



PRELIMINARY PHYTOCHEMICAL ANALYSIS, HPTLC STUDIES AND ANTIPYRETIC ACTIVITY OF ALCOHOL AND AQUEOUS EXTRACT OF *HELICTERES ISORA* L. ROOT

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ABSTRACT

Roots of *Helicteres isora* L. (Sterculiaceae) are considered as one of the botanical source of the drug *Murva* (Controversial drug) which is usually prescribed in Ayurveda, for intermittent fever. The present study was carried out to investigate the effect of ethanol and aqueous fractions of *Helicteres isora* L. Yeast induced pyrexia model was used for evaluation of antipyretic activity on albino rats and fall in body temperature of febrile rats was taken as indication of antipyretic activity. Antipyretic activity of *Helicteres isora* L. at the dose of (200 and 400 mg/kg) was found to be significant.

Key words: *Helicteres isora*, Antipyretic activity, Yeast induced pyrexia, Sterculiaceae.

INTRODUCTION

Helicteres isora L. (Sterculiaceae), considered as one of the botanical source of *Murva* in Punjab, commonly called as Mrigshringa in Sanskrit is a large shrub or small tree, occur often gregariously, throughout India and in dry deciduous forests, up to 1500 m on the hill slopes¹. Accepted source of *Murva* is *Marsdenia tenacissima* (Roxb.) Moon (Asclepiadaceae)². *Murva* is used in several Ayurvedic formulations as an ingredient for the treatment of intermittent fever, abdominal colic, laxative, urinary diseases, pruritus, diabetes mellitus, epilepsy, piles, typhoid, sterility, rigidity in lower limbs and skin diseases³.

The root juice of *H. isora* is claimed to be useful in fever, cough, asthma, stomach infections, intestinal infections, diabetes and as a cure for scabies. Fruits are demulcent, mildly astringent and useful in gripping and flatulence^{4,5}. The decoction of the root is mixed with turmeric powder and applied externally, to treat cuts and wounds, by the ethnic people of Rayalseema of Andhra Pradesh⁶. The presence of cucurbitacin B and isocucurbitacin B were reported in roots⁷. Aqueous, ethanol and butanol extracts of *H. isora* root have been reported to possess significant anti-hyperglycemic activity in both alloxan⁸ and glucose⁹ induced hyperglycemic rats at a dose of 250 mg/kg. Ethanol extract of roots caused significant reduction in plasma glucose, triglycerides and insulin resistant in diabetic mice¹⁰. The potent inhibitory activity of aqueous extract of *H. isora* fruits was reported against avian myeloblastosis virus¹¹ and human immunodeficiency virus¹².

MATERIALS AND METHODS

Plant material

The plant material was collected in flowering condition during January 2008 from Kalakkad forest surroundings of Tirunelveli district of Tamil Nadu. It was identified by Dr. S. N. Yoganarashiman, Dept. of Pharmacognosy, M.S.Ramaiah College of Pharmacy, Bangalore. Voucher specimen no. Varsha 030 and crude drugs are deposited at the Crude drug Museum.

Preparation of Extracts

Alcohol extract was prepared by extracting dried root of *H. isora* powder (100g) with 70%v/v ethanol in a soxhlet apparatus by continuous heat extraction. The ethanol extract was concentrated to a small volume and evaporated to dryness. The alcohol extract for experimental purpose was prepared in distilled water containing 2% v/v Tween 80 (as suspending agent).

Aqueous extract was prepared by macerating with chloroform water (0.2%), followed by filtration and concentrating the extract to small

volume, evaporated to dryness. The aqueous extract was prepared in distilled water containing 2% v/v Tween 80 (as suspending agent).

Preliminary Phytochemical analysis

1.8 kg of the fresh roots was collected from Tirunelveli district, Tamil Nadu. They were dried in shade and preserved in plastic container for phytochemical studies.

Physical constants were determined following *Indian Pharmacopoeia* (1996)¹³, phytochemical tests were carried out following Brain and Turner (1975)¹⁴ and Kokate (1999)¹⁵. Chromatographic studies were carried out following Raaman (2006)¹⁶, Krebs (2005)¹⁷, Wagner (1996)¹⁸ et al and Harborne (1998)¹⁹.

