Original Research Article

Evaluation of Analgesic Activity of Methanolic Extract of *Pouzolzia zeylanica* (L.) Bennett & R. Brown Leaves in Albino Rat

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Abstract: *Pouzolzia zeylanica*, herb is traditionally used for treatment of dysentery, fevers, toothaches, urinary problems and abdominal pain by the tribal communites of Arunachal Pradesh. Juice and decoction of leaves and roots are used for various purpuses. The present investigation was done to evaluate the analgesic activity of methanolic extract of *Pouzolzia zeylanica* leaves (MEPZL) in albino rat model. Methanolic crude extract was administered through oral route to the rats divided in different groups for analgesic test. The analgesic effect of MEPZL was recorded using Eddy's Hot Plate Test and Tail Immersion Test done on albino rats of Wistar Strain. The extract was given to the animals in three different doses of 100, 200 and 300 mg/kg respectively. The test results have shown that pain of the rats were significantly reduced in the treated rats than that of the controls. Treatement of diclofenac sodium was used as the standard drug of test against the plant extract for analgesic activity. The qualitative phytochemical screening on the MEPZL revealed the presence of carbohydrates, alkaloids, flavonoids, tannin and saponin in the extract. The compound(s) having the analgesic property in the extract is yet to be identified. It is speculated that certain compound(s) present in the leaves of the plant tested could be responsible for present analgesic activities of extract. Mechanism of action of these compounds need to be studied. Detail pharmacological investigation may lead to the development of a plant derived useful crude drugs for the treatment and management of acute and chronic pain condition.

Key words: Analgesic tests, Analgesiometer, Phytochemicals, *Pouzolzia zeylanica*

Introduction

Medicinal plants have been used as a major source for curing human ailments since time immemorial. Wild edible plants in particular have been playing a major role as food and medicinal security in developing countries such as Africa, Latin America and Asian nations since the dawn of human civilization (Afolayan and Jimoh, 2009). It is no wonder that one-fourth of the global population are dependent on traditional medicines for the treatment of various ailments (Reddy, 2004). Nutritional potentials of some wild edible plants have been proven as significant which is even greater than that of some cultivated green vegetables species (Yildirim*et al.,* 2001; Thayer, 2006). *Pouzolzia zeylanica* (L.) Bennett & R. Brown, commonly known as *oyik-yekyik* among the *Adi* community of Arunachal Pradesh is a perennial herbaceous flowering plant belonging to Urticaceae family widely distributed along roadside, forest margin and waste places without agronomic care. It is found in the entire North East India, Pakistan, Nepal, South Asian countries, Australia, Japan, China and Mesoamerica (Wilmot-Dear &Friis, 1996). Juice is used to treat boils, dysentery, fevers, toothaches and urinary problems. Decoction of leaf and roots are used against diarrhea, indigestion, abdominal pain, infantile

malnutrition, edema, dysuria, bruises, hemoptysis, hematemesis, and traumatic hemorrhage (Manandhar, 2002). Ethnobotanical field investigation done by the present author in 2015-2016 revealed that the whole plant is traditionally used by the local communities of Arunachal Pradesh and Assam for the treatment of indigestion, constipation, skin inflammation and for healing the freshly cut wound. Perusal of literature revealed that a preliminary study on *Pouzolzia zeylanica* was done which reported to possesses antibacterial activity (Anowara *et al.,* 2012). However, no studies have been made on phytoconstituents and pharmacological activities of the candidate plant (*Pouzolzia zeylanica)*. Therefore, present investigation has been intended to qualitatively screen the phytoconstituents of the methanolic fraction of *Pouzolzia zeylanica* leaves, and to further evaluate the analgesic activities of the methanolic extract of *Pouzolzia zeylanica* leaves (MEPZL) in albino rat model.

