ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In vivo Antiarthritic activity of aqueous extract of *Enicostemma axillare* against Formaldehyde induced Arthritis

KAS Mohammed Shafeeq*, R Senthamarai, and S Karpagam Kumara Sundari

Periyar College of Pharmaceutical Sciences,Periyar Centenary Educational Complex, K. Sathanoor Main Road, Trichy-21

ABSTRACT

Arthritis is inflammation of one or more joints, characterized by swelling, warmth, redness the overlying skin, pain and restriction of motion. The aim of the present study was to evaluate the effect of Aqueous extract of Enicostemma axillare in a dose of 200mg/Kg and 400mg/Kg against formaldehyde induced arthritis using albino rats, the results of present study indicates that the selected extract has produced dose dependent reduction in edema, swelling and inflammation in rats.

Keywords:Arthritis, Enicostemma, axilare, Formaldehyde, edema

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Rheumatism is one of the oldest systemic known inflammatory diseases of mankind and primarily affects the joints and surrounding tissues and may also affect other organs, it affects three times more in women than man, and no substantial progress has been made in achieving a permanent cure. The greatest disadvantage in the presently available potent synthetic drugs lies in their toxicity, side effects and reappearance of symptoms after discontinuation [1].

Herbs have in use for thousands of years, in one form or another, under the indigenous system of medicines like Ayurvedha, siddha and Unani. The herbal drugs have been used throughout the world received greater attention in now a days, Because of its diversity of curing diseases, safety and well tolerated remedies compared to the conventional medicine [2].

Enicostemma axillare Linn (Synonym *Enicostemma littorale* Blume) belonging to the family Gentianaceae is a perennial herb found throughout India and common in coastal areas. The plant traditionally used for diabetes mellitus, rheumatism, abdominal ulcer, hernia swelling, itching and insect poisoning in folk medicine [3]. The present study was aimed to document the anti-arthritic effect of aqueous extract of *Enicostemma axillare* using albino rats.

MATERIAL AND METHODS

Plant Collection

The whole plant of *Enicostemma axillare* Linn were collected full bloom in September from Trichy district, Tamil Nadu and authenticated by Dr. V.Nanda Gobalan, Associate Professor, Department of Botany, National College, Trichy, Tamil Nadu

Extraction

The whole plant were shade dried, powdered and extracted (250gm) with 3litres in a narrow mouthed bottle for three days. After completion of extraction, it was filtered and solvent was removed by distillation under reduced pressure. The extract was then stored in dessicator. A brownish green powder was obtained.

Ethical Committee Approval

The Institutional Ethics Committee (IAEC) has approved the experimental protocols for the Anti-arthritic activity and approval number is PCP/IAEC/009/2008

Acute Toxicity study

The aqueous extract of *Enicosteema axillare* subjected for acute toxicity study using OECD guidelines 423 (Acute Toxic Class Method) in female albino rats in a dose of 2000mg/Kg. The dose200mg/Kg and 400mg/Kg selected for the anti-arthritic study.

ISSN: 0975-8585

Anti Arthritic Activity

The acclimimatized animals were kept fasting for 24hrs with water *ad libitum.* The animals were divided in to four groups six animals each. First group served as control received normal saline (10ml/kg) P.O., Second group served as standard received Indomethacin (10mg/kg) P.O., (4) Third and Fourth group served as Test I and Test II received Aqueous extract of *Enicostemma axillare* 200mg/kg and 400mg/Kg respectively [5].

Arthritis was induced by injecting 0.1ml of 2% formaldehyde solution in normal saline to subplantar region of left hind paw of each rat regardless of weight on the first and third days of the treatment [6]. Drugs were administered to animals for 10days, the size of the hind paw measured in alternate days, change body weight and blood parameters were measured at the end of 10days treatment. Change in paw edema was measured by Digital Plethysometer (Ugobasile, Italy) [7]. The Percentage of edema inhibition can be calculated as shown below

% Edema inhibition =Control (Paw volume at the end of10 days treatment) – Test (Paw volume at the end of 10 days treatment/Control (Paw volume at end of 10 days of treatment) × 100

RESULT and DISCUSSION

Administration of 2% formaldehyde on day 1 and day 3 produced ankle joint swelling in the injected limp of all the animals. This joint swelling was sustained throughout the observation period of 10 days (Table-2). The increase in paw volume was less in aqueous extract and Indomethacin treated groups as compared to the control and this difference was significant (P<0.0001) on all observations days.