Preparation of extract

The roots of *H. isora* were collected, washed and dried at room temperature. After complete drying it was powdered and passed through a sieve (60-40) and stored in a tight container. Dried powdered drug was used to prepare the extract.

Successive solvent extraction

About 50g of the air dried powdered plant material was extracted successively with Petroleum ether (60-80^o), followed by benzene, chloroform, acetone, and ethanol (70%) in a Soxhlet apparatus. Finally, the marc was macerated with chloroform water for 24 hours to obtain an aqueous extract. The extracts were filtered, the solvent was evaporated and accurate weight of the extracts was taken. The extractive value (%) was calculated with reference to air dried drug. The colour and consistency of the extracts were noted down. Extract were tested for confirming the presence or absence of different plant constituents.

HPTLC studies

Chromatographic studies were carried out following Harborne (1998), Stahl (2005) and Wagner (1996) et al.

In the present work Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by WinCATS- 4 software were used. All the solvents used were of HPTLC grade obtained from MERCK. All weighing were done on Precisa XB 12A digital balance.

Preparation of extract

10mg/ml solution was used for chloroform, alcohol and aqueous extract of the drug. Exactly 100mg extract was dissolved in the respective solvent 10ml for preparation of 10mg/ml solution.

Preparation of mobile phase for Chloroform, alcohol and aqueous extracts

Mobile phase for aqueous extract was n-butanol: Glacial acetic acid: water (7:2:1). 10 ml of the solution was prepared by measuring 7ml of n-butanol, 2ml of glacial acetic acid and 1ml of water. Mobile phase for alcohol extract was Toluene: Chloroform: Ethanol (28.5: 57: 14.5). 10 ml of the solution was prepared by measuring 2.85ml of Toluene, 5.7ml of Chloroform and 1.45ml of Ethanol. Mobile phase for Chloroform extract was Chloroform: Methanol (95:10). 10.5ml of the solution was prepared by measuring 9.5 ml of Chloroform and 1ml of methanol.

Chamber used for mobile phase

Camag twin trough chamber (10 x 10 cm)

Chamber saturation

Chamber saturation was done for 18h.

Stationary phase

TLC aluminum sheet precoated with silica gel 60 F₂₅₄, (10 X 10 cm) was used as stationary phase, obtained from MERCK.

Procedure

The Chloroform extract, alcohol extract and aqueous extract solutions were prepared. The TLC plate was activated by heating at 120°C for about 30 min prior to use. Alcohol extract solution (2 µl), standard Chloroform extract solution (5 µl), and Aqueous extract solution (2 µl), each were applied in duplicate, as tracks 1-6, with a band length of 7 mm each on a precoated silica gel 60 F₂₅₄ TLC plate, with Linomat V applicator using a Hamilton syringe. The mobile phase used for chloroform extract was Chloroform: Methanol (95:10). No prewashing of the plate was done. Chamber saturation time was 18 h. The TLC plate was kept for development to a migration distance of 77 mm. Post derivatization has been done with vanillin-phosphoric acid. The derivatized plate was dried in hot air oven at 108°C for 10 min. and scanned at 254 nm and 366 nm, band length 7 mm, slit dimension, scanning speed and source of radiation was Deuterium and Tungsten lamps respectively. The mobile phase used for aqueous extract is n-butanol: Glacial acetic acid: water (7:2:1) and for alcohol extract was Toluene: Chloroform: Ethanol (28.5:57:14.5). No prewashing of the plate was done. Chamber saturation time was 18. TLC plates were kept for development, to a migration distance of 66mm for aqueous and for alcohol extract was 80mm. No post derivatization was done. The developed plates were dried and scanned successively at wavelengths of 254nm, 366nm and 425nm, band width, slit dimension, scanning speed and the source of radiation was deuterium, tungsten and mercury. The R_f and peak area of the spots were interpreted by using software. The derivatized plate was photo documented under 254 nm and 366 nm light using Camag Reprostar 3, equipped with 12 bit CCD camera.

Pharmacological activity

Animals

Healthy Swiss albino mice (6-8 weeks) of either sex weighing 20-25 g and albino rats (Wistar strain) of either sex in the weight range of 170-190 g were selected for the study. Animal house was well maintained under standard hygienic conditions, at a temperature (22±2°C), room humidity (60 % ±10%) with 12 hours day and night cycle, with food and water *ad libitum*. All pharmacological studies were carried out after obtaining approval from the Institutional Animal Ethics Committee of M.S. Ramaiah College of Pharmacy.

