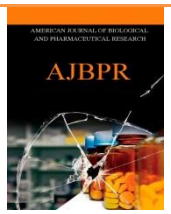
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



EVALUATION OF ANALGESIC ACTIVITY OF METHANOL AND CHLOROFORM EXTRACTS OF *ANTIGONON LEPTOPUS*

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Article Info

Received 29/09/2015
Revised 10/10/2015
Accepted 19/10/2015

Key words: -
Antigonon leptopus, Analgesic activity, Methanol extract, Chloroform extract, tail immersion model.

ABSTRACT

To investigate the analgesic activities of methanol and chloroform extracts of *Antigonon leptopus* (MEAL and CEAL respectively) leaves in albino rats. Analgesic activity was evaluated using tail immersion techniques, by measuring the reaction time of the animals treated with either standard or extract at dose levels of 50, 100, 150, 200 and 250 mg/kg p.o. Tramadol HCl (10 mg/kg) was used as reference standard. In the experimental study, the extracts showed reaction times of 6.15($p < 0.05$) and 7.00 ($p < 0.05$) sec at the doses of 100 and 150 mg/kg of MEAL after 30 min and 5.00($p < 0.05$) and 5.50 ($p < 0.05$) sec at the doses of 150 and 100 mg/kg of CEAL after 60 min, respectively in the tail immersion model. All results occur in dose dependent manner. Based on the findings, it can be concluded that *Antigonon leptopus* possesses analgesic property and this lends some support for its use in traditional medical practice.

INTRODUCTION

Pain is an unpleasant sensory as well as multidimensional and emotional experience associated with actual or potential tissue damage or described by such damage which can interfere with general daily life functioning. Pain is common factor that warns of the danger of the bodily harm and alerts to trauma and injury and it also indicate the primary reason for seeking medical attention. According to the research analysis, over one-third people of world's population suffer from persistent or recurrent pain. The most useful strategies for the treatment are the management of the pain. Many of the current available drug therapies against pain show either inadequate or deleterious adverse effect, so many researches are identify new antinociceptive agents with lesser side effect [1- 5]. So that it is need for the identification of bioactive compounds from natural plant mainly from medicinal plants which are employed as analgesic activity with less or no side effects. Therefore the aim of the study was to evaluate the Analgesic activity of *Antigonon leptopus* [6].

Antigonon leptopus is commonly known as coral vine or Mexican creeper belonging to the flowering plant family polygonaceae is a tender perennial vine [7]. It is the native of Mexico and commonly found in the Caribbean, America, Tropical Asia and Africa [8]. Previous studies have reported that different extracts of these plant possess anti-diabetic [9], analgesic and anti-inflammatory [10], anti-thrombin [11], anti-microbial [12], hepatoprotective [13], anthelmintic [14], lipid peroxidation inhibitory activity [15]. Traditionally, it is used to treat cough and throat constriction [16], asthma, liver and spleen disorder [17], reduce swelling and it's leaves tea are used to treat hypertension, diabetes and menstrual pains [18].

Previous studies have reported that methanolic extract of *Antigonon leptopus* roots extract exhibited a dose-dependence and significant protective response on chemical visceral (writhing) and cutaneous thermic (hot

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plate) pain stimuli for the evaluation of analgesic activity. The extract showed significance anti-inflammatory activity which was comparable to the reference drug diclofenace sodium in carrageenan induce paw edema model. From further study, the methanolic extract of roots of A. leptopus possess a significant migration of neutrophils and leucocytes induced by carrageenan and inhibition of granuloma induced by cotton pellet, vascular permeability induced by acetic acid in mice or rats [18-19]. So that our present study was targeted to evaluate the analgesic activity of methanol and chloroform extracts of *Antigonon leptopus* leaves.

MATERIAL AND METHODS

Plant Sources

The leaves of *Antigonon leptopus* were collected from Azamgarh city, Uttar Pradesh, India during 4 Oct 2013 to prepare the herbarium and botanical identification was done by Dr S.L. Gupta, a Scientist 'E' and Head of Office, Botanical Survey of India, C.R.C., Allahabad-211002.