Table no. 2: Anti-arthritic Activity of whole plant *Enicostemma axillare* Extracts Against Formaldehyde Induced Arthritis in Albino Rats

S. No.	Treatment	Paw Edema (ml)					
		0 day	2nd day	4th day	6th day	8th day	10th day
1	Control (normal saline, 10ml/kg, p.o.)	3.283 ± 0.031	4.033 ± 0.033	4.617 ± 0.031	5.133 ± 0.033	5.583 ± 0.031	5.883 ± 0.031
2	Aqueous extract (200mgkg, p.o.)	3.333 ± 0.033***	3.883 ± 0.031***	4.617 ± 0.031***	4.967 ± 0.049***	4.517 ± 0.031***	4.057 ± 0.021***
3	Aqueous extract (400mgkg, p.o.)	3.193 ± 0.017***	3.724 ± 0.039***	4.423 ± 0.037***	4.249 ± 0.077***	3.873 ± 0.075***	3.338 ± 0.021***
4	Standard-Indomethacin (10mg/kg, p.o.)	3.3 ± 0.037***	4.217 ± 0.031***	4.817 ± 0.031***	5.033 ± 0.042***	4.817 ± 0.031***	3.967 ± 0.033***

n = 6 values as mean ± S.E.M

*** P < 0.0001 Vs Control by two way ANOVA

ISSN: 0975-8585

The aqueous extract at the test dose of 200mg/Kg and 400mg/Kg reduced the edema induced by 2% formaldehyde by (31.04%) and (43.26%) respectively (Table 3) at 10th day, where as the standard Indomethacin showed (32.57%) of Inhibition as compared to the control group. In addition the animals showed reduction in decrease in body weight in treated group as compared to arthritis control, changes in body weight have also been used to assess the course of the disease and response to therapy of Anti-inflammatory drugs [8]. As the incidence of severity of arthritis increased, the change in body weight of the rats also occurred during the course of the experimental period. The loss of the body weight during arthritic condition was also supported by earlier observations [9], on alterations in metabolic activities of diseased rats.

Table no. 3: Percentage Inhibition of Paw Edema by whole plant of *Enicostemma axillare* Extracts Against Formaldehyde Induced Arthritis in Albino Rats

S. No.	Treatment	% Inhibition on 10th day
1	Aqueous Extract (200mg/kg, p.o.)	31.04
2	Aqueous Extract (400mg/kg, p.o.)	43.26
3	Standard- Indomethacin (10mg/kg, p.o.)	32.57

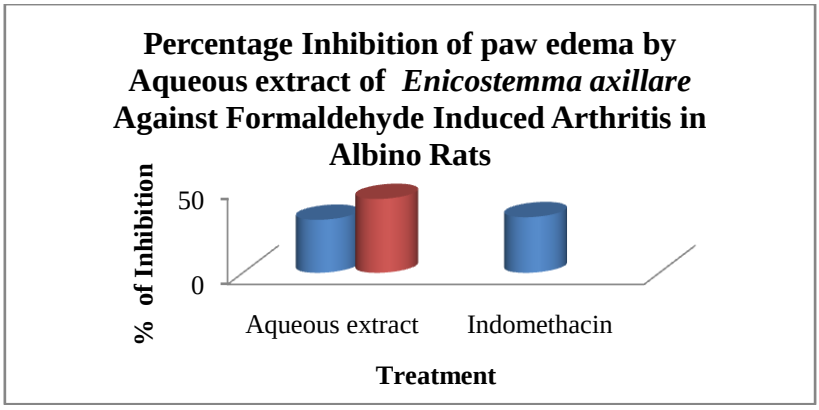

Figure 1

Earlier findings suggest that absorptions of ^{14}C glucose and ^{14}C leucine in rats intestine was reduced in the case of inflamed rats [10]. Treatment with Anti-inflammatory drugs the decrease in absorption was nullified [11] and it shows that the anti-inflammatory drugs have corrected the decreased absorption capacity of intestine during the inflammation. The increased body weight during the treatment of standard drug and extract might be due to restoration of the absorption capacity of intestines (Table 1).