Acute toxicity studies

The extracts were administered orally, at doses of 30, 100, 300, 1000, 3000 mg/kg b.w. to the overnight fasted animals. Further the doses of 2000 and 2500 mg/kg b.w. were also tried. Animals were observed closely for the first three hours, for any toxic manifestation (for example increased motor activity, sedation, acute convulsion, coma and death). Thereafter, the observations were made at regular

intervals for 24 hrs. The animals were under observation for one week^{20,21}.

Antipyretic activity

Yeast induced Hyperthermia model

For induction of fever in rats, 20% w/v of brewer's yeast in distilled water was administered by subcutaneous injection. All animals were induced pyrexia by injection of 10 ml/kg of brewer's yeast solution under the skin in between the shoulder blades. The site of the injection was massaged in order to spread the suspension beneath the skin. Basal rectal temperature was measured before the injection of yeast, by inserting digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded 19 hrs. after yeast injection. The febrile rats were divided into six groups, each containing 6 animals. Thereafter, treatment was carried out as follows:

Group1: Vehicle control (distilled water containing 2% Tween 80) p.o.

Group2: Standard group (Paracetamol 150mg/kg body weight) p.o.

Group3: Aqueous extract (200 mg/kg body weight) p.o.

Group4: Aqueous extract (400mg/kg body weight) p.o.

Group5: Alcohol extract (200mg/kg body weight) p.o.

Group6: Alcohol extract (400 mg/kg body weight) p.o.

The different groups of febrile rats were orally administered with the respective drugs and rectal temperature was recorded at 30, 60, 120, 180 and 300 minutes post treatment. Decrease in rectal temperature post treatment indicated antipyretic effect. The difference in body temperature was recorded^{22, 23}. The data were expressed as mean ± S.E.M values and tested with one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.

RESULTS

Preliminary phytochemical screening of root extract gave positive test for the Presence of tannins, phenolic compounds, amino acids, carbohydrates, phytosterols, triterpenoids and alkaloids.

HPTLC studies

In this study, 2µl of aqueous extract revealed 6 phytoconstituents at R_f 0.17, 0.24, 0.31, 0.61, 0.71 and 0.85 (fig.1) quenched fluorescence at 254nm, out of these, spots at R_f 0.24, 0.31, 0.71 and 0.85 were pronounced and 0.17, 0.61 were comparatively less pronounced with black colour.

Aqueous extract revealed 7 phytoconstituents at R_f 0.11, 0.18, 0.31, 0.36, 0.69, 0.72 and 0.76 (2) respectively, out of these 0.31, 0.36, 0.69, 0.72 and 0.76 were pronounced with blue colour and 0.11 and 0.18 were less pronounced under 366nm.

Aqueous extract revealed three phyto constituents in white light at 425nm at R_f 0.62, 0.69 and 0.85 (fig.3) out of these two 0.85 and 0.69 were more pronounced whereas spots at 0.62 was less pronounced comparatively.

Alcoholic extract (3µl) in toluene: Chloroform: ethanol (28.5: 57: 14.5) revealed 7 spots at R_f 0.16, 0.47, 0.54, 0.63, 0.73, 0.80 and 0.90 (fig.1) quenched fluorescence at 254nm, out of these 0.80, 0.63, 0.54 were pronounced whereas 0.16, 0.47, 0.73 and 0.90 were less pronounced.

Alcohol extract revealed 6 spots under 366nm at R_f 0.55, 0.59, 0.65, 0.71, 0.86, and 0.94 respectively, out of these 0.59, 0.65, 0.71, 0.86 and 0.94 (fig.2) were more pronounced whereas 0.55 was less pronounced.

All spots quenched fluorescence at 254nm, showed blue and violet fluorescence at 366nm.

