Analgesic activity of various extracts of *Holoptelea integrifolia* (Roxb.) Planch leaves

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Abstract: The various extracts of leaves *Holoptelea integrifolia* (*Ulmaceae*) were investigated for analgesic activity in mice by tail flick method. The fresh plant leaves of *H. integrifolia* were collected, dried, cleaned, weighed and chopped into small pieces and percolated in ethanol. The fractionation of crude extract, followed by the addition of distilled water, ethyl acetate and *n*-butanol to an aqueous portion of each solvent, to obtain the dried masses of each four layers. Qualitative chemical examination indicates the presence of secondary metabolites such as alkaloids, flavones, phenol, steroids, tannins and triterpenoids. No acute oral toxicity was observed and extracts considered being safe at the dose of 50-2000 mg/kg body weight. At the dose of 500 mg/kg various extracts of leaves of *H. integrifolia* were found statistically significant ($P<0.05$). A maximum effect was established at 150 min, after drug administration. Diclofenace sodium used as a standard.

Keywords: Analgesic activity, *Holoptelia integrifolia*, ethanol, ethyl acetate, butanol and aqueous extracts.

INTRODUCTION

Holoptelea integrifolia (Roxb.) Planch belongs to the family *Ulmaceae,* is distributed in deciduous forest up to an altitude of about 600 m in tropical area including Myanmar (Burma), Indo China and Sri Lanka (Parrotta, 2001). *Holoptelea integrifolia* is a synonym of *Ulmus integrifolia,* and locally called as *Chilbil* or *Papri* in Hindi and in English as Indian elm or Jungle cork tree, naturally found up in Australia and cultivated in Pakistan only for ornamental purposes and represented by 3 genera *Ulmus, Trema* and *Celtis* It is found in different places of Pakistan especially in Karachi and some other parts of Sindh province (Nasir and Ali, 1985). Bark and leaves of plant are used medicinally in Ayurvedic system of medicine, chiefly in allergic disorders and also for treating oedema, diabetes, intestinal disorders and piles. The seeds and stem bark paste are used externally to treat ringworm, and the latter for scabies. Leaves of the plant are used as an external application for wound. The fruit pulp pounded with black salt is recommended for the treatment of menstrual disorders (Anon, 1959). Pollen grains of plant are utilized in various immunological preparations (Singh *et al*., 1992; Sharma *et al*., 2005), The number of chemical constituents were isolated such as sterols (β-sitosterol, stigmasterol), triterpenes, (friedelin, β-amyrin, oleanolic acid, hederagenin), and hydrocarbons (hexacosanol) from the leaves and other parts of plant (Gupta *et al*., 1969: Misra *et al*., 1977: Sing, 1983). Our research study protocol seeks to search such agents in the ornamental plant which can be utilized as curative agent along with its beautifying perspective and to validate for health care system. In this regard the various extracts of leaves of *H. integrefolia* was examined for analgesic activity by the tail flick method.

MATERIAL AND METHODS

Collection

The leaves of *H. integrifolia* were collected in the month of March 2004 from the premises of the University of Karachi and authenticated by taxonomist Prof. Dr. Surrya Khatoon, Chairperson, Department of Botany, University of Karachi, Pakistan. Voucher specimen number 013 was deposited in the Museum of Herbal Medicine, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

Extract preparation

The fresh leaves of *Holoptelea integrifolia* were collected, cleaned, dried and weighed (2 kg) properly. Chopped into small pieces and percolated in absolute ethanol (Merck) at room temperature for 15 days. The percolate was filtered through Whattman filter paper No.1 and the process was repeated thrice to get the maximum yield. Ethanol percolate was evaporated under reduced pressure at 40°C on the rotary evaporator (Eyela, Japan) to obtain ethanol extract of leaves (20 g, 1% w/w). The portion (15 g) of ethanol leaves extract were partitioned with equal quantity of distilled water (500 mL) and ethyl acetate (500 mL) and after subsequent repeated fractionation with more solvents with same ratio and vigorously shaking, organic ethyl acetate and aqueous layers were obtained. Then partitioned further followed by the addition of

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presaturated *n*-butanol (500 mL) to aqueous layer and allowed to separate the layers. After the effective fractionation these three organic solvents were concentrated by using the rotary evaporator and yield of dried semi-solid masses ethyl acetate (6 g, 40%), *n*-butanol (5.5 g, 36.66%) and aqueous (3.5 g, 23.33%) were calculated and fraction of all these extracts were utilized for qualitative phytochemical screening, acute toxicity and analgesic activity.

