

**ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF  
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Andhra Pradesh, India.\*Corresponding Author: [rkhemamalini@yahoo.com](mailto:rkhemamalini@yahoo.com)**ABSTRACT**

Antinociceptive activity of methanolic extract of leaves *Anogessius acuminata* was studied by peripheral/ non \_narcotic model of nociception like acetic acid induced writhing syndrome test and central/ narcotic models like tail flick tests. The methanolic extract of the plant, administered orally ( 300 mg/kg, body weight) and the standard drug (piroxicam: 10mg/kg body weight, P.O) produced significant analgesic activity in acetic acid induced writhing syndromes as compared to the vehicle treated control group. In the tail flick test, the plant extract produced increase in reaction time which was significantly higher in the test and standard group compared to the control group. The plant possesses significant antinociceptive property as evidenced in all the animal models of nociception. It might possibly exert its effect through diverse mechanism that may involve both central and peripheral pathways. The preliminary phyto chemical investigation revealed the presence of steroids, alkaloids and triterpene in the methanolic extract of leaves of *Anogessius acuminata* which may be responsible for its antinociceptive activity.

**Keywords:** *Anogessius acuminata*, Nociception, tail flick test writhing syndrome.**INTRODUCTION**

Pain is universally understood as a signal of disease and it is most common symptom that brings a patient to a physicians attention, requiring treatment with analgesic agents<sup>1</sup>. Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. A medicinal plant is factually any plant which in one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of direct therapeutic agents. Use of medicinal plant is increasing in many countries where 35% of drugs contain natural products<sup>2</sup>. Most of the drugs used at present for analgesic effect are synthetic in nature, prolong use of which cause several side and

toxic effects like respiratory depression, Constipation, kidney damage, physical dependence as well as gastrointestinal irritation. As these drugs are not commonly available to the rural folks that constitute the major populace of the world, it is therefore essential that effort should be made to introduce new medicinal plants to develop cheaper drugs. The floral richness of the north east region cannot be neglected in context to its medicinal importance. Considering the rich diversity of this region, it is expected that screening and scientific evaluation of plant extracts for their analgesic activity may provide new drug molecule that can combat various side effects of the commercially available synthetic drugs, more over reducing the cost of medication.

Anogessius acuminate commonly known as button tree belonging to the family combretaceae is found in abundance in india mainly in tirupathi hills, which is used for different activities.

#### MATERIAL AND METHODS

**Plant materials:** Leaves of the plant were collected from the medicinal garden of the Department of Botany, S.V University during the month of September 2009, identified by taxonomist of S.V University and a voucher specimen was deposited.

**Preparation of methanol extract:** Leaves of A.A were dried in shade and powered about 1000 g of leaves are soaked 2500ml of methanol for 1 hr in beaker and mixture was extracted by continuous hot percolation for 24 hrs by using soxhlet apparatus and distillation was performed to separate the solvents .

**Phytochemical screening:** Phyto chemical screening of the extract was carried out by standard method .<sup>4</sup>

**Chemicals:** Pentazocine and piroxicam were purchased from sigma respectively, methanol, acetic acid were purchased from merck limited.

**Animals:** Healthy adult swiss albino mice of either sex, approximately of same age weighing between 25-30g and adult male Sprague dawley rats weighing between 180-200g were used for the study. They were housed under controlled conditions at 25± 3° C 50± 5% RH and kept under 10/14 h light /dark cycles with food and water ad libitum. Animals were group housed in polypropylene cages containing sterile paddy husk bedding. The study was conducted after obtaining the approval of the institutional animal ethics committee 51/01/C/CPCSEA. The animals were fasted for 14h before test to achieve better drug absorption through gastro intestinal tract.

#### Analgesic activity:

##### Acetic acid induced writhing syndrome

The intra peritoneal injection of acetic acid results in constriction of abdominal muscle together with stretching of hind limbs known as writhing syndrome. In this test the antinociceptive activity of crude methanolic extract of A.A leaves was studied on

chemically induced pain sensation in female nonpregnant albino mice<sup>5</sup> plant extract standard drug or the vehicle was administered orally 30 min prior to intra peritoneal injection of acetic acid ( 10ml /kg of 0.6 % v/v solution). Total number of stretching episodes for 20 min immediately after acetic acid injection in all the groups were recorded and antinociception was expressed as the percent reduction in writhing numbers compared between the vehicle treated control and animals pretreated with methanolic extract of A.A or piroxicam.

#### Screening of analgesic activity by tail flick

**method:** In this model Nichrome wire analgesiometer (rolex) was used<sup>6</sup>. Individually the tail of each rat was placed over the radiant heat source of the apparatus and the tail withdrawal from the heat (flicking response) was taken as the end point. The reaction time in seconds of each rats in each group was determined at 0, 30, and 60 mins following administration of the test compound (300mg/kg P.O ) or the standard drug (Pentazocine 1.5 mg/kg, P.O) and compared with the control.