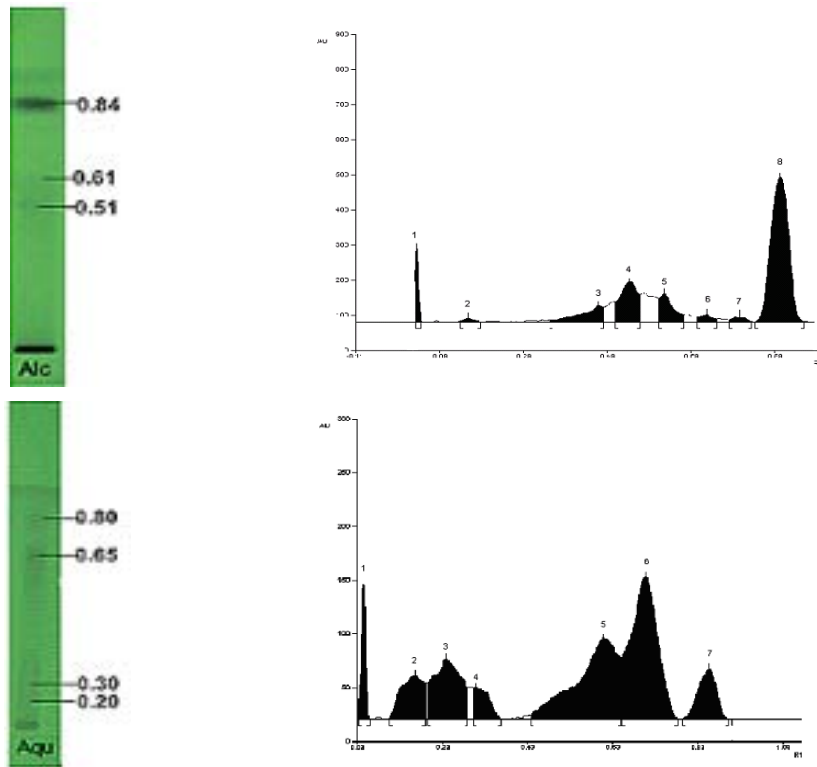


Fig. 1: It shows Chromatogram of Alcohol and aqueous extract at 254nm.

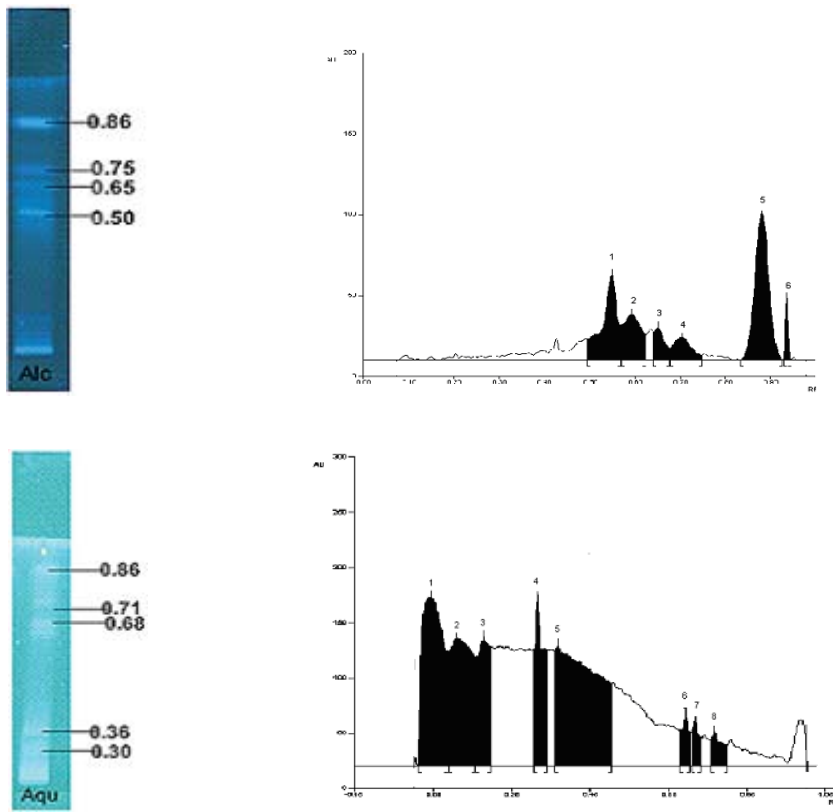


Fig. 2: It shows Chromatogram of Alcohol and aqueous extract at 366nm.