Materials and methods

Plant material

The leaves of *Pouzolzia zeylanica* (L.) Bennett & R. Brown were collected from the Kebang forest area under Pasighat forest division of Siang district (27°43′ to 29°20′ N latitude and 44°42′ to 95°35′ E longitude), Arunachal Pradesh, India, during March to December 2015-August 2016. Herbarium specimen were collected, identified and authenticated at Herbarium of Botanical Survey of India, Arunachal Pradesh Regional Centre, Itanagar (ARUN). A voucher specimen No. LJ/058/HAU/2016 was deposited in the Herbarium of Department of Botany, Rajiv Gandhi University (HAU), Rono Hills, Doimukh, Arunachal Pradesh, India for future reference. The leaves collected were dried under shade, grinded to powder (1kg), and passed through 40 mesh sieve, and stored in air tight container for further use.

Preparation of extract

The powdered plant material (1kg) was extracted by using cold extraction method with 90% methanol as solvent for 72 hours at room temperature. The whole extract of powdered leaves containing solvent was separately collected and filtered with Whatman No. 1 filter paper. The solvent was then evaporated to dryness under reduced pressure in rotary evaporator at 50°C. The concentrated methanol extract of *Pouzolzia zeylanica* leaves (MEPZL) of yield ±110 g (11.1%, w/w) was stored in a desiccator for further use. A weighed amount of the dried extract (prepared in normal saline) was used in the present analgesic studies.

Chemicals and drugs

Chemicals used in the present study such as propylene glycol, pethidine, normal saline, diclofenac sodium were purchased from Sigma-Aldrich Chemicals. All the chemicals and drugs used in the study were of analytical grade.

Animal used

Healthy adult albino rat (Wistar strain) of either sex weighing between 150-250g were used for the present study. The rats were initially acclimatized to the laboratory environment for seven days prior to the experiment. The rats were housed in polypropylene cage, maintain under standard condition (25±2)°C, 12 hours light and dark, and relative humidity 60–70%. The rats were provided normal pellet diet with water *ad libitum*. The animal experiments were carried out according to the guidelines of Institutional Animal Ethical Committee (IAEC), RGU approved vide Memo No. IAEC/RGU/17/13 Dated 12 Dec. 2017 which is in line with national guidelines for animal experimentation.

Phytochemical screening

The crude extract which was freshly prepared was qualitatively tested for the presence of active phytoconstituents. For qualitative screening of phytochemicals of the crude extracts, the manuals and methods suggested by Trease and Evans (1985) and Kokate (1994), and other relevant literatures were referred. Alkaloids test were performed with Dragendorff's reagents developed by Waldi (1965); phenolic compounds (flavonoids) and tannin contents were tested with the methods suggested by Mace (1963), Evans (1997), and Harborne (1998); steroid was confirmed with Libermann-Burchard's reagent method suggested by Finar (1986); and presence of saponin was confirmed with method suggested by Kokate (1999). Presence of reducing sugars were

tested with Benedict's reagent, and Molish reagent suggested by Ramakrishnan *et al.* (1994) and presence of glycosides were tested with Borntrager's test suggested by Evans (1997). Gums and Mucilages were tested with the method suggested by Whistler and BeMiller (1993).

Evaluation of analgesic activity

Hot plate method

The Hot plate test method proposed by Eddy & Leimbach (1953) was employed to assess the analgesic activity of the methanolic extract of *Pouzolzia zeylanica* leaves. The albino rats were first screened by placing them on hot plate (Orchid Scientific Made Digital Analgesiometer, Model No. HC-01). Temperature of Analgesia Meter was maintained at 55±0.5°C and the reaction time was recorded in seconds. When animals showed the signs of paw licking or jumping was considered to be reached in threshold pain. Only those rats which reacted within 30 seconds and which did not show large variations when tested on four separate occasions, each 15 minute apart, were used for the test. The time for paw licking or jumping on the hot plate was recorded as a reaction time. The experimental rats were divided into five groups and each group contains five rats. The reaction time following the oral administration of the MEPZL with doses of 100, 200 and 300 mg/kg body weight, diclofenac sodium 5mg/kg body weight as a standard, and saline as a vehicle control was recorded. Various responses such as paw licking or jumping were recorded before and after 30, 60, 90, 120 and 150 minutes after a latency period of 30 minutes (Chattopadhyay *et al.*, 2004). From the measurement, the percentage of analgesic activity was calculated and recorded.

