

PRE-CLINICAL SCREENING OF INDIGENOUS MEDICINAL PLANT *PHYLLANTHUS
AMARUS* FOR ITS ANALGESIC ACTIVITY

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ABSTRACT: Background: *Phyllanthus amarus* aqueous extract was investigated for its central and peripheral analgesic activities. **Objectives:** To evaluate the central and peripheral analgesic activities of aqueous extract of *Phyllanthus amarus*. **Materials and Methods:** The aqueous extract of *Phyllanthus amarus* was prepared using Soxhlet apparatus. An in vivo study using Swiss albino mice was done to screen the central and peripheral analgesic activity of *P.amarus* extract. The extract was administered at a dose of 100 mg/kg body weight I.P. The peripheral analgesic activity was assessed using acetic acid induced writhing test. The central analgesic activity was assessed using Eddy's hot plate apparatus. **Results:** The aqueous extract of *P.amarus* showed significant ($p < 0.05$) peripheral and central analgesic activity. **Conclusion:** This study demonstrated that *P.amarus* aqueous extract exhibited significant analgesic activities.

Key words: *Phyllanthus amarus*, aqueous extract, central, peripheral, analgesic.

INTRODUCTION

We all know what pain is. We have all suffered from it. Sometimes, we hardly notice it. Sometimes, it's unbearable. Pain is a useful alarm signal that something is wrong (British pain society, 2014). Pain is a sensory and emotional experience. The emotional component is variable from person to person and in the same person from time to time (Rajagopal MR et al). Pain can come from any part of your body: skin, muscle, ligaments, joints, bones (nociceptive pain), injured tissue (inflammatory pain), nerves (neuropathic pain), internal organs (visceral pain) or a combination of these types of pain (mixed pain). One difficult thing about pain is that it can't be measured. Pain is defined by the International Association for the Study of Pain (IASP) as, "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (British pain society, 2014). Right from his birth, man has thrived to relieve pain. Modern man has made a huge leap in the field of pain management. Discovery of analgesic agents, such as aspirin and morphine, that interact with the transmitters and modulators of the pain system are helpful for many people with pain. Even though they are the main stay in the treatment of acute and chronic pain, these agents do have a lot of demerits. The abuse liability of the central acting agents like morphine and the hepatotoxic potential of the peripherally acting drugs like NSAIDs is a concern for the medical fraternity (Craig AD et al, 2011). Considering the above facts, there is a need for an analgesic, which is devoid of the deleterious effects of the conventional analgesics used in the modern medicine. Plants or plant products can play a major role in the discovery of a new analgesic agent. Majority of the people living in developing countries still depend on the plant based therapy due to their lost cost and minimal side effects. *Phyllanthus amarus* (Syn. *Phyllanthus niruri*) is a perennial herb distributed throughout the tropical and subtropical regions of both hemispheres. It belongs to *Euphorbiaceae* family. *Phyllanthus amarus* has a long history in herbal medicine systems all over the world. This important medicinal herb is used for curing a wide range of ailments like jaundice, asthma, hepatitis, urogenital problems, dysentery, dyspepsia, arthritis, malaria, etc. Various pre-clinical studies have shown that this plant possess antiviral, hepatoprotective, hypoglycaemic, hypolipidemic, antioxidant, antimicrobial, anti-amnesic, antitumour activities (Rashmi Mathur et al, 2011; Aruna Kumar R et al, 2010; Rajeshwar Y et al, 2008; R. Bhattacharyya, 2003; Bhattacharjee R, 2007; Shetti AA et al, 2012; Hanumanthachar Joshi et al 2007; Priyanka Sharma, 2009).

MATERIALS AND METHODS

Plant materials

Fresh whole plants of *Phyllanthus amarus* leaves were harvested, properly washed in tap water and then rinsed in sterile distilled water and left to air-dry for several weeks. The different parts of the plants were reduced to powdered form using electric blender. The powders were stored in air-tight containers until required.

Sample Preparation and Extraction

Phyllanthus amarus aqueous extract (PAAE): A weighed quantity (500 g) of the coarse powder was taken and extracted with distilled water in a Soxhlet extractor. The extract was concentrated on a water bath at a temperature not exceeding 60°C (yield 16 % w/w). The aqueous extract was dissolved in distilled water.