Preparation of Plant Extract and Reference Drugs

The leaves were shade dried, ground into coarse powder and passed through 40 meshes. Powder of *A. leptopus* was extracted with using solvent system such as methanol and chloroform (Sd Fine- Chem Limited Mumbai) separately in soxhlet apparatus at the temperature 40º C to 60ºC. After 72 hr it was filter with Whatman filter paper and both extracts was concentrated in vacuum under pressure using rotator flash evaporator [20,21].

There are the 50, 100, 150, 200 and 250 mg/kg doses of both methanolic and chloroform extracts of *Antigonon leptopus* leaves were prepared by suspending the extracts in 2% aqueous Tween 80 (Sd Fine- Chem Limited Mumbai). Both the extracts and vehicle were administered orally (p.o.) to the animals. The reference drug which is used in the study was Tramadol HCl (10 mg/kg) that was prepared by dissolving in distilled water [22].

Phytochemical screening

Freshly prepared both methanol and chloroform extracts were subject to phytochemical screening for constituent identification using a standard protocol [23].

Experimental Animals and treatment regimens

The experiments were performed by using either sex of Albino rats (150-200g) obtained from the animal house of Pharmacy College Azamgarh, Uttar Pradesh. The animals were kept in ventilation department at random and assigned to treatment groups in polypropylene cage with paddy husk as bedding. Animals were housed in controlled condition at a temperature of 23 ± 2ºC, relative humidity is 30 to 70% and a day-night light cycle was provided. The animal in all experimental protocol has been approved by the institutional animal ethical committee accordance with prescribed guidelines of CPCSEA, Government of India.

The acute toxicity study was performed by using as per OECD guidelines AOT No 425. Percentage mortality in each group was recorded within the duration of 24 hr and all animals were observed for any signs for delayed toxicity for further 14 days. The extract of *Antigonon leptopus* leaves in oral doses of 2000 mg and 5000 mg/kg body weight did not shown any lethal dose effects and had good margin of safety in the animals [24].

Animals were divided into seven groups for evaluation methanolic and chloroform extract respectively six animals in each. The first group (Group I) was used as a control group. The second group (Group II) has served as reference standard. Five groups (Group III, IV, V, VI and VII) received chloroform and methanolic extract of *A. leptopus* leaves respectively at five different doses such as 50, 100, 150, 200 and 250 mg/kg per orally. These five doses were selected on the basis of our preliminary screening [25].

Evaluation of Analgesic Activity

Both chloroform and methanolic extracts of *A. leptopus* leaves was tested for Analgesic activity using tail immersion model followed by Vogel HG (2002) with some modification. The animals are kept into separate means one animal in one restraining cages for leaving the tail hanging out freely and adopt for 30 min before testing. They are randomly selected and divided into seven groups six animals in each. Group I is served as control and received vehicle (distilled water 5ml/kg). Group II used to serve as reference standard and received tramadol HCl (10mg/kg). The next five groups III, IV, V, VI and VII served as test and received chloroform and methanolic plant extract separately at the five different doses 50, 100, 150, 200 and 250 mg/kg p.o. respectively. For the experiment, the rat was the lower 5 cm portion of all groups animals was marked and immersed in cup of freshly filled water. The temperature of water that is used was maintained exactly at 55 ºC. The reaction time was noted by withdrawing the tail by the animal. The tail was carefully dried after each determination. The results were evaluated by calculating the mean reaction time of per group and Analgesic activity was exhibited by withdrawing of the tail by animal [26].

Statistical Analysis

All the results were presented as mean ± S.E.M. The data were analyzed for Statistical significant by using one- way analysis of variance (ANOVA) followed by Dunnett multiple comparison test by using Graphpad Prism 6 Demo software. All the response ware regarded as significant at p-value < 0.05.

RESULT

Phytochemical Screening

The preliminary phytochemical screening of *Antigonon leptopus* leaves was carried out and it revealed the presence of carbohydrates, alkaloids, tannins, steroids, terpenoids, saponin glycosides and flavonoids glycosides.

Toxicity study

In preliminary acute toxicity study, oral administration of the plant extract of *A. leptopus* did not produce any sign of toxicity at dose of 2000mg/kg and no any mortality occurred to dose of 5000mg/kg within 24 hours after administration and after seven days observation in rat.

