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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANALGESIC ACTIVITY OF *ECLIPTA PROSTRATA*. L (*ASTERACEAE*)

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ABSTRACT

Present study was designed to investigate the pharmacognostical, phytochemical and analgesic activity of *Eclipta Prostrata*. L (*Asteraceae*). Methanolic and ethyl acetate extracts of *Eclipta Prostrata* were prepared by using soxhlet extraction method. The resultant extracts were subjected to pharmacognostical parameters like water soluble, acid soluble extractives and qualitative phytochemical investigations. Further extracts were screened for analgesic activity by using Eddy's hot plate and heat immersion methods, at a dose level of 50 and 100 mg/kg body weight. Ethyl acetate extract showed significant amount of flavonoids and phenolics when compared to methanolic extract. Ethyl acetate extract showed a dose dependent and significant ($p \leq 0.01$) analgesic activity in all the tested methods compared to diclofenac sodium treated mice. Methanolic extract also exhibited analgesic activity. Altogether results suggest that the ethyl acetate extract of *Eclipta Prostrata* and its biologically active constituents could be used as potential analgesic agents.

KEY WORDS: *Eclipta prostrata*, ethyl acetate extract, methanolic extract, analgesic activity.

INTRODUCTION:

Eclipta prostrata (EP) belongs to *Astraceae* family is very common in tropical and subtropical regions, the chemical constituents of the plant are found to be Alkaloids(ecliptine), saponins, proteins, amino acids, flavonoids, tannins. Wedelolactone and dimethyl wedolactone isolated from *Eclipta prostrata* found to possess potent hepatoprotective activity. The herb is rich source of ascorbic acid and it is used to treat a diverse number of ailments including malaria, poisonous animal or insect bites, vomiting tendency, indigestion, ulcer, burning sensations in hand or feet, scabies, abscess, wounds, and to ease pain of delivery[1]

Various endogenous mediators like histamines, seratonins, and prostaglandins are most abundant in inflammatory cell and among them prostaglandins are ubiquitous substance that indicate and modulate cell and tissue responses involved in inflammation. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythma, edema and pain. Hence for treating inflammatory diseases analgesics and anti inflammatory agents are required [2, 3]. Non steroidal anti inflammatory drugs (NSAIDS) are the most clinically important medicines used for the treatment of inflammatory related diseases like arthritis, asthma and cardiovascular disease [4]. Having various and severe adverse effects like gastric lesions for NSAIDS, adverse cardiovascular thrombotic effects for selective cyclooxygenase-2(Cox-2) inhibitors [5] and tolerance and dependence induced by opiates, use of these drugs as anti inflammatory and analgesic have not been successful in all the cases. Therefore new anti inflammatory and analgesic drugs lacking those effects are being searched all over the worlds as alternatives to NSAIDS and opiates. The research into with folkloric use as pain relievers, anti inflammatory agents, therefore is viewed as a fruit full logical research strategy in the search for new analgesic and anti inflammatory drugs (6). During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs.

The reported works on EP revealed that leaf extracts were used in the treatment of infective hepatitis in India [7] and snake venom poisoning in Brazil [8]. It has been reported that the leaves of this herb are used in the case of gastritis and respiratory disorders like cough and asthma [9]. In addition, the crude form of the herb is reported to have anti-inflammatory, anti fungal and anti hepatotoxic properties. Anti bacterial and antioxidant activities of leaves of EP [10] were also reported. It is found to possess HIV-1 inhibitory activity [11], used for skin and stomach problems and shows larvicidal effect [12]. Present study was designed to investigate the preliminary phytochemical screening, pharmacognistical and analgesic activities of methanolic and ethyl acetate extracts of leaves of EP (L).

MATERIALS AND METHODS

The leaves of EP were collected from the open fields of Deshmukhi, Nalgonda, Andhra Pradesh and were authenticated by department of Botony, Osmania university, Hyderabad.

EXTRACTION PROCEDURE

Collected plant material was shade dried and made in to a coarse powder with the help of mechanical grinder. 120 gm of the powdered plant material was extracted successively using solvents like petroleum

ether, chloroform, ethyl acetate, methanol and water in a soxhlet apparatus for the period of three siphons. The extracts were concentrated by distillation to yield a solid residue.

Preliminary phytochemical screening [13-16]

Qualitative chemical screening was conducted for ethyl acetate and methanolic extracts of EP for the presence of active chemical constituents. Results were shown in table l.

Pharmacognostical studies:

Determination of water soluble Extractives:

About 5g of leaf powder was added to 50ml of water at 80^{o}C and 2g of keisulghur was added to it and filtered. 5ml of the filtrate was transferred to a tarred evaporating dish, the solvent was evaporated on a water bath, drying was continued for half an hour, finally it was dried in a hot a air oven for two hours and weighed. The percentage of water soluble extractive was calculated with reference to air dried drug and data was shown in table 2.

Determination of loss on drying

About 2g of the powdered leaf was accurately weighed in a glass stoppered weighing bottle which is previously dried for 30mins in the drier. Then the sample was gently shaken side wise for even distribution and dried in an oven at 100-105^{o}C by removing the stopper. It was cooled in a dessicator and again weighed. The loss on drying was calculated with reference to the amount of air dried powder taken and data shown in table 3.