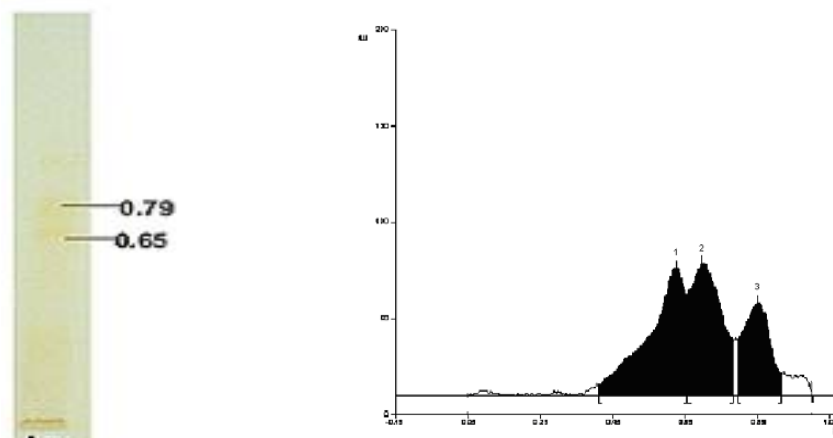


Fig. 3: It shows Chromatogram of Alcohol and aqueous extract at 366nm.

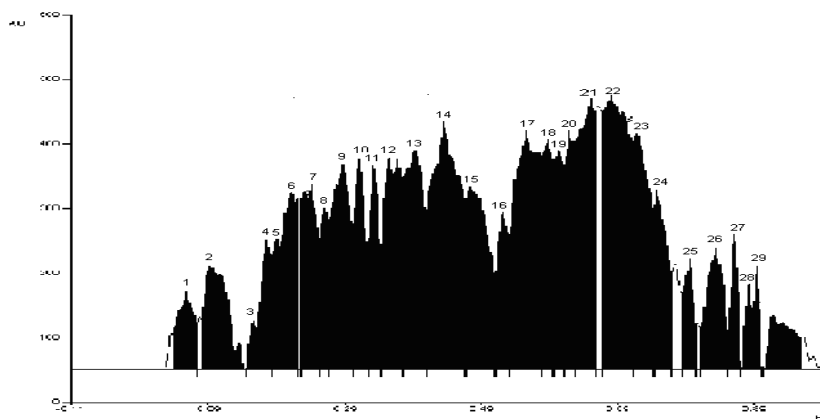


Fig. 4: It shows Chromatogram of Chloroform extract at 254 nm.

Identification of Cucurbitacin B in chloroform extract

Chloroform extract (5 μ l) revealed 28 peaks at R_f 0.13, 0.17, 0.21, 0.24, 0.26, 0.29, 0.31, 0.33, 0.35, 0.37, 0.39, 0.43, 0.47, 0.52, 0.56, 0.59, 0.60, 0.62, 0.65, 0.68, 0.72, 0.75, 0.79, 0.83, 0.86, 0.89 and 0.92 respectively and quenched fluorescence at 254 nm out of these spots. R_f at 0.29 corresponds to that of Cucurbitacin B in mobile phase Chloroform: Methanol (95:10)¹⁸.

Pharmacological studies

Acute toxicity studies

No mortality was observed up to dose of 2000 mg/kg b. w. But animals treated with 1000, 1500, 2000 mg/kgb.w. of either extract showed tremors, decreased motor activity and sedation.

Yeast induced hyperthermia

The reduction in rectal temperature of febrile rats, treated with different doses of aqueous and alcohol extracts of the drug, were recorded at 30, 60, 120, 180 and 300 min after drug administration. Paracetamol was used as the standard. The reduction in rectal temperature of treated animals at each interval was compared with that of untreated febrile rats. Both extracts exhibited significant antipyretic effect, within 30 min of administration, as evidenced by the significant (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) fall in rectal temperature, compared with febrile control animals. The results were comparable with the standard drug paracetamol. Febrile animals treated with higher doses of alcohol extract exhibited greater fall in rectal temperature than those treated with paracetamol. The results are presented in table 1 and histogram.

DISCUSSION

Antipyretics prevent rise in body temperature generally in response to microbial or endogenous pyrogens, as excessive rise in body temperature may cause irreversible tissue damage and possibly death. Pyrogens either activate the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandin (PG), or make available the substrate for the enzyme. In these activities, synthesis of prostaglandin E, especially PGE₁ is thought to be increased in the hypothalamus. Antipyretics compete with arachidonic acid at the active site of cyclooxygenase. During fever, arachidonic acid synthesis may be inhibited by antipyretics. Most of the currently available antipyretics inhibit both cyclooxygenase 1 and cyclooxygenase 2 (COX-1 and COX-2, respectively), inhibiting the synthesis of prostaglandins and thromboxanes. Inhibition of COX-2 is thought to mediate, at least in part, the anti-pyretic action of aspirin and related antipyretic drugs while inhibition of COX-1 results in the unwanted side effects associated with this drug²⁴. The anti-pyretic action of *H. isora* may thus be dependent on its inhibition of PGE₁ synthesis.