Tail Immersion Test Result

The analgesic activity of the methanol and chloroform extracts with tramadol HCl in the tail immersion test are shown in Table I and II respectively. At all doses, the effect of the extract was more pronounced but the extracts showed highest reaction times of 6.15($p < 0.05$) and 7.00 ($p < 0.05$) sec at the doses of 100 and 150 mg/kg of MEAL after 30 min and 5.00($p < 0.05$) and 5.50 ($p < 0.05$) sec at the doses of 150 and 100 mg/kg of CEAL after 60 min , respectively. Tramadol HCl (10 mg/kg) produced a significant analgesic effect at all observation times when compared to control, with a maximum increase in reaction time latency at 60 min which declined with time.

Table 1. Analgesic effect of MEAL by Tail Immersion method (mean ± SEM)

Group	Dose (mg/kg)	Reaction time (in second)									
		Pre Treatment	I 0.30hr	II 1.0hr	III 1.30hr	IV 2.0hr	V 2.30hr	VI 3.0hr	VII 3.30hr	VIII 4.0hr	IX 24.0hr
I	50	2.01± 0.01**	5.57± 0.53*	3.02± 0.01**	3.56± 0.47*	2.03± 1.01*	5.50± 1.50*	2.02± 0.01**	1.06± 0.01**	1.04± 0.01*	2.01± 0.01**
II	100	1.59± 0.50*	6.15± 0.99*	4.03± 0.01**	3.02± 1.0*	2.52± 0.50*	3.07± 0.03*	2.05± 0.03**	2.02± 0.15**	1.56± 0.48*	2.04± 0.04**
III	150	3.52± 0.50*	7.00± 1.47*	5.55± 0.47*	5.04± 0.01*	4.53± 1.49*	2.55± 0.49*	2.03± 0.01**	2.52± 0.52*	2.52± 0.52*	4.01± 1.0*
IV	200	5.02± 1.0*	5.05± 0.03*	5.59± 0.49*	5.52± 0.50*	3.52± 0.48*	2.55± 0.49*	3.02± 0.01*	2.52± 0.52*	3.01± 0.10*	5.0± 1.0*
V	250	3.51± 1.51*	4.03± 1.03*	4.57± 0.49*	5.02± 0.99*	3.58± 0.50*	3.57± 0.49*	2.55± 0.47*	2.01± 0.01**	1.56± 0.47*	3.52± 1.52*
Std.	10	2.53± 0.48	5.02± 1.01	6.52± 0.49	5.51± 1.50	5.15± 0.96	5.51± 0.50	4.55± 0.54	3.54± 0.54	3.51± 0.49	3.01± 0.01
Cont.	10	4.53± 0.53	4.06± 1.97	4.54± 0.48	4.50± 1.50	4.52± 1.50	5.56± 1.47	5.52± 0.50	4.02± 0.01	4.00± 1.00	4.54± 0.52

All values are expressed in mean ± standard error mean (n=7).

All data were found to be significant at 5% level of significance and non-significance where $**p<0.05$ and $*p>0.05$ respectively.

Table 2. Analgesic effect of CEAL by Tail Immersion method (mean ± SEM)

Group	Dose (mg/kg)	Reaction time (in second)									
		Pre Treatment	I 0.30hr	II 1.0hr	III 1.30hr	IV 2.0hr	V 2.30hr	VI 3.0hr	VII 3.30hr	VIII 4.0hr	IX 24.0hr
I	50	3.50± 0.50*	3.50± 0.50*	4.00± 1.00*	2.50± 0.50*	3.50± 0.50*	3.50± 0.50*	5.50± 1.50*	2.50± 0.50*	2.50± 0.50*	3.50± 0.50*
II	100	2.50± 0.50*	2.50± 0.50*	4.00± 1.00*	5.00± 2.00*	3.50± 0.50*	3.50± 0.50*	4.00± 1.00*	2.50± 0.50*	2.50± 0.50*	3.50± 0.50*
III	150	2.50± 0.50*	3.50± 0.50*	5.00± 0.00**	3.50± 0.50*	4.50± 0.50*	2.50± 0.50*	6.50± 3.50*	2.00± 0.0**	2.50± 0.50*	3.00± 0.00*
IV	200	3.00± 0.40*	4.50± 0.50*	5.50± 0.50**	6.00± 2.00*	4.50± 2.50*	5.50± 1.50*	5.00± 3.00*	3.00± 1.00*	3.00± 0.00*	2.50± 0.50*
V	250	3.50± 0.50*	3.50± 0.50*	3.50± 0.50**	8.50± 0.50*	5.50± 0.50*	3.50± 0.50*	4.00± 0.00*	3.00± 0.00*	2.50± 0.50*	3.00± 1.00*
Std.	10	2.53± 0.48	5.02± 1.01	6.52± 0.49	5.51± 1.50	5.15± 0.96	5.51± 0.50	4.55± 0.54	3.54± 0.54	3.51± 0.49	3.01± 0.01
Cont.	10	4.53± 0.53	4.06± 1.97	4.54± 0.48	4.50± 1.50	4.52± 1.50	5.56± 1.47	5.52± 0.50	4.02± 0.01	4.00± 1.00	4.54± 0.52