ANALGESIC ACTIVITY [17]

Swiss albino mice weighing 20-30g of either sex were obtained from the animal house of Nizam institute of Pharmacy, Deshmukhi, Nalgonda and housed in polycarbonate cages. The rats had free access to standard pellet chow and water *ad libitum* throughout the experiment with the exception of some experiments (see below) in which the animals were deprived of food, but not water, for 18-24 h before the experiments were performed. After procurement, all the animals were divided into different groups and were left for one week for acclimatization to experimentation room and were maintained on standard conditions (23^{0}, 60%-70% relative humidity and 12 h photo period). There were six animals in each group for observational screening and acute toxicity studies. All experimental protocols described below were approved by the ethical board.

Eddy's hot plate method

The animals were grouped into 4 each of six animals

Group I received distilled water, which was served as control

Group II received methanolic extract (100 and 200mg/kg)

Group III received ethyl acetate extract (100 and 200mg/kg)

Group IV received Diclofenac sodium (100 and 200mg/kg)

Extracts were administered orally and the standard drug was administered intraperitonially.

Sixty minutes after oral administration of extract and 30min after i.p. injection of diclofenac sodium, animals were individually placed on the hot plate (maintained at 55^{o}C) and the response such as paw licking or jump response, which ever appeared first were noted. Cut off period of 15 sec was maintained and results were shown in table 4.

Heat conduction method

The animals were grouped into 4 groups of six animals each

Group I received distilled water, which served as control

Group II received methanolic extract (100 and 200mg/kg)

Group III received ethyl acetate extract (100 and 200mg/kg)

Group IV received diclofenac sodium (100 and 200mg/kg)

The exracts were administered orally and the standard drug administered intraperitonially. Sixty minutes after oral administration of extract and 30min after i.p. injection of diclofenac sodium, the tail tip of individual animals were dipped up to 5cm into hot water (maintained at 58^{o}C) and the response time was noted as the sudden withdrawal of the tail from the hot water. Cut off period of 10 sec was maintained. Results were shown in table 5.

RESULTS AND DISCUSSION:

Eclipta prostrata is widely used in traditional chinese herbal medicine and in ayurveda. It is considered to be the best remedy for the hair and also used as a rejunevative and liver tonic. To carryout the present study the plant was collected, authenticated and extracted using different solvents. The primary and secondary metabolites were analyzed in methanolic and ethyl acetate extracts and found that steroids, alkaloids, flavonoids, tannins, reducing sugars and proteins were present.[13-16] Under physicochemical parameters extractive values and loss on drying were determined. Even though water extractive value was found to be high, methanolic and ethyl acetate extracts were selected for the analgesic activity as they found to possess significant levels of flavonoids. [12]

Methanolic and ethyl acetate extracts of *Eclipta prostrata* showed significant analgesic activity as evidenced by the reaction time to the pain stimulus. The results were significant at $p \leq 0.01$ for both the methods. On preliminary phytochemical screening the extracts was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain conception. Flavonoids may increase the amount of endogenous serotonin or may interact with 5-HT2A and 5-HT3 receptors which may be involved in the mechanism of central analgesic activity.

Previous researchers reported the presence of several therapeutically valued flavonoids from the EP. Moreover, ethylacetate extract showed highest analgesic activity in the entire experimental model similar to standard drug diclofenac sodium when compared with methanolic extract, which may be due to its highest flavonoid and phenol contents present in ethyl acetate extract [12].

Table 1: Preliminary phytochemical screening of leaf extract of *Eclipta prostrata*

Chemical test	Methanolic extract	Ethyl acetate extract
Alkaloids	+	+
Anthraquinones	-	-
Tannins	+	+
Saponins	+	+
Steroids	+	+
Proteins	+	+
Glycosides	-	-
Reducing sugars	+	-
Flavonoids	+	+

+=present, -=absent

Table 2: Physicochemical parameters of EP

Parameters	%W/W
Loss on drying	1.17
Water soluble extractives	22.6
Methanolic extractives	4.37
Ethylacetate extractives	6.12

Table 3: Analgesic activity of EP by eddy's hot plate method and hot immersion method

Grouping	Reaction time (seconds)	
	Eddy's hot plate method	Hot immersion method
Control	2±0.16	1.8±0.16
Methanolic extract		
100mg/kg	2.8±0.22	2±0.2
200mg/kg	3.2±0.18*	2.8±0.06*
Ethyl acetate extract		
100mg/kg	5.8±0.42*	4.8±0.8*
200mg/kg	6.7±0.38*	7.5±0.7**
Diclofenac (Std)		
100mg/kg	6.8±0.32	5.8±0.2
200mg/kg	8.2±0.46	8.9±0.7

Values are expressed as mean±SEM, n=6, p≤0.01,*significant, **highly significant

CONCLUSION:

Oral administration of methanolic and ethyl acetate of *Eclipta prostrata* extracts shown significant analgesic activity. Ethyl acetate extract found to have more analgesic activity than methanolic extract may be due to presence of much significant flavonoid content. Further studies may reveal the exact mechanism of action responsible for the analgesic and anti inflammatory activity.

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