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Analgesic effects of methanolic extracts of *Anogeissus latifolia* wall on swiss albino mice

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ABSTRACT

The main objective of my present research work is to find out the good pharmacological properties from medicinal plants with their preliminary phytochemical study and also to evaluate the analgesic activity of Anogeissus latifolia belongs to combretaceae family on mice by acetic acid and formalin induced pain model. Dose was selected from the literature and the dose chosen for the study is 300 mg/kg, a small trial on acute toxicity was used to confirm the dose by using 3000 mg/kg, and there was no mortality found, so 1/10th of this dose was chosen for analgesic activity. Normally herbal drugs are free of side effects and available in low cost. The methanolic extract of Anogeissus latifolia at 300 mg/kg showed a significant analgesic activity in acetic acid and formalin induced model when compared to that of standard drug.

Key words: *Anogeissus latifolia*, leaf, methanolic extract, acetic acid induced and formalin induced pain model.

INTRODUCTION

In the history of medicine for the relief of pain many medicinal herbs has been used as natural products which contain many active principles are believed to have their potential therapeutic value. Taking into account that the most important analgesic prototypes (Salicylic acid and morphine) were originally derived from plant sources, the study of plants species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic drugs. Early human used to seek remedies from any materials due to the problem of uncontrolled pain. Herbal drugs are considered to be as effective as synthetic drugs with lesser side effects. Herbal medicines are in line with nature, with less hazardous reaction¹. Pain usually occurs when peripheral nociceptors are stimulated during tissue injury, visceral distension and other factors. During such situation, pain perception is a normal physiologic system which is mediated by healthy nervous system. The world health organization defined health as a complete state of mental, physical and social well being and not merely the absence of disease. In recent studies focusing on plant research has an increase and non steroidal anti inflammatory drugs constitute most widely used classes of drugs. Since past decades, traditional system of medicine has become a topic of global importance. Medicinal plants form the backbone of traditional systems of medicine in India.

MATERIALS AND METHODS

Anogeissus latifolia DC belonging to combretaceae family is a large or moderate sized tree which is available in dry deciduous forests and available throughout India. The tree has been studied for antioxidant activity, hydrogen donating ability, nitric oxide, super oxide scavenging activity and hydrogen peroxide decomposition activity². Leaves are opposite or sub-opposite. Bark is smooth with grey- white colour and exfoliating in irregular thin scales. A variety of substance which contributes to hepatoprotective activity has been identified in the extracts of *Anogeissus latifolia* which includes tannins³, gallic acid, ellagic acid and flavanoids such as leutin, quercetin which are known as potential antioxidants. The bark of the plant has also reported to have several biological activities such as anti ulcer, anti microbial and wound healing activities⁴. The hydroalcoholic extract of *Anogeissus latifolia* has reported to have chemoprotective activity in paracetamol induced toxicity in rat model. Thus, the present study was undertaken for the investigation of analgesic activity of methanolic extract of *Anogeissus latifolia*^{5,6}.

Collection and authentication of plant materials : The plant material was collected in the month of June 2011 from Srichalam hills and a specimen was dropped in the herbarium and the leaves was authenticated by Professor Dr. Madhavachetty S. V. University, Trupathi. The collected powdered material was shade dried and pulverized.

Solvent used for extraction: Petroleum ether and methanol

Preparation of the extract: The dried powders of leaf of *Anogeissus latifolia* were defatted with petroleum ether (60-80°C) in a Soxhlet Apparatus by continuous hot- percolation. The defatted powder material (marc) thus obtained was further extracted with methanol with same method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Phytochemical Screening : The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

Test for reducing sugars (Fehling's test): The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for anthraquinones: The individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for terpenoids (Salkowski test): To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for flavonoids: A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins: To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins: About 0.5 g of the individual extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (FeCl₃) was added and observed for brownish green or a blue-black coloration

Test for alkaloids: 0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test): To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated H₂SO₄. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Procurement of Experimental Animals: Swiss albino mice (20-25 g) of either sex and of approximate same age are used in the present studies were procured from listed suppliers of NIN, Hyderabad, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (1447/PO/9/11/CPCSEA) after scrutinization. The animals received the drug treatments by oral gavage tube.

Acute Toxicity Study: The study was carried out according to OECD (Organization of Economic Co-operation and Development) guidelines 423. Nine female Wistar albino rats weighing 150-200 g were taken and extracts were administered orally to animals at a dose of 3000 mg/kg in 0.3% w/v Carboxy Methyl Cellulose Sodium. Then the animals were observed for mortality and morbidity at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hr. Food was given to the animals after 4 hr of dosing and the body weight was checked at 6 hr after dosing. Morbidity like convulsions, tremors, grip strength, lethargy, ptosis and pupil dilation were observed. The animals were observed twice daily for 14 days and body weight was noted.

Analgesic Activity:

Analgesic activity was assessed by acetic acid and formalin induced model.