Phytochemical analysis

Qualitative phytochemical analysis of the ethanol and aqueous extracts were carried out by using different reagents and determined various phytoconstituents (Harbone, 1998).

Animal

The adult male Wistar Albino mice strain (weighing 30 ± 2 g) was utilized for the study. These animals were housed in experimental room of the animal house of Baqai Medical University and maintained on balanced laboratory diet. Total numbers mice's were 30 maintained on group of six at 22 ± 1°C with light/dark cycle of 12 h. All experimental protocols were approved from the Institutional Animal Ethical Committee.

Acute toxicity

Acute oral toxicity of extracts was determined in adult Wistar Albino rats of either sex weighing 150-200gm at a dose of 50-2000 mg/kg body weight. Changes in various autonomic and behavioral responses were noted up to 4 hr with the gap for 15 minutes. The animals were kept under observation for 7 days, gross effect and mortality was observed during this period (Ghosh, 1984).

Analgesic activity

The analgesic activity was assessed by tail flick method according to Janseen, followed by Distasi (Janseen *et al*., 1963 and Distasi *et al*., 1988) with slight modification. Briefly mice were divided into six groups, each group containing six animals. Each mouse was kept in a suitable restrainer with the whole tail extending out. An area of the tail 2-3 cm in length was marked and immersed in water bath at 51 ± 1°C temperature. The withdrawal time of the tail from hot water was noted as the reaction time. Control animals received 10 mL/kg of 0.9% saline orally while extracts (ready in 10% DMSO) in dose of 500 mg/kg body weight were given orally by intubations. The observations were taken immediately and then at intervals of 30, 60, 90, 120, 180, 210 min. for each extracts i.e. ethanol, ethyl acetate, butanol and aqueous. Diclofenac sodium (50 mg/kg) was used as standard. The pain inhibition percentage (Tin Wu *et al*., 2003) was calculated according to the following formula:

$$\% \text{ Pain inhibition} = T_1 - T_0 / T_0 \times 100$$

Where, T_1= Post drug Latency and T_0 = Pre drug Latency

STATISTICAL ANALYSIS

The results were subjected to statistically analyze by One-way ANOVA followed by LSD multiple comparison tests applied for multiple comparisons amongst different groups. $P < 0.05$ was regarded as statistically significant. All values are expressed as mean ± SEM.

RESULTS

Qualitative phytochemical examination of crude extract revealed the presence of alkaloids, flavonoids, phenol, steroids, tannins and triterpenoids (table 1). No acute oral toxicity was observed and extract considered to be safe at the dose of 50-2000 mg/kg body weight. The results of various extracts of *H. integrifolia* leaves, analyzed for analgesic activity by tail flick method are given in (table 2) and found statistically significant *(P<0.05)* at the dose of 500mg/kg body weight as compared to the control. Maximum activity exhibited by crude ethanol extract control. The ethylacetate extract gives moderate but transient analgesic effect. The extracts show greater activity after 60 min of drug administration and ethanol extract exhibited 180.56 % inhibition at 150 min of drug administration (fig. 1).

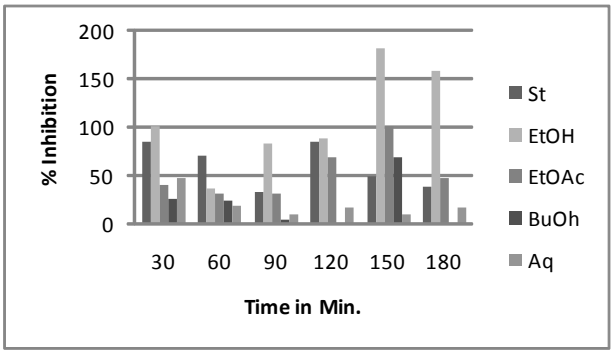

Fig. 1: Percent inhibition of various extracts of *Holoptelea integrifolia* as compared to standard drug (St.), ethanol extract (EtOH), ethyl acetate (EtOAc), butanol (BuOH) and aqueous (Aq).

