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Analgesic and Anti-Arthritic Effect of *Enicostemma littorale* Blume

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Abstract

***Enicostemma littorale* (Blume), the folk medicine is used for rheumatic pain and it was selected for scientific validation. The 85% methanolic extract obtained from the whole plant of *Enicostemma littorale* has been assessed for analgesic and anti-inflammatory activity. The analgesic activity has been evaluated by hot plate and tail immersion method and the anti-inflammatory and antioxidant activities are evaluated by Complete Freund's adjuvant induced arthritic model. The results of the evaluation of analgesic activity in hot plate and tail immersion method revealed that the extract exhibits significant activity at 150 mg/kg body weight and the effect is found to increase dose dependently. In Freund's adjuvant induced arthritis, *Enicostemma littorale* is found to decrease the paw volume (15.81%). Significant protection is also observed by elevating antioxidant enzymes. In conclusion, the 85% methanolic extract of *Enicostemma littorale* possesses significant analgesic and anti-inflammatory activities in Freund's adjuvant induced arthritic model in rats.**

Keywords

Complete Freund's Adjuvant, Hot Plate Oxidative Stress, Tail Immersion

1. Introduction

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory disease of unknown etiology and is marked

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by synovial hyperplasia with local invasion of bone and cartilage leading to joint destruction [1]. RA affects ~1% of the adult population with more women being afflicted than men [2] [3]. Recent reports suggest that patients with RA not only have a higher chronic disease burden but also may have increased morbidity and mortality from cardiovascular disease compared with persons without RA [4]. However, despite optimal use of currently available anti-rheumatic agents, most RA patients live with chronic pain and severe functional decline because these therapies focus primarily on preventing joint inflammation and soft tissue swelling, but are not effective in preventing cartilage breakdown and the joint destruction associated with RA [5]. They also have undesirable side effects such as causing peptic ulcers [6].

Herbal medicines have been described in traditional texts and used as antimicrobial, anti-inflammatory and antiviral medicine for the cure of allergies, RA, infections, wound healing and fever [7] [8].

Enicostemma littorale Blume, (EL) a small herb of family Gentianaceae is commonly used by rural folks of Gujarat, India. It is cited in ancient literature as an anti-malarial, anti-pyretic and as a laxative [9]. Vasu *et al*. (2003) has confirmed the presence of phenols, tannins, flavonoids, glycosides, anthroquinones and sterols in EL and also reported its hypoglycemic, antioxidant and hypolipidemic potential in newly-diagnosed NIDDM patients [10]. The presence of alkaloid gentianine and the bitter glycoside swertiamarin in EL has been identified [11]. Recently aerial part of *Enicostemma littorale* is reported to show hypolipidemic effect in *p*-dimethylaminobenzene (*p*-DAB) induced hepatotoxic animals [12].

Sadique *et al*. (1987) have investigated the anti-inflammatory activity of *Enicostemma littorale* (EL) [13]. They have observed that 100 mg/100g body weight of EL's alcoholic extract exhibits 54% anti-inflammatory activity in carrageenan induced paw edema. They have also evaluated the effect of the same extract in cotton pellet granuloma model. In our present study we have made an attempt to evaluate the analgesic, anti-inflammatory and antioxidant activity of the 85% methanolic extract of EL. Analgesic activity is confirmed by hot plate and tail immersion method. Likewise we have performed pharmacological investigation on the anti-inflammatory activity of EL using adjuvant induced animal models.

2. Materials and Methods

2.1. Plant Material

The *Enicostemma littorale* (EL) was collected from Madurai region, Tamilnadu, India. The plant was identified and authentified at Department of Pharmacognosy, Centre for Advanced Research in Indian System of Medicine, SASTRA University, Tamilnadu, India.

2.2. Extraction

The material was dried in the shade. One kg of crushed root of the plant was extracted with seven liters of 85% methanol for 72 hours. The extraction was repeated for three times. The extract was concentrated by distillation. The final a trace of solvent was dried in a vacuum oven at 50˚C. The yield of extract was 3.34%.

