions in mice. In the tail flick model, the ethanol extract (400mg/kg) showed a significant ($P < 0.001$) increase in the pain threshold to the heat stimulus. The ethanol extract (400 mg/kg) of both root and stem inhibited both phases of the formalin-induced pain with a more pronounced effect on the second than the first phase. In carrageenan induced rat paw oedema method, the ethanol extracts of both stem and root (400 mg/kg) exhibited significant anti-inflammatory activity. In cotton pellet induced granuloma method, the maximum percent inhibition was exhibited by ethanol extract (400 mg/kg) of the stem and root, which was 23.27% and 27.25% respectively. The ethanol extracts of both the parts at the doses of 400 mg/kg, produced a pronounced antipyretic effect in hyperthermic rats in a dose dependent manner when compared with untreated rats. Moreover the ethanol extracts were more active than the aqueous extracts of both the parts in terms of activities shown in this paper. The phytochemical analysis of the ethanolic extracts of both the parts showed the presence of anthraquinone glycosides, tannins, carbohydrates, saponins and steroids. The above results have furnished the pharmacological evidence that supports the folklore claim of the drug as an analgesic, antiinflammatory and antipyretic drug.

Keywords: *Rumex hastatus*; antinociceptive; antiinflammatory; antipyretic

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INTRODUCTION

The West Himalayan biogeographic zone is known for its rich genetic diversity in several wild plants of food value and some of these plants possess potential medicinal properties[1,2]. *Rumex hastatus* D. Don (Polygonaceae), commonly known as 'khatimal' is a bushy shrub or under shrub 30-90cm high. It occurs chiefly on dry rocks and hillsides of western Himalayas from Kumaun to Kashmir, at altitudes between 300 and 2400 meters[3]. Due to pleasant acidic taste, the leaves and shoots are used in chutneys and pickles[4]. Traditionally, the decoction of the roots of the plant is used in rheumatism, backache[5], asthma[5,6], cough and fever[6]. The leaves and young shoots are used as carminative, purgative, diuretic and in stomach problems[7]. This plant is also used in traditional

systems of medicine for prevention and treatment of sexually transmitted diseases including acquired immune deficiency syndrome (AIDS) [8]. The main chemical constituents which are reported from the plant belong to various classes *viz;* anthraquinones, naphthalenes, flavonoids and phenolic compounds[9]. The plant has been screened for various pharmacological activities like anti-viral[10], anti-bacterial, antifungal[11], anti-diarrheal[12] and antioxidant[13]. The present study was designed to evaluate the antinociceptive, antiinflammatory and antipyretic potential of *Rumex hastatus* stem and root as claimed by the traditional uses.

MATERIALS AND METHODS

Plant material

The whole plant of *Rumex hastatus* D. Don was collected in the month of September, 2010 from Darlaghat, Distt Solan, Himachal Pradesh (India). The plant specimen was identified and authenticated by Dr.H.B.Singh, Head, Raw Material, Herbarium and Museum Division, National Institute of Science Communication and Information Resources, New Delhi (Ref.NISCAIR/RHMD/Consult/-2010-11/486/84).

Preparation of extract

The air dried stem and root parts of the plant were ground to coarse powder individually. The powdered plant material was extracted with 90% ethanol in soxhlet apparatus for the preparation of ethanol extract and for aqueous extract preparation, the plant material was extracted with distilled water by cold maceration method. All the extracts were further dried at low temperature under reduced pressure and used for the present study. The suspensions of ethanol and aqueous extracts were prepared by using 1% carboxymethylcellulose (CMC) in distilled water.

Phytochemical screening

The phytochemical screening of ethanol and aqueous extracts of stem and roots of *Rumex hastatus* D. Don was performed to detect the presence of various phytoconstituents in the plant[14].