Table 1: Preliminary phytochemical investigation of *Holoptelea integrifolia* leaf extracts

S. N.	Chemical test	Color indication	Result
1	Test for Steroids	Bluish green ring	+
2	Test for triterpenoids	Purple ring	+
3	Test for tannins	(a) Bluish Black Precipitation (b) Precipitation	+
4	Test for Flavonoids	Pink tomato red colour	+
5	Test for Alkaloids	Orange precipitate	+
6	Test for Saponnins	Frothing Persistence	+

+ = present and - = Absent

Table 2: Analgesic effect of various extracts of *Holoptelea integrifolia* (Roxb.) Planch leaves

Treatment	Time in min.							
	0	30	60	90	120	150	180	210
Control (Normal Sline)	7.33 ± 0.21	8.33 ± 0.42	10.66 ± 1.9	9.50 ± 0.22	8.50 ± 0.71	7.16 ± 0.16	7.00 ± 0.44	8.00 ± 0.63
Diclofenic Sodium (Standard)	7.33 ± 0.21	15.33 ± 1.62	18.16 ± 2.38	12.66 ± 1.28	15.66 ± 2.74	10.66 ± 0.80	9.66 ± 0.55	9.66 ± 0.91
Extracts								
Ethanol	13.33 ± 0.95**	16.66 ± 2.66**	14.50 ± 0.22	17.33 ± 1.45**	16.00 ± 0.63**	20.16 ± 2.65**	18.16 ± 1.55**	15.83 ± 1.03**
Ethylacetate	7.66 ± 0.21	11.66 ± 0.88	13.83 ± 1.62	11.83 ± 1.27	14.33 ± 1.08**	14.33 ± 1.6**	10.33 ± 0.95**	10.50 ± 0.92*
Butanol	15.00 ± 1.59**	10.50 ± 0.61	8.16 ± 0.47	9.83 ± 0.3	8.50 ± 0.22	12.00 ± 0.93*	6.50 ± 0.61	7.83 ± 0.87
Aqueous	10.66 ± 1.68	12.16 ± 1.68	9.00 ± 0.96	8.66 ± 0.84	9.83 ± 0.83	7.83 ± 0.47	5.83 ± 0.47	7.16 ± 0.7

Values are given in Mean ± SEM, N= 06 in each group. *P<0.05, **P<0.01 as compared to control
Control animals dose = 10 ml/kg of 0.9% saline. Extracts in doses of 500mg/kg body weight

DISCUSSION

The present study indicates that the extract has shown significant analgesic action in mice, by increasing the latency period in the tail flick test. Preliminary phytochemical screening revealed the presence of flavonoids compound in *Holoptelia integrifolia* and they are recognized as powerful antioxidants. In the analgesic activity, flavonoids primarily target prostaglandins which are involved in controlling pain perceptions (Vogal *et al*., 1997; Ranjnarayana *et al*., 2001; Jin-Won *et al*., 2011). Therefore, it can be assumed that *Holoptelia integrifolia* extracts might suppress the formation of prostaglandins by inhibiting or antagonizing the enzyme cyclo-oxygenase (Das *et al.,* 2010*)* and also makes the nociceptors more sensitive to pain producing agents such as bradykinin (Curtis *et al*., 1990) and ultimately relieve the sensation of pain.

CONCLUSION

From the whole exercise, it may be concluded that the crude ethanol extract of plant *H. integrifolia* Planch was found to possess significant analgesic activity, while ethyl acetate extracts showed moderate activity, *n*-butanol and aqueous extract of the plant showed mild analgesic effects which indicates its use in the traditional system of medicine in order to support to reduce pain but further studies are required to evaluate the mechanism of action and activity directed bioassay for the new source of drug development.

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