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## Antinociceptive Screening of Methanol Extract of *Anogeissus Accuminate*

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### ABSTRACT

Findings of this study may help efficacy and potency of herb as an analgesic. The methanol extract of *Anogeissus acuminate* was examined for its central analgesic activity by using Tail immersion, and Formalin induced pain in albino mice. The doses administered were 300mg/kg P.o. The pentazocin 10mg/kg I.P was taken as standard drug. Data analyzed by One way ANOVA followed by Dunnett's test. All the results were expressed as mean  $\pm$  SEM  $P < 0.05$  was considered as significant. The animal that administered of 300mg/kg leaf extract has shown the maximum analgesic activity comparable to pentazocin ( $P < 0.001$ ). The analgesic was observed after 30 min of drug administration and showed wearing off after 120 min, and methanol extract of *Anogeissus acuminate* has central analgesic activity involving spinal as well as supra spinal mechanisms. This activity may be due to saponins found in the extract. Extra polation of findings in clinical situation how ever needed to develop it as novel analgesic.

**Keywords:** *Anogeissus acuminate*, Analgesic, Tail immersion, and Formalin.

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## INTRODUCTION

Pain is universally understood as a signal of disease and it is the most common symptom that brings a patient to a physician attention, requiring treatment with analgesic agents[1]. 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[2]. The present investigation was to scientifically prove the analgesic potential of *Anogeissus acuminata*. Other species of *Anogeissus* genus have been scientifically proven for analgesic activity. But no such scientific data is available for *Anogeissus accuminata*. Traditionally *Anogeissus accuminata* has been used in the treatment of head ache's menstrual pain, rheumatoid arthritis, tuberculosis, ulcers, etc. so, *Anogeissus accuminata* was selected for the pharmacological evaluation of analgesic activity.

### ***Anogessius accuminate***

*Anogeissus accuminate* Roxburgh in commonly called ass button tree belonging to family combretaceae. Cultivated in south Asia, Arabian peninsula and Africa leaves contain tannin 9.32% and non tannin 9.32%. Its medicinal value has been known for time .It is found in the northwest region, the flowering occurs in April-june and but at other seasons also[3].

## MATERIAL AND METHODS

Present studies have been conducted in the Department of Pharmacology at T.R.R.C.P. The experimental protocol was approved by institutional animal ethics committee (IAEC).

### **Plant Material**

The leaves of *Anogeissus accuminata* were procured from S.V. University, Tirupathi and a specimen voucher was placed in the library. The leaves were washed under running water, shade dried and the dehydrate leaves powdered to a fine texture and 100g of the dried leaves was repeatedly extracted with 95% methanol for 24hrs.

### **Phyto-Chemistry**

The plant contains triterpenoid saponins possessing various major chemical constituents. Other constituents of the plant are water soluble base and alkaloids. The methanol extract of the plant contain flavonoids and tannins.

### **Experimental Animals**

Wistar albino mice and rats of either sex weighing 35-40gms bred in NIN facility were used for the study. The animals were housed under standard laboratory conditions maintained at natural light & dark cycle and had free access to food and water. They were acclimatized to



laboratory conditions before the experiment. Each animal was used once in every experiment and all the experiments were carried out in day light.

### **Acute Toxicity Study**

Extract was given in the dose range 300mg-3000mg/kg p.o and acute toxicity was carried out in albino mice by method of OECD (organization for economic co-operation and development, guideline No. 423).

### **TEST METHODS**

Animals were divided into various groups in such a way that 6 animals were there in each group. Animals treated with 5% Gum acacia suspension (0.1ml P.o) served as control, Group I Pentazocin 10mg/kg bodyweight i.p. Served as standard Group II and animals in test groups III were treated with 300mg/kg p.o of methanolic extract of *Anogeissus accuminata* respectively. Each animal was treated with respective drug 30min before experimentation. Following are the details of experiments performed.

#### **Tail Immersion Test**

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice. The animals of the control, positive control and test group were treated with Pentazocine 10mg/kg body weight, 5% acacia as control 0.1ml, p.o & test samples at the dose of 300mg/kg of *Anogeissus accuminata* respectively [4]. 1-2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 sec was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail flick response was taken as the index of antinociception and was determined before and 0, 30, 60 & 90 min after the administration of drugs.