Tail-immersion test

Albino rats (Wistar strain) of either sex were divided into five groups and each group contains five rats. The methanol extracts of the plant at 100, 200, 300 mg/kg body weight were administered orally to the first groups of animals. While others remaining groups of rats received 5 mg/kg bodyweight of propylene glycol as vehicle control, and 5 mg/kg bodyweight of Pethidine as drug Standard. The tail was then dipped into a pot of water maintained at 55±0.5 ÚÙC. The time in seconds to withdraw the tail out of the water was taken as the reaction time. The reading was taken after 30 minutes of administration of the test drugs (Olaleye *et al.*, 2000).

Statistical analysis

The results were expressed as Mean ± Standard Error (SE) and percentages (%). Statistical analysis of the data was done using Dunnett's test and one way analysis of variance (ANOVA), in Graph Pad Prism5 biostatistics software package.

Results

Phytochemical constituents

The result of qualitative phytochemical analysis of the methanol extract of *P. zeylanica* (L.) Bennett & R. Brown (MEPZL) is shown in Table 1. It is shown that the MEPZL possess carbohydrates, alkaloids, tannins and gums, flavonoids and saponin as major active constituents.

Table 1. Phytochemical constituents of methanol extract of *Pouzolzia zeylanica* leaves.

Sl. No.	Constituents	Detection
1	Alkaloid	+
2	Carbohydrates	++
3	Flavonoids	+
4	Glycosides	-
5	Saponin	++
6	Steroids	-
7	Tannin	+
8	Gums	+

Values are mean of triplicate experiments and represented as present (+), absent (-).

Analgesic activity

Hot plate method

In hot plate method of analgesic activity evaluation, the result shows that all the test and standard drugs are significantly ($p<0.001$) increases the reaction time as compare to the control group [Table 2, Fig. 1(A-D)]. By applying Dunnett's test, it was revealed that there is significant effect of MEPZL on test groups 3, 4 and 5 as compare to the standard (test group 2) at 60 and 90 minutes at doses 100, 200 and 300 mg/kg. The effect of MEPZL on test group 5 become more at 120 minutes at 300 mg/kg.

Table 2. Analgesic effect of MEPZL in albino rats recorded through Hot plate test method (Wistar Strain)

Animals Groups	Treatment	Doses	Reaction in second at time(minutes) (mean ± SE)			
			0	60	90	120
Group 1	Normal saline	10 ml/kg	9.6 ± 0.316	9.82 ± 0.517	9.94 ± 0.361	9.6 ± 0.268
Group 2	Diclofenac sodium	100 mg/kg	10.84 ± 1.588	23.02 ± 1.989***	22.04 ± 2.366***	17.16 ± 1.647***
Group 3	MEPZL	100 mg/kg	9.58 ± 0.241	11.42 ± 0.920	18.06 ± 1.076**	13.08 ± 0.561*
Group 4	MEPZL	200 mg/kg	10.08 ± 0.149	19.32 ± 1.760***	23.38 ± 1.690***	17.82 ± 0.823***
Group 5	MEPZL	300 mg/kg	9.7 ± 0.533	22.24 ± 1.805***	29 ± 0.915***	26.2 ± 0.635***

Each value in the mean ± SE for 5 rats, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with control.

