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Full Length Research Paper

Phytochemical screening and analgesic properties of ethanol extract of the leaves of *Hugonia mystax* L.

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Hugonia mystax (H. mystax) **has been used in the siddha and ayurvedha for various ailments. In this study ethanolic crude extract (EEHM) of the leaves were studied for various analgesic methods. The ethanolic extract of *H. mystax* was spiked into the male swiss albino mice (weighing 20 to 25 g) and male wister rats weighing (150 to 200 g) and analyzed the analgesic activity by hot plate and acetic acid induced method. The phytochemical analysis of ethanolic extract of *H. mystax* showed the presence of carbohydrates, flavonoids, steroids, saponins, terpenoids and absence of alkaloids, proteins and amino acids. For acute toxicity test, mice were injected different doses of each extract by intraperitoneal route and the LD_{50} values were determined. The analgesic effect was evaluated in mice by the hot plate method and acetic-acid writhing test. The extracts have produced significant analgesic effects by the acetic acid writhing test and by the hot plate method ($p < 0.01$) and a dose-dependent inhibition was observed. The overall results indicate the significant analgesic activity and also its justification for further traditional uses *H. mystax* leaves.**

Key words: *Hugonia mystax*, analgesic activity, toxicity.

INTRODUCTION

The genus *Hugonia* L. of family Linaceae comprise about 40 species in the world; of which *Hugonia mystax* L. was reported from India and Srilanka (Santapau and Henry, 1983; Pullaiah and Chennaiah, 1997). This plant *H. mystax* is locally named Modirakanni. Ethno botanically, the fruits were used by the tribals of Kalakad Mundanthurai for the treatment of rheumatism (Sutha et al., 2009). The literature study reveals that the roots of *H. mystax* were used as anthelmintic, astringent and also used for dysentery, snake bite, fever, inflammation and rheumatism. Biological activities such as analgesic, anti inflammatory and ulcerogenic were also reported (Balasubramaniam et al., 1997; Guha et al., 2001). The anti-oxidant activity was confirmed by the studies on the leaf (Rajeswari et al., 2013). Antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of root extracts showed significant activity against various human pathogens (Vimalavady et al., 2012). Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. The bioactive components are identified through by the gas chromatography-mass spectrometry (GC-MS) analysis (Kaneria et al., 2007).

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The drug compendium (Rastogi et al., 2002; Yoganarasimhan, 2000) showed the leaves of *H. mystax* has an analgesic activity but there is no study reported. With addition to the fact that the compound containing analgesic activity nature was phytochemically identified in the ethanol extracts of leaf of *H. mystax* (Rajeswari et al., 2012) by GC-MS analysis and the result showed the *H. mystax* leaves containing the isoprenoid compound. Many of the studies showed that the chemical nature of isoprenoid have the analgesic character (Damiao, 2011; Magdalena et al., 2013; UIC, 2014). The compounds of squalene and vitamin E (Rajeswari et al., 2012; Damiao, 2011; Magdalena et al., 2013; UIC, 2014) have the isoprenoid units which was present in the leaves of *H. mystax*. So we can make attempt by this analgesic character, later on the leaves extract showed the good result in the trail works. So, we take this as the consideration and also on the medicinal value and utility. The present study was aimed to explore the analgesic activity of the medicinal plant named *H. mystax*. The objective of the work was to prove the analgesic activity of *H. mystax* leaves through the techniques of hot plate and acetic acid induced method.

MATERIALS AND METHODS

Collection, identification and preparation of plant materials

Fresh leaves of *H. mystax* were collected from velliangiri hills from Coimbatore, Tamilnadu. It was identified by a scientific officer, Dr. P.Samydurai Assistant Professor, Department of Botany, Kongu Nadu Arts and Science College, Coimbatore. The identification was confirmed with Botanical Survey of India (BSI), Coimbatore, TamilNadu, India. The reference number was: BSI/SRC/5/23/10-11/Tech-1522. The herbarium specimen of *H. mystax* was prepared and deposited in the department of Pharmacology, Nandha College of Pharmacy and Research Institute, Erode, India for future reference.

Animals

Male swiss albino mice weighing 20 to 25 g and male wister rats weighing 150 to 200 g was used for this study. The animals were obtained from animal house, Nandha College of Pharmacy, Erode, Tamilnadu. The experimental procedures and protocols used in this present study were reviewed by institutional animal ethical committee (688/2/C-CPCSEA) of Nandha College of Pharmacy and the proposal number was (NCP/IAEC/PG-40/2009) and also in accordance with the guidelines of Institute for Animal Care Education (IACE). Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30 to 70%. A 12:12 light: day cycle was followed. All the animals were allowed free access to water and fed with standard commercial pelleted chaw (M/s. Hindustan Lever Ltd., Mumbai). The present work was conducted with an effort to minimize the usage of number of animals and the suffering caused by the used procedures in the study.

Preparation of extracts

Leaves of *H. mystax* were dried in shade for two weeks. Dried leaves were coarsely powdered, sieved (#40) and stored in an air tight container at room temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform and ethanol using soxhlation method. The extracts were concentrated to dryness using rotary evaporator. The yields of various extracts were found to be 4.5% w/w (petroleum ether), 4.7% w/w (chloroform) and 10.5% w/w (ethanol). All the extracts were preserved in a refrigerator at 4°C. However, only ethanolic extract of the leaves was selected for further studies.