Writhing Test (Chemical stimulus)

Aspirin like non-narcotic analgesic activity of the test extracts was investigated by the ability to protect a painful writhing syndrome in mice. The syndrome is characterized by abdominal torsion, drawing up of hind limbs to the abdominal wall, marked contraction of the abdominal area and periodical arching of the back to rub the abdominal wall on the glazed surface on which the mouse is kept. Writhing was consistently produced in mouse by an intraperitoneal injection of 0.6% aqueous acetic acid. Overnight fasted, healthy adult male albino Swiss mice weighing between 18 to 25gm in groups of six each were taken for present investigation. Control and the test extracts were administered orally in a dose of 300mg/kg body weight respectively to the test groups animal. The control group of animals were given normal solution in the dose of 10ml/kg body weight. One group of animal was administered with Diclofenac sodium as standard, orally in a dose of 5mg/kg (b.w). After a gap of 30 minutes of the administration of the test extracts, all the groups of mice were given the writhing agent, 0.6% aqueous acetic acid, in a dose of 1ml/100gm (b.w) intraperitoneally. Five minutes after administration of acetic acid the number of writhing produced in these animals were counted for next 10 minutes and the number of writhing produce in the treated groups were compared with those in the control group and the percentage protection was calculated as show below^{7,8}.

Formalin induced model:

The method used was similar to that described previously (Hunskar S, Hole K, 1987)⁹. The control group received normal saline (0.1ml/10g) and standard group Aspirin (100mg/kg). MEAL(300mg/kg) was orally administered and after 30 minutes of treatment, 20µl of 1% Formalin solution was injected subcutaneously in the right hind paw of the mice. The time spent in licking and biting of the affected paw was noted. The total paw licking response was measured as early phase (0-5mins) and late phase (15-20mins) after formalin injection^{10,11}. The percentage of pain inhibition was expressed by the given formula:

Percentage inhibition = [(Control mean-Treated mean)/Controlmean] x 100

RESULTS

In the preliminary phytochemical investigation on the *Anogeissus latifolia* it reveals the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides and sugars in both the extracts.

Acetic acid induced writhing test

The methanolic extract used orally at a dose of 300mg/kg showed significant ($P < 0.05$) inhibition of pain responses which was compared with control group (Table 1). The number of writhing during the 15mins period showed by the all the three groups was calculated and compared with the control. The percentage inhibition of pain by 5mg/kg diclofenac sodium was found to be more significant (76.62%) and the methanolic extract showed 73.59% as compared to the control.

Table .1 : Effect of the Methanolic extract of leaves of *A.latifolia* wall (300 mg/kg) on acetic acid induced writhing in rat

S.No	Group	Dose (mg/kg)	No.of writhings	Inhibition (%)
1	Control	--	46.2 ± 1.2	--
2	Diclofenac sodium	5	10.8 ± 2.1*	76.62
3	MEAL	300	12.5 ± 1.7*	73.59

* $P < 0.05$, ** $P < 0.01$ vs. control. Values are mean ± SE from 6 animals in each group.

Formalin induced pain

The formalin induced test produces a inhibition of pain responses. The extract with dose 300mg/kg produced significant ($P < 0.05$) inhibition in the early phase of the pain. In the late phase, the extract at a dose of 300mg/kg showed a significant ($P < 0.01$) inhibition of pain (Table 2). Acetyl salicylic acid (100mg/kg) produced significant reduction in pain response when compared with control.

Table .2 : Effect of the Methanolic extract of leaves of *A.latifolia* wall (300 mg/kg) formalin induced nociception in mice

Group	Dose	Licking time (Sec)		% Inhibition	
		1 st phase	2 nd phase	1 st phase	2 nd phase
Control	10ml/kg	49.7 ± 5	82.5 ± 5.5	--	--
Aspirin	100	40.8 ± 5	23.2 ± 3.3	18	72**
MEAL	300mg/kg	29.4 ± 5	28 ± 4	40	66**

* $P < 0.05$, ** $P < 0.01$ vs. control. Values are mean ± SE from 6 animals in each group.

DISCUSSION

The results obtained from the study revealed that the Methanolic extract of *Anogeissus latifolia* produced a significant inhibition of pain response. The standard drugs however showed greater effect than the extract. The acetic acid induced writhing is basically a revelation of peripheral pain^{12, 13}. The acetic acid induces release of different endogenous chemical pain mediators like prostaglandin E2 (PG)^{14,15}, substance P, serotonin, histamine, bradykinin which further causes stimulation of nociceptive neurons^{16,17}. The extract caused inhibition of this pain, which is thought to be due to inhibition of release of prostaglandins, the mechanism quiet similar to Aspirin and other non steroidal anti inflammatory drug (NSAIDS). MEAL also exhibited activity in the formalin test which can differentiate between the central and peripheral pain component. Formalin induced pain is biphasic having an early phase (0-5mins) and the other late phase (15-20mins)¹⁸. Centrally acting drugs show good response in both the phases, but the peripherally acting drugs act only on the late phase¹⁹, which is due to inhibition of prostaglandin synthesis.

CONCLUSION

In conclusion, we can confirm that the methanolic extracts of *Anogeissus latifolia* are endowed with both central and peripheral analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

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