Animals

Adult Swiss Albino mice (20 - 30 g) and Adult Wistar Albino rats (180 - 200 g) of either sex were obtained from Animal House, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. The animals were kept under standard conditions in the animal house of the institution and were fed standard diet and water, *ad libitum*. The Institutional Animal Ethical Committee, Guru Jambheshwar University, Hisar, approved the experimental protocol (Endst.No. IAEC/2010/13-25).

Drugs and Chemicals

The drugs and chemicals used in the present study were obtained from following sources, diclofenac sodium (Novartis India Ltd, Mumbai), pentazocine (Vardhman Labs, Haryana), paracetamol (GlaxoSmithkline, Mumbai), indomethacin (Jagsonpal Pharmaceuticals Ltd, Haryana) and glacial acetic acid (Qualigens, Mumbai). All other chemicals/solvents used were of analytical grade.

Acute toxicity study

Healthy adult mice of either sex were treated with graded dose of *Rumex hastatus* stem and root extracts (50, 100, 200, 400, 600 mg/kg/day). The mice were observed continuously for 8 hours daily for behavioural, neurological and autonomic profile for 30 days to study any possible toxic effects[15,16].

Antinociceptive activity

The antinociceptive activity was evaluated by using acetic acid induced writhing method[17,18], tail flick method[19] and formalin-induced pain in mice[20,21]. Swiss Albino mice (20 - 30 g) were divided into 10 groups of six animals each. All the animals were fasted overnight prior to the experiment, but water was provided *ad libitum*. Animals of group I served as blank control and animals of group II received the standard drug. Mice of group III to VI received ethanol and aqueous extract of *Rumex hastatus* D. Don stem respectively, at a dose of 200 mg/kg and 400 mg/kg. Mice of group VII to X received ethanol and aqueous extract of *Rumex hastatus* D. Don root respectively, at a dose of 200 mg/kg and 400 mg/kg. This experimental protocol was followed in the three models, used in screening analgesic activity in mice.

In acetic acid induced writhing method, writhings were induced by intraperitoneal injection of 0.6% v/v aqueous acetic acid (10 ml/kg) in Albino mice. The test samples were given orally, 30 mins prior to acetic acid injection and

the number of writhes or abdominal stretchings were counted for 15 minutes. Animals of control group were administered 1% CMC solution at the dose of 10 mg/ml. The standard drug, diclofenac sodium (10 mg/kg) was given orally to the animals of standard group.

In tail flick method, analgesia in experimental animals was assessed with a tail flick apparatus (Analgesiometer). Baseline latency (reaction time) was observed prior to drug (extracts) treatment and half-hour, one hour, two hours, and three hours after drug (extracts) administration. Animals which served as blank control were administered 1% CMC solution at the dose of 10 mg/ml and pentazocine (5mg/kg intraperitoneally) was administered as a standard.

In formalin-induced pain in mice, pain was induced by injecting 0.05 ml of 2.5% formalin along with distilled water in the sub planter region of right hind paw. The animals (six per group) were administered test extracts (200 and 400 mg/ml), indomethacin 10 mg/kg (positive control) and 5 ml/kg of 1% w/v aqueous carboxy -methylcellulose suspension (negative control), orally 1hour prior to formalin injection. These mice were individually placed in a transparent plexiglass cage (25cm×15cm×15cm) for observations. The time (seconds) that was spent licking (or) biting the injected paw, was indicative of pain. The first period (earlier or neurogenic phase) was recorded 0-5 minutes after formalin injection and the second period (later or inflammatory phase) was recorded 15-30 minutes after the injection.