2.3. Animals

In this present study, six month old male Wistar albino rats weighing 180 - 210 g were used. The animals were obtained from Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tamilnadu, India were used. The rats were fed with standard laboratory chow and water before the experiment. The animal house was equipped with temperature 22˚C ± 1˚C at 14/10 h light/dark cycle. All the animal testing was performed after getting clearance from Institutional Animal ethical committee (Clearance No. 11/SASTRA/IAEC/RPP).

2.4. Experimental Protocol for Hot Plate Test

In the hot plate test [14], rats were divided into different groups. Each group consists of six animals. Group 1 animals were treated with 5% Tween 80 in water, Group 2 animals were treated with extract at the dose of 150 mg/kg body weight. Group 3 animals were treated with extract at the dose of 250 mg/kg body weight. Group 4 animals were treated with extract at the dose of 350 mg/kg body weight. All groups of animal received the single dose of extract 30 min prior of nociceptive stimulation. In this test, animals were individually placed on a hot

plate maintained at a constant temperature (55˚C ± 0.3˚C). The latency to first sign of hind paw licking or jump response to avoid heat nociception was taken as an index of nociceptive threshold with cut off time of 15 sec. The nociceptive threshold was observed at 1, 2, 3 and 4 hours after wit treatment with EL and the vehicle.

2.5. Experimental Protocol for Tail Immersion Method

In the tail immersion method [15], rats were divided into different groups. Each group consists of six animals. Group 1 animals were treated with 5% Tween 80 in water, Group 2 animals were treated with extract at the dose of 150 mg/kg body weight. Group 3 animals were treated with extract at the dose of 250 mg/kg body weight. Group 4 animals were treated with extract at the dose of 350 mg/kg body weight. All groups of animal received the single dose of extract 30 min prior of nociceptive stimulation. The anti-nociceptive effect was determined in rats using the tail-flick test by analgesiometer. The responses were elicited at 15, 30, 45 and 60 min after treatment with EL and the vehicle.

2.6. Experimental Protocol for Freund's Adjuvant Induced Arthritis

Animals were divided in to three groups. Each group consists of six animals. Group 1 animals were treated with 5% Tween 80 in water, Group 2 animals were administered with Complete Freund's adjuvant (CFA), Groups 3 animals were treated with extract at the dose of 150 mg/kg body weight and CFA. The right foot pad of each rat was injected subcutaneously with single dose of 0.05 ml of CFA with emulsified in an equal volume of phosphate buffered saline [16]. The animals were treated with extract once a day for the period of 45 days. On 46th day all the animals were sacrificed by cervical dislocation under ether anesthesia. Liver and kidney was excised from animals and washed with saline. 10% tissue homogenate was prepared in Tris buffer and various biochemical parameters like thio barbituric acid reactive substances (TBARS) [17], reduced glutathione (GSH) [18], glutathione peroxidase (GPx) [19] and catalase [20] were estimated.

2.7. Statistical Analysis

The values were expressed as Mean ± SD. Significant difference have been observed using One Way Analysis of variance (ANOVA) by SPSS software version 12.0. $p < 0.05$, was considered as significant difference. Groups having different alphabets are differ significantly at $p < 0.05$.

3. Results

On treating animals with EL (150 mg/kg body weight.) significant differences have been observed in the 1st hour itself and maximum effect has been observed in the 2nd hour which has extended up to 3rd hour. Enhancing the dose of extract one can increase the effect and maximum effect has been observed 1st hour at 250 and 350 mg/kg body weight of extract (**Table 1**). Likewise treating animals with EL extract increased the response time significantly ($p < 0.05$) at 15 minutes itself. Significant increment in response time has not been observed along with the dose of the extract (**Table 2**).

From **Table 3**, the paw volume of CFA administered and EL treated animals. The paw volume is found to be decreased from the 22nd day onwards and EL decreased the paw volume by 15.81% at the end of 45th day (**Table 3**).

Administration of Complete Freund's adjuvant causes an increased level of TBARS in liver tissue and not in the kidney in diseased animals ($p < 0.05$, **Table 4**). Treatment of animals with EL significantly decreased the TBARS by $p < 0.05$ in liver tissue. Likewise, catalase level is observed to be decreased in the liver tissue and not in the kidney in treated group. EL treatment increased the level of catalase (**Table 4**).