Qualitative phytochemical analysis

The leaves of *H. mystax* was extracted by the continuous hot percolation method. The ethanolic extract of *H. mystax* was subjected to preliminary phytochemical screening to identify the different phytoconstituents like flavonoids, phenols, saponins, steroids, tannins and terpenoids.

Acute toxicity study

Acute toxicity study was carried out as per stair case method (as per Organisation for Economic Co-operation and Development (OECD) guidelines 425). Albino mice of either sex 20 to 25 g were used. The animals were fasted overnight prior to the acute experimental procedure. The animals were administered with aliquot doses of 100 to 250 mg/kg extracts orally, suspended in Tween 80 (1% w/v). The dose which caused no mortality and was tolerated was determined in a stepwise manner and the effective dose was found to be 100 mg/kg b.w. so that 100 and 200 mg/kg b.w. was selected for further studies.

Analgesic activity

Hot plate method

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging to the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate method was employed for the purpose of preferential assessment of possible centrally mediated analgesic effects of the ethanolic extract of *H. mystax*. The central analgesic drug pentazocine was used for positive control group. In this experiment, four groups (n = 6) of swiss albino mice (20 to 25 g) were placed on a hot plate maintained at room temperature for 15 min. The controlled temperature of commercially available Eddy's hot plate is 55 to 56°C (Rajeswari et al., 2014).

Grouping of animals

Group 1 – Received normal control (0.5% CMC p.o.)
Group 2 – Received Pentazocin (30 mg/kg i.p.)
Group 3 – Received Ethanolic extract of *H. mystax* (100 mg/kg, p.o.)
Group 4 - Received Ethanolic extract of *H. mystax* (200 mg/kg, p.o.)

The observations were recorded and the time interval of 15, 30, 45 and 60 min, respectively. The results of hot plate method in swiss albino mice were tabulated in Table 1.

Acetic acid induced writhing in mice

Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic

Table 1. Analgesic activity of ethanolic extract of *H. mystax* by hot plate method.

Treatment	Reaction time (min)			
	15	30	45	60
Control (0.5% CMC)	2.2 ± 0.2	2.4 ± 0.1	2.5 ± 0.2	2.4 ± 0.4
Pentazocine (30 mg/kg)	4.4 ± 0.4[b]	5.4 ± 0.2[b]	6.2 ± 0.4[b]	8.2 ± 0.6[b]
EEHM (100 mg/kg)	4.2 ± 0.3[b]	5.0 ± 0.2[b]	5.8 ± 0.3[b]	7.4 ± 0.3[b]
EEHM (200 mg/kg)	4.3 ± 0.3[b]	5.3 ± 0.2[b]	6.0 ± 0.1[b]	8.0 ± 0.2[b]

The data represent the Mean ± SEM (n=6), p<0.05[c], p<0.01[b], p<0.001[a] when compared to control. (One way ANOVA followed by Tukey T test).

Table 2. Analgesic activity of ethanolic extract of *H. mystax* by acetic acid induced writhing method.

Treatment	Number of writhing	% Inhibition
Control (0.5% CMC)	65.67 ± 0.5	-
Indomethacine (5mg/kg)	19.57 ± 0.5[b]	72
EEHM (100 mg/kg)	35.32 ± 0.5[b]	47
EEHM (200 mg/kg)	23.65 ± 0.7[b]	65

The data represent the mean ± SEM (n = 6), p < 0.05[c], p < 0.01[b], p < 0.001[a] when compared to control.

activity. An irritating agent such as acetic acid is injected intra-peritoneally to mice and stretching reaction is evaluated (Shanmugasundaram and Venkataraman, 2005).

Grouping of animals

Group 1 – Received normal control (0.5% CMC p.o.).
Group 2 – Received Indomethacin (5 mg/kg p.o.).
Group 3 – Received Ethanolic extract of *H. mystax* (100 mg/kg, p.o.).
Group 4 - Received Ethanolic extract of *H. mystax* (200 mg/kg, p.o.).

Swiss albino mice of male sex were divided into four different groups each containing six animals. Food was withdrawn 12 h prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. The central analgesic drug indomethacine was used for positive control group. After 60 min, writhing was induced by intraperitonial injection of 1% acetic acid in volume of 0.1 ml/10 g body weight. The writhing episodes were recorded for 30 min; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted. The result of acetic acid induced writhing method in mice was tabulated in Table 2.

RESULTS AND DISCUSSION

The analgesic activity of ethanolic extract of *H. mystax* by hot plate method test indicated a significant increase in reaction time ($p < 0.01$) at the dose of 100 and 200 mg/kg comparable to control. In acute toxicity study, no toxic symptoms were observed for the drug up to 2000 mg/kg body weight. The activity produced by the standard pentazocine was found to be the highest reaction time among the group tested. Prostaglandins and bradykinins were suggested to play an important role in pain. The hot plate test was selected to investigate central anti-nociceptive activity because it had several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. The ethanolic extract of *H. mystax* showed significant analgesic activity by acetic acid induced writhing method. The oral administration of ethanolic extract of *H. mystax* induced a dose dependent analgesic activity. Injection of acetic acid into control mice produced 65.67 ± 0.5 writhes. Pretreatment with ethanolic extract of *H. mystax* at doses of 100 and 200 mg/kg reduced the number of writhes by 35.32 ± 0.5 (47% protection) and 23.65 ± 0.7 (65%), respectively.

Conclusion

From the investigation, the ethanolic extract of *H. mystax* leaves possesses potent analgesic effect against differrent stimuli. This is evidenced by significant increase in the reaction time by stimuli in different experimental models.

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Conflict of interest

There is no conflict of interest as regard this study.

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