Table no. 1: Changes in Body Weight in Formaldehyde Induced Arthritis after treatment with whole plant of *Enicostemma axillare* extracts

S. No.	Treatment	Body weight (gms)	
		Before Induction	After treatment
1	Control (normal saline-10ml/kg, p.o.)	191.667 ± 2.108	147.50 ± 2.141
2	Aqueous Extract (200mg/kg, p.o.)	185.833 ± 2.386***	180.833 ± 3.270***
3	Aqueous Extract (400mg/kg, p.o.)	187.242 ± 2.742***	189.277 ± 2.015***
4	Standard – Indomethacin (10mg/kg, p.o.)	186.667 ± 2.108***	180.833 ± 2.007***

n = 6 values as mean ± S.E.M

*** P < 0.0001 Vs Control by two way ANOVA

In arthritic condition, there is a mild to moderate rise in WBC count that may be due to the release of IL-IB inflammatory response, IL-IB increases the production of granulocyte and macrophages colony stimulating factors. In the present study, migration of leukocyte in to the inflamed area is significantly suppressed by extract when compared to the standard drug Indomethacin, as seen in from the significant reduction in the total WBC count (Table 4).The acute phase proteins in ESR and C-Reactive proteins (CRP) share the property of showing elevation in concentration in response to stress or inflammation like injection, injury, surgery and tissue necrosis. The ESR count significantly increased in arthritic control group, where as these counts were remarkably counteracted in the standard Indomethacin and extract treated groups and thus justifying its significant role in the arthritic condition.

Table no.4: Effect on hematological parameters by whole plant of *Enicostemma axillare* Extracts Against Formaldehyde Induced Arthritis in Albino Rats

S.No.	Treatment	Hb (gm%)	Total WBC Count (cells/cu.mm)	RBC Count (million/cu.mm)	ESR (mm/1/2hr)
1	Control (normal saline, 10ml/kg, p.o.)	11.8432±0.212	8429.326±97.234	4.916±0.220	14.54±0.323
2	Aqueous extract (200mg/kg, p.o.)	12.883±0.342***	7124.334±45.321***	5.217±0.065***	10.167±0.032***
3	Aqueous extract (400mg/kg, p.o.)	13.432±0.142***	6543.425±66.324***	5.433±0.067***	8.423±0.412***
4	Standard-Indomethacin (10mg/kg, p.o.)	13.817±0.158***	6426.667±50.837***	5.383±0.048***	5.50±0.428***

n = 6 values as mean ± S.E.M
*** P < 0.001 Vs Control by two way ANOVA

In traditional system of medicine, many plants and their products were known act as potential medicinal agent in the treatment of various ailments, but unless corroborated by Clinical and experimental evidence, the therapeutic effect of the plants cannot be confirmed, the results of present study indicates that the aqueous extract of *Encostemma axillare* at a dose of 200mg/Kg and 400mg/Kg has significant Anti-arthritic activity which is comparable with standard Indomethacin.

ACKNOWLEDGEMENT

The author wish to thank the Management of Periyar College of Pharmaceutical Sciences for providing facilities to complete this research work in due time

REFERENCES

[1] Kiritikar KR. Basu BD. Indian Medicinal Plants. Bishen Sing Mahendrapal Sing, Dehradun, 1999, pp. 1655-1656
[2] Sudhersan C. Curr Sci 1998;74(12):1099-100
[3] Wealth of India. National Institute of Science Communication, CSIR, New Delhi, pp.80
[4] Gupta MP, Bhalla TN, Gupta GP, Mitra CR. Japan J Pharmacol 1971;21(3):377-382
[5] Biren NS, Jalpure, Nayak SS, Seth AK. Int J Pharma and Bio Sci 2009;2(1):39-43

[6] Suryawanshi JS, Karande KM, Dias RJ, Patil KS. Ind J Nat Prod 2008;24(2): 20-25
[7] Almedia FR, Rao VS, Matos ME. Braz J Med Bio Res 2007; 22(11): 1397-99
[8] Winder CV, Lembke LA, Stephens MD. Arthritis Rheum 2005;12(5): 472-482
[9] Walz DT, Dimartino MJ, Misher A. J Pharmacol Exp Ther 1971; 178(1):223-231
[10] Somasundara S, Sadique J, Subramaniam A. Clin Exp Pharmacol Physiol 1983;10(2): 147-152
[11] Somasundara S, Sadique J, Subramaniam A. Biochem Med 1983;29(2):259-264