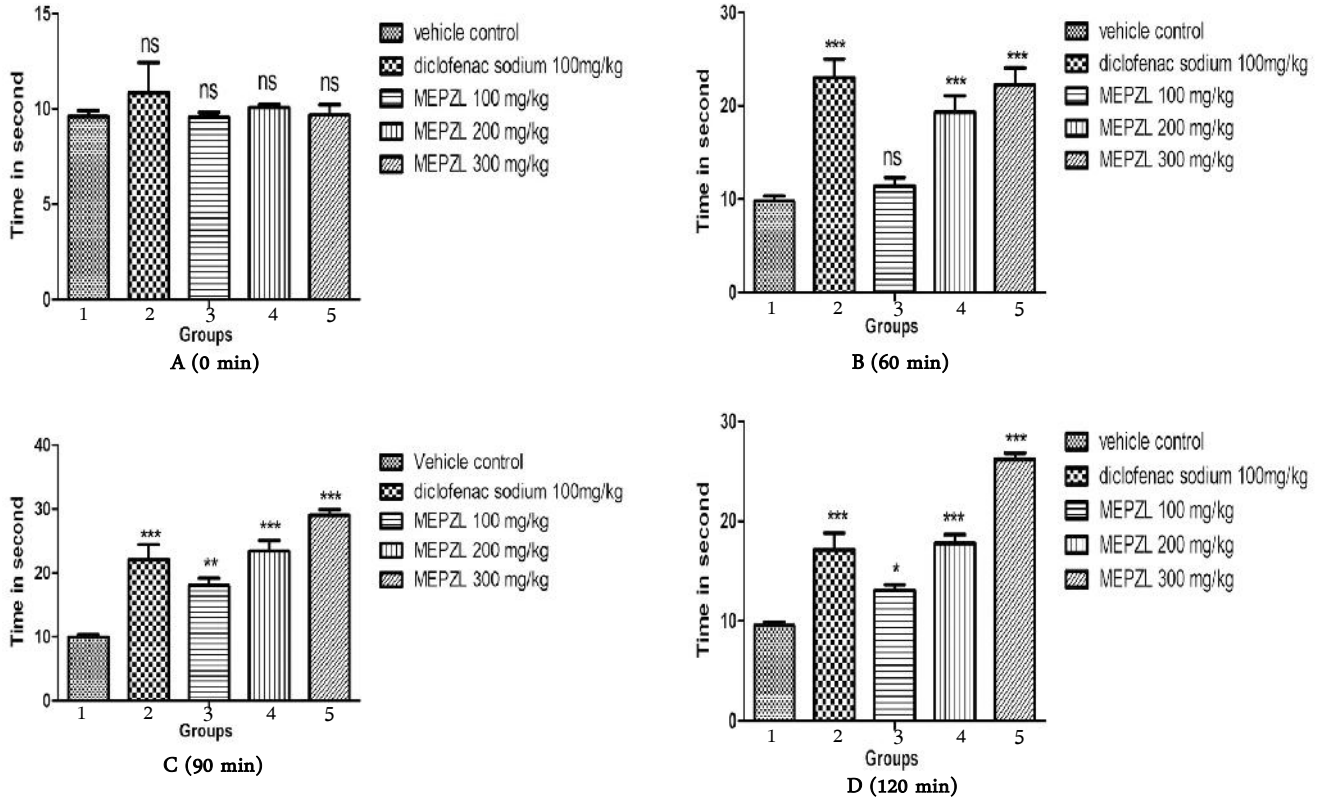

Fig. 1 (A-D) represent the reaction time at 0 minute, 60 minutes, 90 minutes and 120 minutes respectively in Albino Rats studied in Hot Plate method. All the data were represented by mean ± SE, . $^{***}p<0.001$, $^{**}p<0.01$ and $^{*}p<0.05$ compare to the control group. ns = non-significant compare to control group. #group1= rat receiving normal saline, #group2=rat receiving Diclofenac sodium 100mg/kg(p.o), #group3= rat receiving MEPZL 100 mg/kg(p.o), #group4= rat receiving MEPZL 200 mg/kg(p.o), #group5= rat receiving MEPZL 300 mg/kg(p.o)

Tail immersion test

The findings of the tail immersion test are presented in Table 3 and Fig. 2 (A-E) The tail immersion test conducted for the evaluation of analgesic activity revealed that the reaction time in seconds for the control group showed the shortest duration of tail movement (withdrawn) in a beaker of hot water as compared to the effect of the standard drug which showed the highest withdrawn time (in second) of tail out of the hot water. By applying Dunnett's test, it was shown that the effect of all the test samples showed significant effects as well as the test group5 (300mg/kg bodyweight) showed effective result as compared to the Standard implicating both spinal and supraspinal analgesic pathways.

