

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

Vijusha M.*¹, Shalini K.², Veeresh K.², Rajini A.² and Hemamalini K.¹

¹Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad, Andhra Pradesh, India

²Sree Dattha Institute of Pharmacy, Sheriguda, Ibrahimpatnam, Hyderabad, Andhra Pradesh, India

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 tail immersion, tail flick and eddy's hot plate method. Dose was selected from the literature and the dose chosen for the study is 200 mg/kg, a small trial on acute toxicity was used to confirm the dose by using 2000 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 200 mg/kg showed a significant analgesic activity in tail immersion, tail flick, and hot plate model when compared to that of standard drug.

Key words: *Anogeissus latifolia*, leaf, methanolic extract, tail immersion, tail flick and eddy's hot plate model.

INTRODUCTION

In the history of medicine for the relief of pain many medicinal herbs has been used .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 and 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 were authenticated by Dr. Madhavachetty. The collected powdered material was shade dried and pulverized.

Solvent 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 2000 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 Tail immersion methods, Tail-Flick method and Eddy's hot plate.

Tail Immersion Method: (Thermal stimulus)

In the Tail immersion method albino rats (180-200g) were divided into four groups each consisting of six animals. Group I served as a control (received vehicle), Group II served as a standard (received Acetyl salicylic acid 640mg/kg p.o) while the Group III received the methanolic extract (As per b/w). The time in second to withdraw the tail clearly out the water was taken as the reaction time. The observations were made before and after administration of respective drugs at 30 min, 60 min, 120 min, and at the end of 180 min.

Tail-flick method: The tail of the mouse was placed on the hot wire (55°C) and time taken by the animal to withdraw its tail from the hot wire was taken as the reaction time. Analgesic activity was measured at 30, 60 and 90 minutes after administration of the drugs. The analgesic activity was said to be complete if the mouse failed to withdraw its tail within 10 seconds of exposure.

Hot Plate Method: (Thermal stimulus)

In the hot plate method albino mice (18-28) were divided into four groups each consisting of six animals. All the animal selected for the studied were under gone the normal basal reaction time and then separated as different groups like Group I served as a control (received vehicle), Group II served as a standard (received Pentazocine 5mg/kg) while the Group III received the methanolic extract (As per b/w). All animals were lowered onto the surface of a hot plate (50±1.00°C) enclosed with cylindrical glass and the time for the animal to jump or lick the fore limb was noted as the reaction time (RT). Cut off time in the absence of a response was 15 sec to prevent the animals from being burnt⁷. The observations were made before and after administration of respective drugs at 30 min, 60 min, 120 min, and at the end of 180 min^{8,9}.

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. While evaluating analgesic activity of different extracts by hot plate method, it was observed that pentazocine 5mg/kg showed significant analgesic effect at 0, 15, 30, 45 and 60minutes. Peak effect was observed at 60 minute. Normal did not have any significant change in basal reaction time. The tail immersion method, it was observed that pentazocine (5mg/kg) showed highly significant analgesic effect at 0, 15, 30, 45, and 60minutes . Peak effect was observed at 60minutes. Normal (Group-1) did not have any significant change in basal reaction time. Methanolic extract at a dose of 300mg/kg showed highly significant activity ($P < 0.001$) at different time interval as compared to control group. In the tail flick method the basal reaction time has decreased with standard and treated groups and the effect was highly significant in 150min.

Table 1: Effect of Methanolic extract of leaves of *A.latifolia* (300 mg/kg) on rat submitted to the tail immersion

S. No	Group	Dose (mg/kg)	Average tail withdrawing time (s)				
			0 min	15 min	30 min	45min	60
1	Control	--	3.93 ± 0.11	3.95 ± 0.18	3.97 ± 0.17	3.97 ± 0.15	3.98 ± 0.18
2	Pentazocine	30	3.92 ± 0.30*	4.09 ± 0.21*	5.31 ± 0.41*	6.54 ± 0.22*	7.10 ± 0.17*
3	MEAL	300	3.91 ± 0.16*	4.14 ± 0.21*	5.82 ± 0.28**	6.25 ± 0.22*	7.18 ± 0.17**

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

Table 2: Effect of the Methanolic extract of leaves of *A.latifolia* wall (300 mg/kg) on tail flick in rat

S.No	Group	Reaction time					
		0	30	60	90	120	150
1	Control	28.8 ± 1.4	31.4 ± 1.0	33.4 ± 1.5	34.8 ± 1.3	36.8 ± 1.5	37.2 ± 1.8
2	Standard	27.6 ± 1.8	68.2 ± 6.6**	76.4 ± 4.8**	87.0 ± 1.6**	89.0 ± 0.8**	48.2 ± 4.6**
3	MEAL	24.2 ± 1.0	65.8 ± 9.2**	69.6 ± 8.5**	75.6 ± 5.6**	81.2 ± 3.8**	45.0 ± 5.5**

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

Table 3: Effect of Methanolic extract of leaves of *A.latifolia* (300 mg/kg) on rat submitted to the hot plate test

S.No	Group	Dose (mg/kg)					
			0 min	15 min	30 min	45min	60
1	Control	--	6.52 \pm 0.25	6.22 \pm 0.21	6.38 \pm 0.31	7.25 \pm 0.38	6.16 \pm 0.54
2	Pentazocine	10	6.56 \pm 0.23*	11.45 \pm 0.38*	14.65 \pm 0.36**	14.79 \pm 0.81**	15.45 \pm 0.56**
3	MEAL	300	6.23 \pm 0.48**	10.10 \pm 0.31**	12.56 \pm 0.23*	12.86 \pm 0.72**	13.67 \pm 0.18**

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

DISCUSSION

In daily clinical practice pain is a condition which we feel regularly. *Anogeissus latifolia* has been traditionally used by the tribals of middle kerala to cure specific ailments. This attempt is to prove the efficacy of the plant extract as a potential analgesic drug and to demonstrated a positive result. The analgesic properties were also studied using sensitive models that could provide different grades of noxious stimuli (in thermal stimulus). In the present study the thermal test was selected because of several advantages including the sensitivity to strong analgesics and limited tissue damage. It has been reported that a number of flavonoids possess analgesic activity. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase. From the preliminary phytochemical study, it observed that MEAL contains flavonoids¹⁰. Pethidine is a centrally acting analgesic and showed significant increase in reaction time.

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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