Animals

Adult Swiss albino mice of either sex weighing 25-30 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC), Yenepoya University. The mice were maintained under standard conditions in the Animal House (CPCSEA approved, Reg No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The mice were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard housing conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water ad libitum. The mice were maintained on a 12:12 hour light-dark cycle.

Assessment of Central analgesic activity of PAAE

Eddy's hot plate test:

In this method, the mice in each group (06 per group) was treated with vehicle (1 ml of distilled water, orally), PAAE (100mg/kg body weight orally), and tramadol (5 mg/kg, Intraperitoneally) serving as positive control. The animals were housed for 10 days. All the animals received the drugs 1 hour before conducting the experiment. The mouse was placed on the Eddy's hot plate, with a temperature of 55°C. Time taken by the mouse to lick its paw or jump was noted as reaction time. The cut off time was kept as 15 seconds to prevent injury to the paw.

Assessment of Peripheral analgesic activity of PAAE

Acetic Acid Induced Writhing Reflex Test in Mice:

Mice in each group was treated with vehicle (1 ml of distilled water, orally), PAAE (100mg/kg body weight orally), and Ibuprofen (30 mg/kg orally). The animals were housed for 10 days. Analgesic activity was then assessed by counting the number of writhes induced by 0.6% acetic acid administered Intraperitoneally one hour after the administration of the test and standard drugs. Numbers of writhes per animal was counted for 10min. A writhe was considered when animal showed contraction of abdomen with simultaneous stretching of at least one hind limb. Protection against writhing was taken as an index of analgesia.

Statistical Analysis

Data was analysed using One Way ANOVA, followed by Tukey Kramer multiple comparison test.

RESULTS

Assessment of Central analgesic activity of PAAE:

Eddy's hot plate test:

The Eddy's hot plate test showed (Table:1) that the withdrawal time of the paw was prolonged in the PAAE group when compared to the normal group thus exhibiting analgesic property of the extract.

Table1: Effect of PAAE on Pain using Eddy's Hot Plate

Groups	Withdrawal of paw in seconds (Day 10)
I.Normal Saline	2.666±0.085
II.PAAE	11.02±0.05 ^a
III. Tramadol	12.28±0.81 ^a

Results were expressed as Mean ± SD.
One Way ANOVA, followed by Tukey Kramer multiple comparison test, n = 6
a: p< 0.001, extremely significant on comparing group II,III with group I.

Table 2: Effect of PAEE on Pain in Acetic acid induced Writhing

Groups	Onset of writhing in minutes	No. Of writhing
I.Normal Saline	1.02 ±0.04	18
II.PAEE	5.34±0.47 ^a	1
III. Ibuprofen	3.07±0.01 ^a	1
Results were expressed as Mean ± SD. One Way ANOVA, followed by Tukey Kramer multiple comparison test, n = 6 a: p< 0.001, extremely significant on comparing group II,III with group I.		

Assessment of Peripheral analgesic activity of PAEE:**Acetic Acid Induced Writhing Reflex Test in Mice:**

It was seen that (Table:2) onset of writhing (stretching behaviour) was delayed and number of writhing after acetic acid administration has reduced in the PAEE group when compared to the normal group thus exhibiting analgesic property of the extract.

DISCUSSION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. All of us have experienced pain. The central and peripheral factors are involved in the pain mechanisms. Usually it wean off by itself. Some time it requires treatment. Nevertheless it is an alarming signal for the underlying problem. The clinical treatment of pain has improved with the advent of analgesic substances, such as aspirin and morphine that interact with the modulators and transmitters of the pain and are helpful for many people with pain. But none of currently used centrally and peripherally acting analgesic agents are devoid of adverse effects (Debasis Mishra et al 2011; Olufunmilayo O Adeyemi et al, 2011; MilindParle et al, 2013). The screening for analgesic activity of an agent in animals is useful in predicting the analgesic activity of an unknown component (MilindParle et al, 2013). The results showed that the aqueous extract of *Phyllanthus amarus* showed analgesic activity in the central and peripheral models of pain. This clearly indicates that this plant has got a morphine like activity and aspirin like activity. This dual analgesic activity can be attributed to the phytoconstituents present in both the extracts. This extract contain lot of phytoconstituents like lignans, tannins, flavonoids, alkaloids, phytosterols etc that might have a role in this analgesic activity.

CONCLUSION

The results from this study revealed the efficacy of the analgesic properties in *Phyllanthus amarus*. Further research is being carried out to pinpoint the active constituent of the plant which shows this dual analgesic property.

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