Discussion

Analgesic activity of MEPZL was evaluated using hot plate model to characterize peripheral and central analgesic activity.

Table 3. Analgesic effect of MEPZL in albino rats (Wistar Strain) recorded through Tail immersion test method

Animals Groups	Treatment	Dose (unit/Kg)	Reaction in second at time(minutes) (mean ± SEM)				
			0	60	90	120	180
Group 1	Propylene glycol	5 mg	1.6 ± 0.202	1.6 ± 0.202*	1.6 ± 0.202*	1.6 ± 0.202*	1.3 ± 0.165*
Group 2	Pethidine	5 mg	1.96 ± 0.073	3.38 ± 0.464*	4.12 ± 0.033*	4.52 ± 0.148**	4.5 ± 0.136***
Group 3	MEPZL	100 mg	1.98 ± 0.143	2.94 ± 0.061	4.32 ± 0.086**	4.66 ± 0.073***	4.52 ± 0.148***
Group 4	MEPZL	200 mg	1.94 ± 0.061	3.02 ± 0.148	4.4 ± 0.084***	4.84 ± 0.067***	4.8 ± 0.069***
Group 5	MEPZL	300 mg	1.92 ± 0.111	3.6 ± 0.141**	4.62 ± 0.103***	5.08 ± 0.096***	5 ± 0.049***

Each value id the mean ± SEM for 5 rats, * *P < 0.05*; ** *P < 0.01*; *** *P < 0.001*compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