Antiinflammatory activity

The antiinflammatory activity was evaluated by using carrageenan induced rat paw oedema method[22,23] and cotton pellet induced granuloma method[24,25]. The experimental rats were divided in a similar way as done in the previous activity. In carrageenan induced rat paw oedema method, oedema was induced by sub planter injection of 0.1 ml of 1% freshly prepared suspension of carrageenan into right paw of each rat. The test groups received ethanol and aqueous extract of root and stem (200 and 400 mg/kg, orally) and the control animals received 1% CMC solution orally at the dose of 10 mg/ml, while animals of standard group received diclofenac sodium (10 mg/kg) by intraperitoneal route. The test samples, control drug and the standard drug was given to the animals 60 minutes prior to the injection of carrageenan. The paw volume was measured by using a plethysmometer before and 1, 2, 3 and 6 hours after the injection of carrageenan. The percent of inhibition was calculated as Vc-Vt/ Vc ×100, where Vc was the average volume of the control group and Vt was the average volume of the test drug group.

In cotton pellet induced granuloma method, the animals were anaesthetized and sterile cotton pellets (50±1mg) were implanted in the axilla region of each rat through a single needle incision. The test groups received ethanol and aqueous extract of stem and root (200 and 400 mg/kg, orally) and the control animals received 1% CMC solution orally at the dose of 10 mg/ml, while animals of standard group received indomethacin (10 mg/kg) by intraperitoneal route for seven consecutive days from the day of cotton pellet implantation. On the eighth day, the animals were again anaesthetized and the cotton pellets were surgically removed. After removing the surrounding fibro-vascular tissues, the pellets were incubated at 37°C for 24 hours and dried. The weight of the granuloma was determined in terms of increase in the weight of the cotton pellet.

Antipyretic activity

The antipyretic activity was evaluated by using the technique of yeast-induced pyrexia in rats[26]. The initial rectal temperatures of the rats were recorded using digital thermometer connected with thermister probe. Hyperthermia was induced by subcutaneous injection of 15% brewers yeast suspension. The rats which showed an increase in temperature of at least 1.5° C after 18 hours of injection of yeast suspension were used for the experiment. The selected animals were divided into 10 groups in the similar manner as done in previous experiments. Group1 acted as control and was given only the vehicle (1% CMC solution, 5ml/kg). The test groups received ethanol and aqueous extract of root and stem (200 and 400 mg/kg) and the standard group received paracetamol at the dose of 150 mg/kg. All the treatments were carried out orally in CMC suspension. The temperature was recorded after 0.5,1, 2 and after 4 hours.

Statistical analysis

The values were expressed as mean ± standard error of mean (S.E.M.) and statistical analysis was carried out by using one-way analysis of variance (ANOVA) method followed by Dunnett's test. $P<0.05$ was considered statistically significant when compared with standard references.

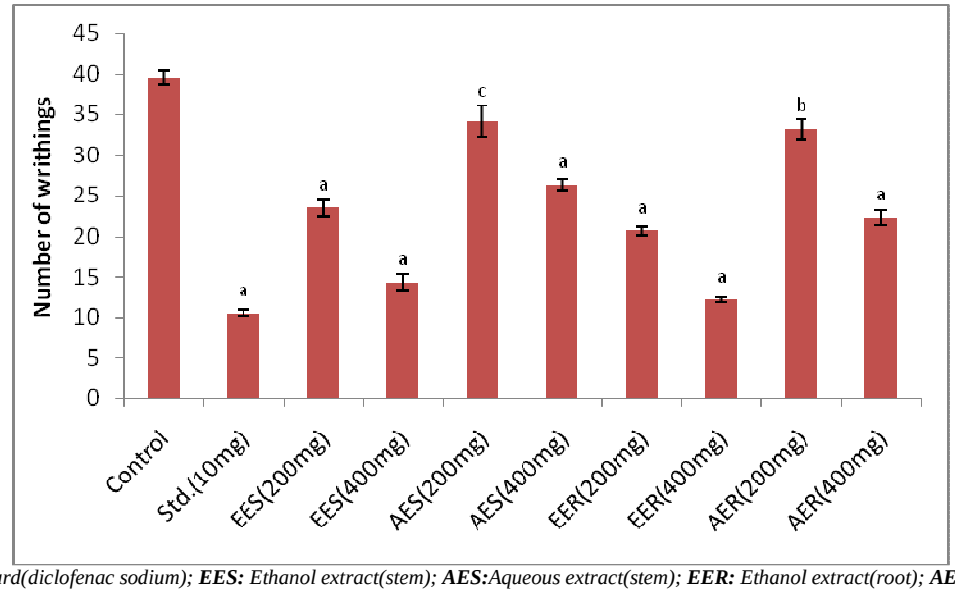
RESULTS

Phytochemical screening: The preliminary phytochemical screening of ethanol extracts of stem and roots of *Rumex hastatus* showed the presence of anthraquinone glycosides, tannins, carbohydrates, saponins and steroids.