**Statistical analysis:** the results were subjected to statistical analysis as per standard statistical method<sup>7</sup>.

#### RESULTS

Phytochemical screening of the methanolic extract of Anogessius acuminate revealed the presence of alkaloid, steroid and triterpenes. In acute toxicity study, there was no change in motor activity and gross behaviour during 24 h of observation and the plant extract was found to be safe up to 3 g/ kg of body weight, P.O. The low toxicity of the plant observed suggests that the plant extract is relatively safe for consumption and did not affect any of the parameters measured in the acetic acid induced writhing syndrome test, there was 84.61% reduction in writhing numbers after single oral administration of Anogessius acuminate for 300mg/kg which were significantly higher ( $p < 0.01$ ) compared to the control group. The standard drug piroxicam showed 91% reduction of the writhing number in acetic acid induced writhing's syndrome test, which was however, higher than the plant extract. In the tail flick test, the reaction time (sec ) showed 20% significancy from 30 to 60min after single oral administration of

Anogessius acuminata and Pentazocine (1.5mg /kg p.o) from 46.58% respectively when compared to the control group, there was no significant increase in the reaction time from 30 to 60 mins of observation period indicating dose and time dependent analgesic activity of the plant.

## DISCUSSION

In the present study, the antinociceptive effect of methanolic extract of the leaves of Anogessius acuminata was evaluated in different experimental models of pain viz non-narcotic model like acetic acid induced writhing syndrome test and narcotic models and tail flick tests. The results of the present study clearly demonstrated that the methanolic extract of Anogessius acuminata possessed a definite antinociceptive activity as observed by significant increase in the reaction time in acetic acid induced writhing syndrome and tail flick test as compared to the control group. Acetic acid causes inflammatory pain by inducing capillary permeability<sup>8</sup> and liberating endogenous substances that excite pain nerve endings<sup>9</sup>. The intensity of anti-nociception of Anogessius acuminata treated group was higher than the control group in acetic acid induced abdominal constricts in mice. NSAID'S can inhibit cox in peripheral tissues and therefore, interfere with mechanism of transduction of primary afferent nociceptors. The mechanism of analgesic effect of methanolic extract of leaves of Anogessius acuminata could probably be due to blockade off the effect or the release of endogenous substances that excite pain nerve endings similar to that of piroxicam and other NSAIDS. The tail flick is most common tests of nociception that are based on a phasic stimulus of high intensity. The nociceptive experience is short lasting and it is well accepted that agonist  $\mu$ -opioid receptors produce analgesia in acute pain models<sup>10</sup>. Therefore, it is believed that

substances are effective in tail flick exert their effects pre dominantly through  $\mu$ -opioid receptors (as the plant under study also contain triterpine as one of its phyto constituent, so it may act through inhibition of leukotriene biosynthesis .The presence of alkaloid in the plant extract supports the claim that this compound has anti nociceptic property since, alkaloid flavonoids and saponis have been found in other natural products with analgesic and anti inflammatory properties<sup>11,12</sup>. It may also be related partly to the presence of steroids that have been shown to exert analgesic effects in animal models of nociception<sup>13</sup>.

Acetyl-11-keto-beta boswellic acid (AKBA) a pentacyclic triterpenic acid present in the acidic extract of the boswellia serrata gum resin is a novel highly specific inhibitor of 5-lipoxygenase, the key enzyme for leukotriene biosynthesis . Leukotriene as well as peptidoleukotriens results in an increase in vascular permeability and chemotaxis of polymorphonuclear leucocytes as well as release of mediators from leucocytes, which sensitize nociceptors<sup>14, 15</sup>. The plant extract exhibited antinociceptive activity in all the animal models of nociception and possibly exerted its effect through diverse mechanism that may involve both central and peripheral pathways. Anogessius acuminata also possesses anti-inflammatory and thus supporting the rationale behind the traditional use of this plant in inflammatory condition<sup>16</sup>. Further pharmacodynamic investigations are required to understand the precise mechanism of antinociception exhibited by the methanolic extract of leaves of Anogessius acuminata.

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**Table 1: Anti-nociceptive activity of Anogeissus acuminata in acetic acid induced writhing and tail flick response in mice**

Group	Dose mg /kg	Methanolic extract of Anogeissus acuminata on acetic acid induced writhing		Tail flick response	
		Total No writhing Mean $\pm$ SEM	inhibition %	Latency of response in sec	%of inhibition
Control	0.3ml /NS	43.33 $\pm$ 2.01	-	6.56 $\pm$ 0.17	-
Standard	Piroxicam/ Pentazocine	34.83 $\pm$ 4.36***	91%	12.28 $\pm$ 0.15	46.58
MEAA	300mg/kg	12 $\pm$ 0.16**	84.61%	5.12 $\pm$ 0.54	20%

N= six animals in each group, values are mean  $\pm$ SEM, P<0.001 where compare to control by dunnett's test NS= normal saline.

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