Table 5 shows the level of glutathione and its dependent enzyme in both liver and kidney homogenate. Administration of Complete Freund's adjuvant brings the deletion of both glutathione and glutathione peroxidase in liver tissue, whereas, significant depletion has not been observed in kidney tissue ($p < 0.05$). Treating animals with EL shows a rise in the level of both glutathione and its enzyme significantly ($p < 0.05$, **Table 5**).

4. Discussion

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation, hyperproliferation of the synovial lining and cartilage destruction [21]. EL treatment is observed to increase the response time significantly in both hot plate and tail immersion method ($p < 0.05$). The elevated pain threshold of rats towards heat

Table 1. Analgesic activity of *Enicostemma littorale* on rats in hot plate method.

Groups	Dose	Response time in sec				
	Reaction time in h	0 h	1 h	2 h	3 h	4 h
Group 1	-	3.1 ± 0.90	3.1 ± 1.50	3.8 ± 0.96	3.1 ± 0.30	4.8 ± 0.50*
Group 2	MLE 150 mg/kg	2.5 ± 0.0*	4.3 ± 1.20*	5.0 ± 0.8*	5.3 ± 2.2*	
Group 3	MLE 250 mg/kg	2.4 ± 0.50*	8.12 ± 0.90*c	7.5 ± 0.60*	8.3 ± 1.50*	4.3 ± 1.20*
Group 4	MLE 350 mg/kg	2.8 ± 0.6*	8.4 ± 2.80*	6.0 ± 1.60*	8.1 ± 1.80*	4.5 ± 1.70*

Values are Mean ± SD. One Way ANOVA was carried out using SPSS software version 12.0. Significant difference ($^{*}p < 0.05$) has been observed between 0 hour and different hours of the same group.

Table 2. Analgesic activity of *Enicostemma littorale* on rats in tail immersion method.

Drug	Dose	Response time in sec				
	Reaction time in min	0 min	15 min	30 min	45 min	60 min
Group 1	-	4.3 ± 0.50	4.0 ± 0.80	3.8 ± 0.50	5.3 ± 0.96	4.0 ± 0.80
Group 2	MLE 150 mg/kg	2.8 ± 0.50*	4.3 ± 0.50*	3.5 ± 1.00*	4.3 ± 0.96*	4.9 ± 0.90*
Group 3	MLE 250 mg/kg	2.6 ± 0.50*	3.5 ± 1.00*	4.8 ± 1.30*	4.1 ± 0.90*	4.0 ± 0.01*
Group 4	MLE 350 mg/kg	3.1 ± 0.30*	4.3 ± 0.50*	4.0 ± 0.80*	4.4 ± 1.10*	4.1 ± 0.90*

Values are Mean ± SD. One Way ANOVA was carried out using SPSS software version 12.0. Significant difference ($^{*}p < 0.05$) has been observed between 0 hour and different hours of the same group.

Table 3. Changes in paw volume of Complete Freund's adjuvant induced arthritic rats treated with *Enicostemma littorale*.

Days	Diseased Animals	*Enicostemma littorale* (150 mg/kg, B.wt)	
	P.V	P.V	P.I
1	4.27 ± 0.08	4.1 ± 0.01	-
8	6.3 ± 0.43	7.07 ± 0.25	-
15	6.68 ± 0.51	6.78 ± 0.38	-
22	6.7 ± 0.87	6.42 ± 0.26	4.18
29	6.7 ± 0.46	6.08 ± 0.15	9.25
36	6.63 ± 0.32	5.9 ± 0.2*	11.01
43	6.45 ± 0.39	5.43 ± 0.07*	15.81

Note: P.V—Paw volume in mm; P.I—Percentage Inhibition as compared to Control; Values are Mean ± SD. $^{*}p < 0.05$ was considered statically significant.

Table 4. Antioxidant effect of *Enicostemma littorale* in adjuvant administered rats.