Acute toxicity study: The treatment of normal mice with graded dose of *Rumex hastatus* D. Don stem and root extracts for 30 days revealed the non-toxic nature of test extracts. There was no change in general behavior or appearance such as restlessness, coma, respiratory distress, hair loss, loss in body weight etc. Toxicity studies showed no mortality up to the selected dose of 600mg/kg body weight till the end of the experiment.

Antinociceptive activity: In acetic acid induced writhing method, the ethanol extract (200 and 400 mg/kg) and aqueous extract (400 mg/kg) of stem and root caused a significant ($P < 0.001$) inhibition of abdominal constrictions induced by acetic acid in a dose dependent manner [Figure 1]. The ethanol extract (400 mg/kg) of root showed maximum inhibition of 69.18% in acetic acid induced abdominal constrictions in mice and the effect was comparable to that produced by indomethacin 10 mg/kg (73.41%). In tail flick method, the ethanol as well as aqueous extracts (200 and 400 mg/kg) of both stem and root exhibited the maximum increase in the baseline latency, after three hours of drug treatment [Table 1]. At 400 mg/kg the ethanol extract of *Rumex hastatus* root exhibited maximum inhibition of 9.07 % in the formalin-induced licking during the first phase, followed by ethanol extract of stem (8.20%) [Figure 2]. The maximum percentage inhibition in the late phase (15-30 min) of the formalin-induced licking activity was exhibited again by ethanol extract (400 mg/kg) of root (39.01%). This percentage inhibition was comparable with percentage inhibition exhibited by the standard drug, indomethacin (10mg) in the late phase (41.19%).

Figure 1: Effect of ethanol and aqueous extract of stem and root of *Rumex hastatus* D. Don on acetic acid - induced writhing in mice



Std: *Standard(diclofenac sodium);* ***EES:*** *Ethanol extract(stem);* ***AES:****Aqueous extract(stem);* ***EER:*** *Ethanol extract(root);* ***AER:*** *Aqueous extract(root).*

Each point represents the mean ±S.E.M., n = 6. [a] *$P < 0.001$,* [b] *$P < 0.01$,* [c] *$P < 0.05$ vs blank control group*