#### **Formalin Induced Pain**

Pain was induced by injection 0.05ml of 2.5% formalin (40% formaldehyde) in distilled water in subplantar region of right hind paw. Rats were individually placed in transparent Plexiglas's cage observation chamber. The amount of time spent licking and biting the injected paw was indicative of pain and recovered in 0-5 min (first phase) and 15-30 min (second phase)[5].The rats were divided into 3 groups each containing 6 rats and were administered with either 5% acacia (0.1ml, p.o), methanolic leaves extract (300mg/kg) i.p or pentazocin (10mg/kg, s.c) 30 minutes after this treatment 50ml of a freshly prepared 2.5% solution of formalin was injected. S.C under the plantar surface of the left hind paw of the each rat. The rats were placed individually in an observation chamber and monitored for one hour. The severity of pain response was recorded for each rat based.

## Statistical Analysis

Statistical analysis was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The  $P < 0.005$  were considered to be statistically significant.

## RESULTS AND DISCUSSIONS

### Acute Toxicity Study

Acute toxicity study for the methanolic extract of *Anogeissus accuminata* showed LD<sub>50</sub> 3000mg/kg. Therefore for this study, animals were screened for doses 300mg/kg body weight of extract. The analgesic effects of methanolic extract of *Anogeissus accuminata* are shown in the Table 1 and 2, the analgesic activity were expressed in Mean  $\pm$  S.E.M relative to control.

**Table: 1 Effects of the methanolic extract of Anogessius accuminata on Tail immersion test in mice (T.I.T)**

S.No	Treatment	Dose mg/kg	Latency period Mean $\pm$ S.E.M	% Protection
1.	Control	0.1ml	3.33 $\pm$ 0.58	—
2.	Standard	10mg/kg	6.00 $\pm$ 0.58**	80
3.	MEAA	300mg/kg	4.33 $\pm$ 0.44**	55

Significant relative to control reading \* $P < 0.05$ , \*\* $P < 0.01$ , n=6, SEM-Standard error mean

**Table: 2 Effects of the methanolic extract of Anogessius accuminata on Formalin induced pain**

S.No	Treatment	Dose mg/kg	Early phase 0-5 min	Percentage protection	Late phase 15- 30 min	Percentage protection
1.	Control	0.1ml	113.50 $\pm$ 3.75	—	134.3 $\pm$ 5.04	—
2.	Standard	10mg/kg	54.6 $\pm$ 1.06**	52.1	34.60 $\pm$ .32**	72.1
3.	MEAA	300mg/kg	62 $\pm$ 0.18**	45.9	20..20 $\pm$ .93**	62.3

Significant relative to control reading \* $P < 0.05$ , \*\* $P < 0.01$ , n=6, SEM-Standard error mean

## DISCUSSION

The analgesic activity of *Anogeissus accuminata* was investigated in the present study. The mechanism for testing analgesic activity was selected such that both centrally and peripherally mediated effects were investigated. The tail immersion methods elucidated peripheral & central activity respectively, while the formalin test investigated both. The extracts 300mg/kg, administered orally, significantly inhibit the pain in both mice and rats. The result strongly suggests that the mechanism of action of extract may be linked to lipoxygenase and/or cyclooxygenase[5].

In the formalin test there is distinctive biphasic nociceptive response termed neurogenic and inflammatory phases. Drugs that primarily act on central nervous system inhibit



both phases equally while peripherally acting drugs inhibit the late phase [6]. The neurogenic and inflammatory phase is due to the release of substance p, histamine, serotonin, bradykinin, prostaglandins and leukotrienes respectively. This test is useful for not only assessing analgesic drugs but also helping in the elucidation of mode of action. The extracts 300mg/kg were able to block both phases of formalin in the second phase.

Tail immersion model of analgesic assessment is best reserved for evaluating compounds for centrally acting analgesic activity. The extracts 300mg/kg shows best effect after a latency period of 6 hrs which is more than other fractions.

### CONCLUSION

In the present study analgesic activity of leaf of *Anogeissus accuminata* was investigated by means of formalin test, tail immersion model of analgesic assessment. The oral administration of methanolic extracts showed analgesic activity by link to lipoxygenase & cyclooxygenase. The result strongly suggests that the extracts can be used efficiently as analgesic agents

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