Groups	TABRS		Catalase	
	Liver	Kidney	Liver	Kidney
Group 1	0.47 ± 0.05	0.29 ± 0.04	17.60 ± 1.59	15.24 ± 1.81
Group 2	0.82 ± 0.03*b	0.34 ± 0.03ns	11.23 ± 0.72*a	12.49 ± 0.56ns
Group 3	0.64 ± 0.22a	0.32 ± 0.07ns	17.52 ± 1.37b	12.36 ± 1.01ns

Values are Mean ± SD; *Refers to significant difference between Group 1 and 2 ($p < 0.05$); nsNo significant difference; a,bSignificant difference between Group 2 vs Group 3 and 4.

Table 5. Effect of *Enicostemma littorale* on Glutathione and its related enzyme in adjuvant administered rats.

Groups	Glutathione peroxidase		Reduced glutathione	
	Liver	Kidney	Liver	Kidney
Group 1	0.1863 ± 0.0007	0.1122 ± 0.0001	25.85 ± 0.39	26.67 ± 2.65
Group 2	0.1068 ± 0.0001^{*a}	0.1124 ± 0.0001^{ns}	19.74 ± 2.45^{*a}	25.72 ± 3.99^{ns}
Group 3	0.1358 ± 0.0005^{b}	0.1056 ± 0.0012^{ns}	26.20 ± 3.37^{b}	25.25 ± 0.36^{ns}

Values are Mean ± SD; *Refers to significant difference between Group 1 and 2 ($p < 0.05$); ^{ns}No significant difference; ^{a,b}Significant difference between Group 2 vs Group 3 and 4.

might be due to the centrally acting effect. The flavonoids present in the EL might be responsible for this analgesic activity [22].

However, some of the earlier reports indicating that inflammation and tissue injury related oxidative stress have been implicated in the pathogenesis of rheumatoid arthritis. Free radicals are enormously produced at the site of inflammation and tissue injuries. Excessive generation of reactive oxygen species (ROS) leads to a variety of pathological processes such as inflammation, diabetes, hepatic damage and cancer [23].

TBARS level of disease animals increased significantly ($p < 0.05$). Free radicals, the highly reactive entity and very short lived molecules, are constantly produced in a wide variety of normal physiological functions. Although free radicals perform some useful functions, they are toxic when generated in excess [24]. The most important characteristic of toxic free radicals generated by CFA administration in either *in vivo* or *in vitro* is peroxidation of lipids resulting in tissue damage and death of affected cells [25]. The profound body of evidence implicating that free radical induced lipid peroxidation in the pathogenesis of various pathological conditions including chronic inflammatory conditions [26].

The harmful effect of ROS is neutralized by a broad class of protective agents termed antioxidants which prevents oxidative damage by reacting with free radicals before any other molecules can become a target. The free radical mediated damage is prevented by various enzymatic antioxidants like catalase, glutathione peroxidase and non-enzymatic antioxidants like reduced glutathione [27].

Glutathione peroxidase prevents the accumulation of oxidized lipids in mitochondrial cell membranes and also detoxifies H_2O_2 by utilizing reduced glutathione as a co-substrate [28] (Hassan *et al*., 2001). The glutathione and glutathione peroxidase levels of CFA administered animals were observed to have a decrease in diseased animals ($p < 0.05$).

The antioxidant activity of EL might be due to the presence of flavonoids compounds. Flavonoids have been reported to have anti-inflammatory, anti-arthritic activity [29]. The present study establishes the therapeutic rationale of using EL extract in various anti-inflammatory polyherbal formulations. Anti-inflammatory effect in chronic model with EL could be because of one of its constituents *i.e*. Betulin, a triterpene sapogenin that is known to have anti-inflammatory activity [30]. The antioxidant and anti-inflammatory activities of EL might be due to the presence of swertimarin.

5. Conclusion

In conclusion, *Enicostemma littorale* increased the response time of animals in both hot plate and tail immersion method. EL increased the antioxidants thereby decreases the oxidative stress. The decreased oxidative stress is associated with a decreased paw volume. Thus, the *Enicostemma littorale* is a potent analgesic, anti-inflammatory and antioxidant herb.

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