Anti-inflammatory activity

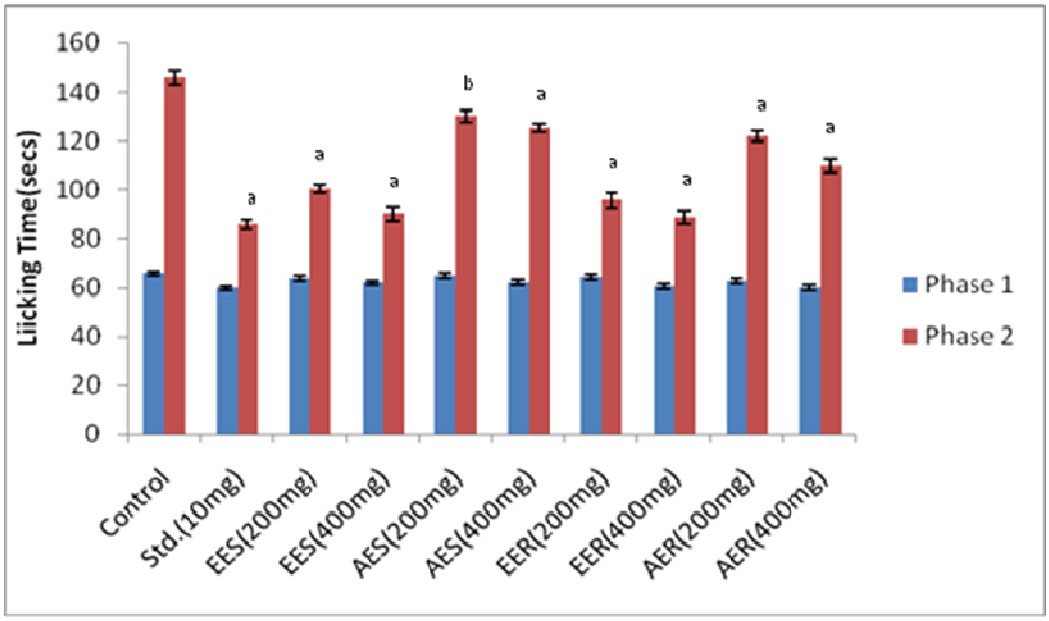
In carageenan induced rat paw edema method, the ethanol extracts of both stem and root (400 mg/kg) of *Rumex hastatus* D. Don exhibited more significant anti-inflammatory activity than 200 mg doses after 3 hours of drug treatment in experimental animals. While after 6 hours, all the test extracts showed significant dose dependent anti-inflammatory activity, when compared with standard drug indomethacin [Figure 3]. Different extracts of stem and roots inhibited the increase in dry weight of cotton pellet induced granuloma, but the maximum percent inhibition was exhibited by ethanol extract (400mg/kg) of *Rumex hastatus* root (27.25%) followed by ethanol extract (400mg) of *Rumex hastatus* stem (23.27%) [Figure 4]. The aqueous extracts were less effective than ethanol extract.

Table 1: Effect of ethanol and aqueous extract of stem and root of *Rumex hastatus* D. Don in tail flick method in mice

Extracts	Dose mg/kg	Reaction time(in secs) after…				
		0 hr	1/2 hr	1 hr	2 hrs	3 hrs
Control	-	4.2±0.2449	4.4+0.2772	4.6±0.2106	4.8±0.3742	4.4±0.3002
Pentazocine	5	4.4±0.4002	6.8±0.3742 [b]	12± 0.3742[a]	12±0.3542[a]	11±0.5831[a]
Ethanolic extract	200	4.2±0.3162	5.8±0.4899	6.2±0.2649	6.8±0.5099[c]	6.6±0.3652[b]
(stem)	400	4.2±0.2449	6.2±0.3746[c]	6.8±0.3652[b]	7.2±0.4010[b]	8.4±0.3162[a]
Aqueous extract	200	4.6±0.4012	5.8±0.4892	6±0.4002	6.4±0.3772	6±0.3653
(stem)	400	4.4±0.5099	5.4±0.4112	6.4±0.3742[c]	6.6±0.3652[c]	6.2±0.3746[c]
Ethanolic extract	200	4.2±0.3742	5.2±0.4899	6.4±0.5099[c]	7.2±0.3981[b]	7±0.3414[a]
(root)	400	4.6±0.6782	6.4±0.3742[c]	8.8±0.4890[a]	11±0.3752[a]	10±0.6647[a]
Aqueous extract	200	4±0.4001	5.4±0.2499	6±0.4002	6.4±0.5097	5.8±0.3742
(root)	400	4.4±0.5099	5.6±0.3074	6.4±0.3742[c]	6.8±0.5831[c]	6.6± 0.3672[b]

Data is shown as mean ±S.E.M., n = 6. [a] P < 0.001, [b] P < 0.01, [c] P < 0.05 vs blank control group.