All values are expressed in mean ± standard error mean (n=7).

All data were found to be significant at 5% level of significance and non-significance where $**p<0.05$ and $*p>0.05$ respectively.

Fig 1. Effect of MEAL in Reaction time

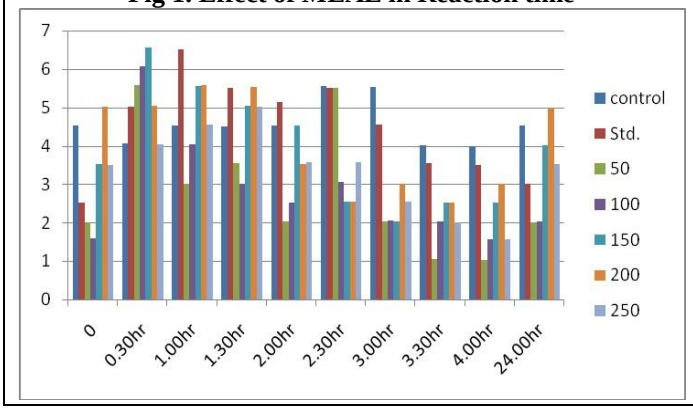
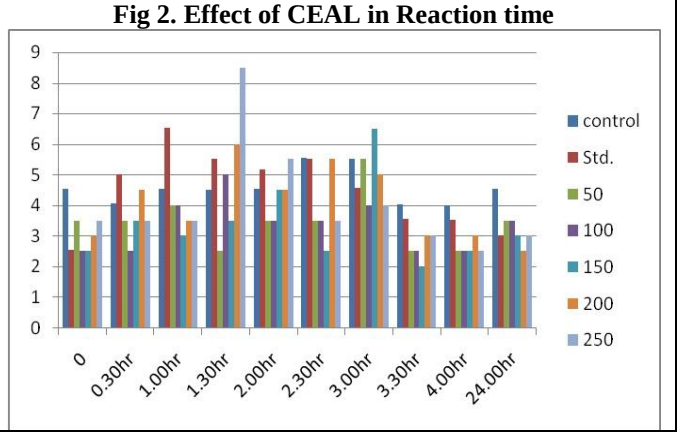


Fig 2. Effect of CEAL in Reaction time



DISCUSSION

The study evaluated the analgesic activity of methanol and chloroform extracts of *A. leptopus* leaves. The analgesic activity was carried out in rodent model of acute pain; such as tail immersion test. This method is classical nociception model used to screen prospective analgesic activity of compounds or plant extracts. Tail immersion test was used to analyse the central mechanism of analgesic activity of the extract. It is known that centrally acting analgesic drugs elevate the pain threshold of rat related to heat [27]. In this model, a thermal stimuli elevate algesia, indicates narcotic involvement [28]. Thermal algesia tests are more sensitive to opioid-μ receptors [29]. The result observed from this test suggests that the analgesic activity of the extract may significantly involve central mechanism of action.

CONCLUSION

From the above study, it can be concluded that methanol and chloroform extracts of *A. leptopus* leaves possesses a significant analgesic activity and it is also observed that methanol is more sensitive than chloroform. However, comprehensive phytochemical and pharmacological studies are essential to reveal the exact mechanism by which the plant extract is exerting these pharmacological actions.

CONFLICT OF INTEREST

None.

ACKNOWLEDGEMENT

I would like to thank Director Prof. Dr. Kishu Tripathi and Prof Bajrang Tripathi, Chairman, Pharmacy College, Azamgarh for providing constant encouragement, valuable guidance and facilities at all stages of this work.

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