Antipyretic activity using yeast induced pyrexia model was performed on Wistar rats of either sex. Pyrexia was induced by subcutaneous injection of 20% w/v of brewer's yeast in distilled water. Both alcohol and aqueous extracts at a dose level of 200 and 400 mg/kg b.w. showed significant antipyretic activity within 30 min. of drug administration.

Preliminary phytochemical screening of root extract gave positive test for the tannins, phenolic compounds, amino acids, carbohydrate, phytosterols, triterpenoids and alkaloids. The presence of tannins, phytosterols, alkaloids, triterpenoids present in the roots of *H. isora* may be responsible for the antipyretic activity^{25, 26, 27, 28, 29}.

Table 1: It shows the effect of *H. isora* extract on yeast induced pyrexia in rats

Groups	Drug and dose	-19 h	0 h	½ h	1h	2h	3h	5h
Control	Distilled water containing 2% Tween 80	34.83 ±0.119	38.40 ±0.248	38.40 ±0.299	38.54 ±0.21	38.52 ±0.202	38.50 ±0.202	38.50 ±0.203
Standard	Paracetamol 50 mg/kg	35.14 ±0.221	37.93 ±0.236	37.16 ±0.153	37.15* ±0.26	36.69*** ±0.328	36.69*** ±0.328	35.48*** ±0.531
Test	Alcohol 200 mg/kg	34.99 ±0.242	39.75 ±0.253	35.58*** ±0.352	35.37*** ±0.32	35.35*** ±0.307	35.26*** ±0.297	35.11*** ±0.331
	Alcohol 400 mg/kg	34.44 ±0.282	38.75 ±0.253	34.41*** ±0.14	34.18*** ±0.264	34.03*** ±0.099	34.32*** ±0.115	34.61*** ±0.239
	Aqueous 200 mg/kg	35.45 ±0.252	39.80 ±0.299	36.73** ±0.397	36.26*** ±0.346	36.09*** ±0.35	35.43*** ±0.358	35.77*** ±0.369
	Aqueous 400 mg/kg	35.03 ±0.307	38.15 ±0.274	36.48*** ±0.308	36.38*** ±0.313	36.13*** ±0.287	35.95*** ±0.319	35.8*** ±0.325

The values are expressed as mean ± SEM; n=6 animals in each group.

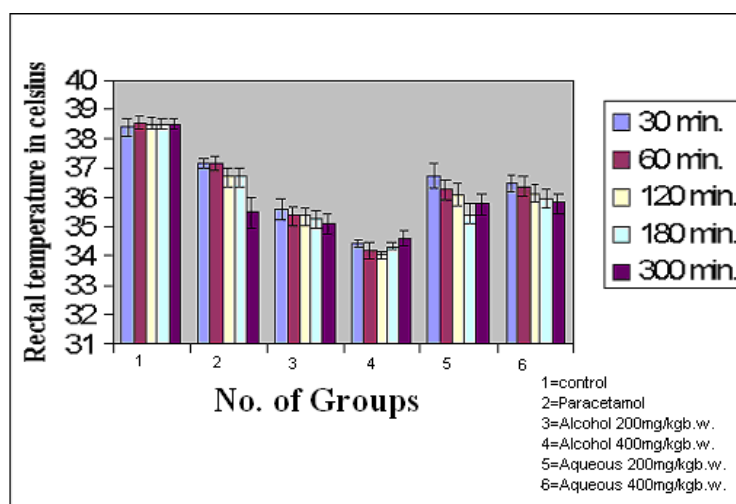


Fig. 5: It shows the effect of alcohol and aqueous extract of *H. isora* on febrile rats.

CONCLUSION

Based on the results of the present study it can be concluded that the alcohol and aqueous extracts of *H. isora* has potential antipyretic activity. Hence the present study substantiates the use of *H. isora* as a botanical source *Murva* for the treatment of fever.

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