Figure 2: Effect of *Rumex hastatus* D. Don ethanol and aqueous stem extracts on formalin-induced licking in mice

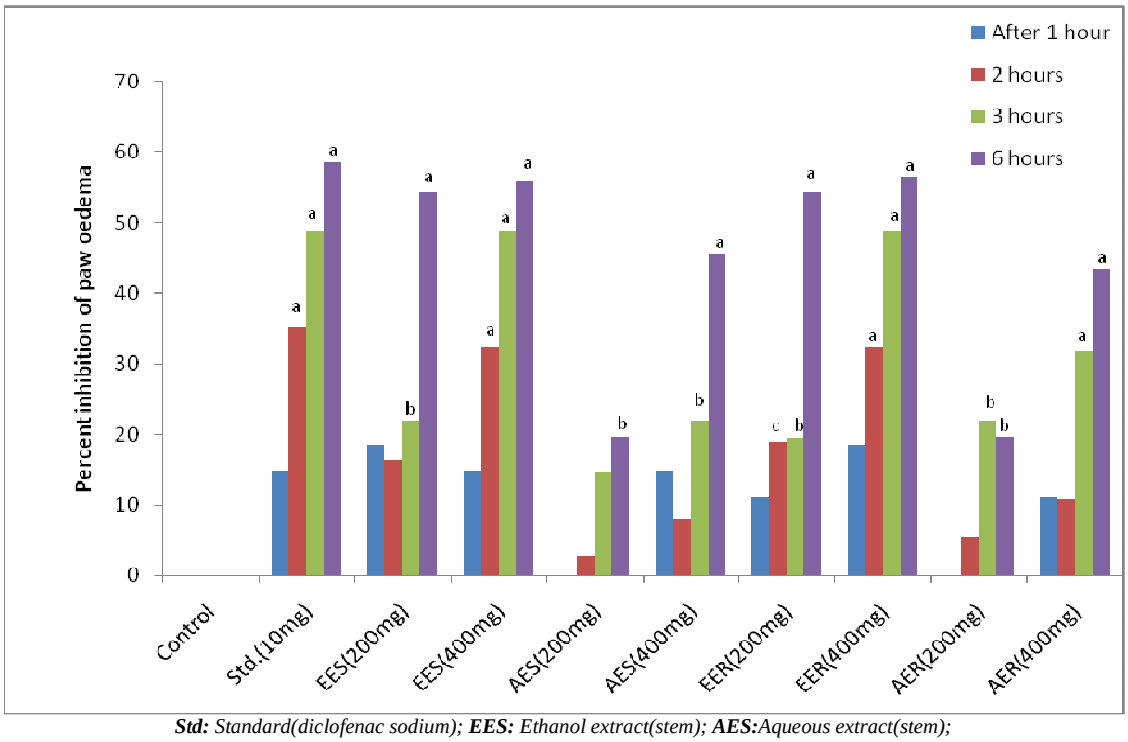


Std: *Standard(indomethacin);* ***EES:*** *Ethanol extract(stem);* ***AES:****Aqueous extract(stem);* ***EER:*** *Ethanol extract(root);* ***AER:*** *Aqueous extract(root). Each point represents the mean ±S.E.M., n = 6. [a] P < 0.001, [b] P < 0.01, [c] P < 0.05 vs blank control group.*