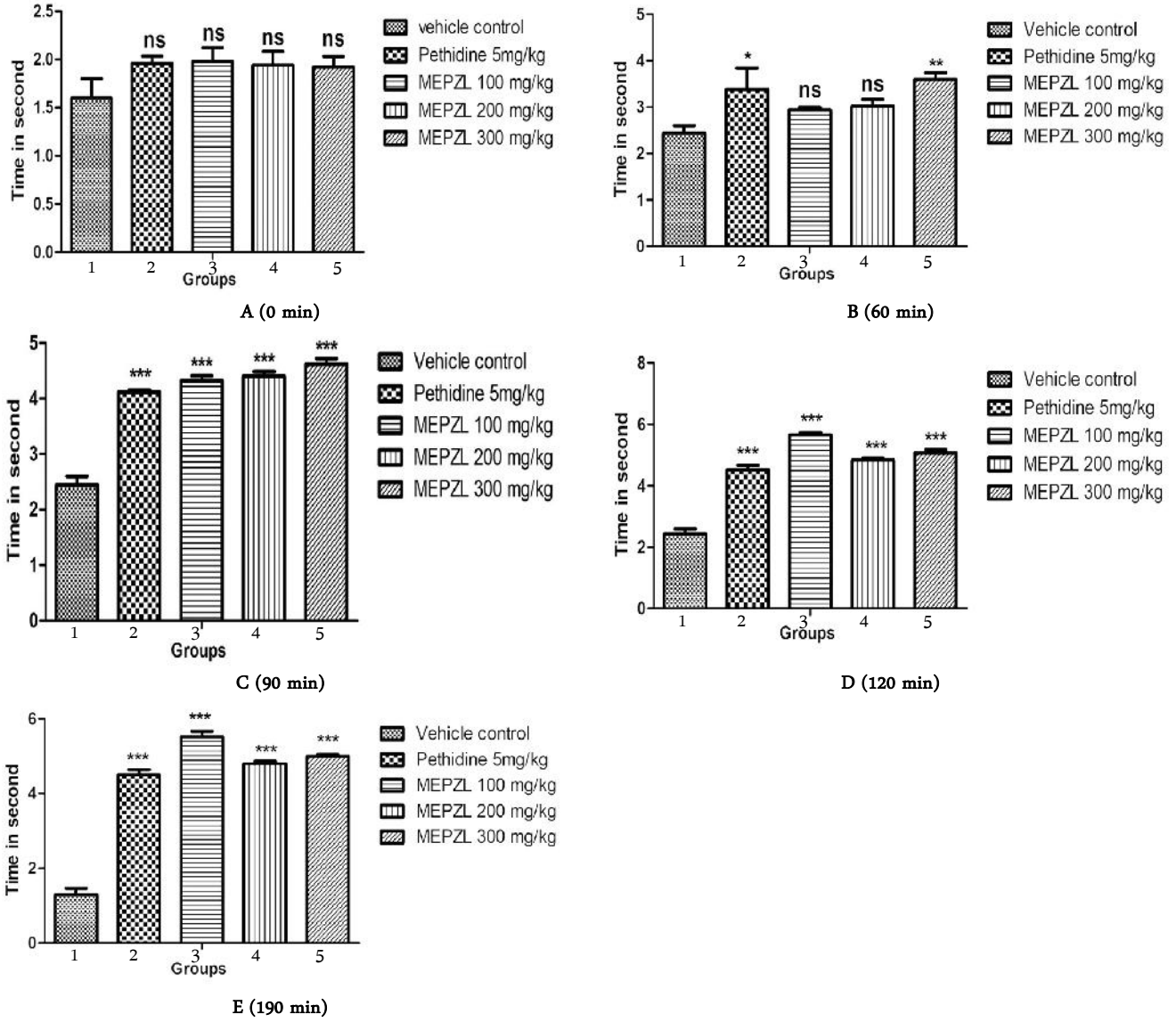

Fig. 2 (A-E) represent the reaction time at 0 minute, 60 minutes, 90 minutes, 120 minutes and 180 minutes respectively in Albino Rats studied in Tail immersion method. Results are mean ± S.E.M. of n=5, where tests and standard Pethidine significantly different vs. control at *P<0.05*, *P<0.01*, *P<0.001*. ns- non significant compare to control group. #group1= rat receiving normal saline, #group2=rat receiving Pethidine 5mg/kg(p.o), #group3= rat receiving MEPZL 100 mg/kg(p.o), #group4= rat receiving MEPZL 200 mg/kg(p.o), #group5= rat receiving MEPZL 300 mg/kg(p.o)

The test revealed that the reaction time for rat was significantly increased in a dose dependent manner after one hour of oral administration which suggests the central analgesic effect of *Pouzolzia zeylanica* leaf extracts. The Tail immersion test are useful in illuminating the centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level. The analgesic effect produced by the tail immersion tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of endogenous prostaglandins, leukotrienes, and other endogenous substances that are key players in pain (Ricciotti and Fitz Gerald, 2011). This means that MEPZL showed both peripheral and central analgesic activity for the transmission of painful message in rats. Qualitative phytochemical screening also revealed that MEPZL contains major phytoconstituents such as alkaloids, polyphenolic compounds, saponin and tannin which might be responsible for analgesic activities being shown in present study. Pal *et al.* (2009) reported analgesic and anti-convulsant effects of saponin on rat in dose dependent manner. Vianna (1998) have studied the analgesic and anti-inflammatory effect of the tannin fraction. Flavonoids are the polyphenolic compound with diverse pharmacological activities. Xiao *et al.* (2016) reported natural flavonoids as promising source for development of analgesic drugs. Borgi *et al.* (2008) reported the anti-inflammatory and analgesic effect of flavonoids compounds. Farouk *et al.* (2008) discussed about analgesic effect of alkaloids and possible mechanism involves.

The present study has shown that the methanol extract of *Pouzolzia zeylanica* leaves (MEPZL) possesses both central and peripheral analgesic activities as well as does have significant antinociceptive effects in laboratory animals at the doses investigated. Therefore, the leaves part of the plant extract could be a suitable candidate for further investigation as an agent for the treatment and management of acute and chronic pain condition. Leaf parts also contains important biologically active constituents such as saponin, tannins, alkaloids and polyphenolic compounds (flavonoids) which might be responsible for present analgesic activities worthy of further investigation.

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