Antipyretic activity

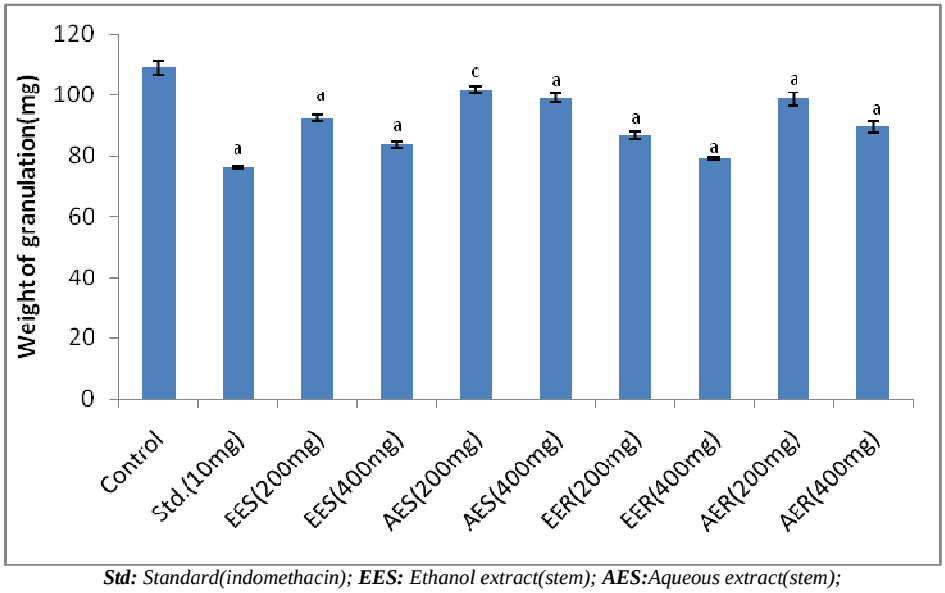
The results of antipyretic effect of the ethanol and aqueous extracts of *Rumex hastatus* D. Don stem and root, on yeast induced pyrexia in rats are shown in Table 2. The subcutaneous injection of yeast suspension in 0.9% normal saline solution, increased the rectal temperature after 18 hours. The ethanol extracts of roots of *Rumex hastatus* D. Don at the dosage of 400 mg/kg started showing its antipyretic effect after 1 hour of dose administration. The ethanol extracts of both the parts at the dosage of 400 mg/kg, produced a pronounced antipyretic effect in hyperthermic rats in a dose dependent manner when compared with untreated rats. The results were comparable with the standard drug, paracetamol (150 mg/kg).

Figure 3: Effect of ethanol and aqueous extract of stem and root of *Rumex hastatus* D. Don in carrageenan induced rat paw oedema method in rats



Std: *Standard(diclofenac sodium);* ***EES:*** *Ethanol extract(stem);* ***AES:****Aqueous extract(stem);*
EER: *Ethanol extract(root);* ***AER:*** *Aqueous extract(root). Each point represents the mean ±S.E.M., n = 6.* [a] *P < 0.001,* [b] *P < 0.01,* [c] *P < 0.05 vs blank control group.*

Figure 4: Effect of ethanol and aqueous extract of stem and root of *Rumex hastatus* D. Don in cotton pellet induced granuloma method in rats



Std: *Standard(indomethacin);* ***EES:*** *Ethanol extract(stem);* ***AES:****Aqueous extract(stem);*
EER: *Ethanol extract(root);* ***AER:*** *Aqueous extract(root). Each point represents the mean ±S.E.M., n = 6.* [a] *P < 0.001,* [b] *P < 0.01,* [c] *P < 0.05 vs blank control group.*

Table 2: Effect of ethanol and aqueous extract of stem and root of *Rumex hastatus* D. Don in yeast induced pyrexia in rats

TREATMENT	Dose	Temperature in °C				
	(mg/kg)	Initial	After Yeast tt	1 hour	2 hours	4hours
Control	1ml	37.52±0.1336	38.65±0.1155	38.96±0.1306	39.23±0.1034	39.98±0.1322
Paracetamol	150	37.40±0.1489	38.54±0.1347	38.10±0.0988 [b]	37.45±0.1136 [a]	37.43±0.0400 [a]
Ethanolic extract	200	37.44±0.1137	38.96±0.0985	38.88±0.1176	38.87±0.1341	39.52±0.1915
(stem)	400	37.73±0.1380	39.38±0.1984	38.28±0.1242 [c]	38.27±0.2520 [b]	37.97±0.1242 [a]
Aqueous extract	200	37.45±0.1136	39.13±0.1414	38.83±0.08341	38.78±0.1043	39.38±0.1291
(stem)	400	37.61±0.0878	39.14±0.1408	38.99±0.1341	38.83±0.0834	39.30±0.0245 [c]
Ethanolic extract	200	37.41±0.1488	38.92±0.2341	38.83±0.0834	38.78±0.1043	38.54±0.0200 [c]
(root)	400	37.76±0.1341	39.26±0.1545	38.27±0.2604 [c]	38.25±0.2628 [b]	37.86±0.1178 [a]
Aqueous extract	200	37.44±0.1205	38.97±0.2722	38.77±0.1043	38.73±0.1286	39.44±0.2304
(root)	400	37.45±0.1136	38.85±0.3176	38.68±0.1332	38.63±0.1605 [c]	39.21±0.0775 [b]

Data is shown as mean ±S.E.M., n = 6. [a] P < 0.001, [b] P < 0.01, [c] P < 0.05 vs blank control group.

DISCUSSION

The above results indicate that ethanol and aqueous extracts of stem and root of *Rumex hastatus* D. Don exerted peripheral and central analgesic activity. The extracts also exerted their effects against acute and chronic inflammations and also showed antipyretic activity. Moreover the ethanol extracts were more active than the aqueous extracts of both the parts in terms of activities shown.

The acetic acid induced writhing method is associated with the release of endogenous substrates like bradykinin and prostanoids. The results show that the analgesic effect of the extracts may be due to inhibition of the synthesis, and/or liberation of these inflammatory mediators [27]. The extracts also had a significant effect in the tail flick test. Centrally acting analgesic drugs elevate pain threshold of animals towards heat. Therefore it shows that the extracts might be exerting central analgesic activity. In the formalin test, there is a distinctive biphasic nociceptive response termed early (phase 1) and late phases (phase 2). Drugs that act primarily on the central system inhibit both phases equally while peripherally acting drugs inhibit the late phase [28,29]. The early phase is probably a direct result stimulation of nociceptors in the paw and reflects centrally mediated pain while the late phase is due to inflammation with a release of serotonin, histamine, bradykinin and prostaglandin [20]. The extract inhibited both phases of the formalin-induced pain with a more pronounced effect on the second than the first phase. This study shows the presence of both central and peripheral effects. This speculation of two fold activity is further supported by the significant activity observed on both the acetic acid-induced abdominal constrictions and tail-flick test.

Carrageenan-induced rat paw oedema is a suitable experimental animal model to study the acute inflammatory activity. Carrageenan, a sulphated polysaccharide obtained from sea weed (Rhodophyceae) is commonly used to induce biphasic acute inflammation. The first phase (1 hour) is due to release of histamine and serotonin. The second phase (over 1 hr) is caused by the release of bradykinin, protease, prostaglandin and lysosome. Prostaglandins play a major role in the development of the second accelerating phase of swelling relation, which is measured at around 3 hours time [30]. Our results showed that the active extracts inhibited the oedema during all phases of inflammation in this model. The cotton-pellet granuloma model is widely used to evaluate the activity of the extract against chronic inflammation. The extracts significantly reduced cotton pellet-induced granuloma, thereby suggesting its activity in the proliferative phase of the inflammation [15].

Mostly, non-steroidal anti-inflammatory drugs produce their antipyretic activity by inhibiting prostaglandin synthetase within the hypothalamus. Thus, the extracts may have exerted antipyretic activity in yeast induced pyrexia in rats through the inhibition of prostaglandin synthesis in hypothalamus [26].

The preliminary phytochemical screening of ethanolic extracts of stem and roots of *Rumex hastatus* showed the presence of mainly anthraquinone glycosides, tannins, saponins and steroids and it is speculated the above proven effects may be due to the action exerted by these constituents either singly or in combined form.

CONCLUSION

The above results have furnished the pharmacological evidence that supports the folklore claim of the drug as an analgesic, antiinflammatory and antipyretic drug. Further studies are required to isolate the active phytoconstituents which are responsible for the reported activity.

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