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Analgesic, anti-inflammatory & central nervous system (CNS) depressant activity of whole plant of *Allmania longipedunculata* (Trimen) Gamble

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Abstract
In the present study, ethanolic extract of Allmania longipedunculata (Trimen) Gamble, whole plants were evaluated for Analgesic, Anti- inflammatory and Central Nervous System Depressant activity in Swiss albino mice. The analgesic activity of the extract of Allmania longipedunculata (Trimen) Gamble (200mg/kg) was evaluated by using eddy's hot plate and Anti-inflammatory activity by formalin method. Central Nervous System Depressant activity was assessed by Actophotometer method. The extract of whole plant of Allmania longipedunculata (Trimen) Gamble in a dose of 200 mg/kg showed significant Analgesic, Anti- inflammatory and slight Central Nervous System Depressant activity.

Keywords: Analgesic, anti-inflammatory, central nervous system depressant, formalin, eddy's hot plate, Actophotometer, *Allmania longipedunculata* (Trimen) Gamble

1. Introduction
Herbs are still widely used for the treatment of diseases, because of its fewer secondary effects [1]. The medicinal plants are compatible with human body and are an important component of the health care system [2]. Several phytochemical have been isolated from different plant species; these components have received considerable attention due to their therapeutic potential as analgesic, anti-inflammatory, anticancer and antioxidant activities [3]. Like the other medicinal weeds Allmania longipedunculata (Trimen) Gamble is also used for the treatment of varying diseases.
The pain is an unpleasant sensory and emotional experience which is associate with tissue damage. So the pain is perception not a sensation [4]. There are several types of pain, including Neuropathic pain, nociceptive pain and psychogenic pain. Neuropathic pain is due to direct injury on dysfunction of peripheral or central nervous system [5]. Nociceptive pain is caused by stimulation of peripheral nerve fibers that respond only to stimuli approaching or exceeding harmful intensity (nociceptors) [6]. psychogenetic pain is that is mostly related to disturbances and without evidence of a disorder that could account for the pain or its severity [7].
The plant *Allmania longipedunculata* (Trimen) Gamble (amaranthaceae family) is a slender erect herb. The leaves are used traditionally for stomach disorder. This plant is commonly known as peddabrrabadhiaku (telugu), and most common in wet waste fields and cultivated lands [8]. The other varieties of *Allmania* are used for nutritive purpose [9]. *Allmania nodiflora* (L.) R.Br. ex Wight Ripe fruits are used for constipation and dysentery Leaves are used as febrifuge [10]. According to literature on *Allmania longipedunculata* (Trimen) Gamble, no any work has been published. Based on this, an attempt has been made to evaluate these activities on ethanolic extract of whole plant of *Allmania longipedunculata* (Trimen) Gamble.

2. Material and method
The whole plant of *Allmania longipedunculata* (Trimen) Gamble (amaranthaceae) were collected from in and around cultivated and waste lands of Raghu college of pharmacy, Visakhapatnam, were authenticated by botanical survey of India (BSI), Hyderabad, Telangana (state), India. All the experiments were carried out using Swiss albino mice (25-30 gm) male of age 12 weeks. All the experimental procedure and protocols used in this study are reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Raghu College of pharmacy contributed in accordance with guidelines of the CPCSEA, government of India.
All the mice were given a period of acclimatization for 14 days before starting the experiments. Mice were fed pellet diet, and water ad libitam, temperature maintaining at 24± 2° and relative humidity OD 60-70%. These male Swiss albino mice were divided into three different groups each containing six animals, the animals were marked individually.

The chemicals used for these experiments were procured from various sources i.e. formalin (Finar chemical private Ltd. Ahmadabad), CarboxyMethyl Cellulose (Oxford laboratory Mumbai) ethanol (Jiangsu Huaxi International trade Co. Ltd) and drugs like aspirin (Reachem Laboratory Chemicals Pvt. Ltd) and chlorpromazine (Sun Pharmaceutical Industries Ltd. Mumbai) were used during the experimental protocol.

2.1 Extraction and preparation of sample

The whole plant were dried under shade and coarsely powdered and subjected to extraction with ethanol by simple maceration process. The extract of whole plant was concentrated to ¾ th of original volume by using rotary evaporator at 40° C under reduced pressure. The concentrated extract of *Allmania longipedunculata* (Trimen) Gamble was subjected to preliminary chemical test for detection of phytoconstituents; give positive result for steroid, triterpinoid and phenols. The dried extract was suspended in 1% sodium CMC in distilled water and used for pharmacological investigation.

2.2 Acute toxicity

The acute toxicity of *Allmania longipedunculata* (Trimen) Gamble was assessed by using up and down method [11]. After the administration of one single dose of *Allmania longipedunculata* (Trimen) Gamble (5, 50, 300, 2000 mg/kg) the survival of animals was observed during 24 hrs. If an animal survived at any given dose, the dose for the next animal was logarithmically increased; if it died, the dose was decreased.

2.3 Hot plate method

The analgesic activity was measured by hot plate method[11]. Swiss albino mice weighing between 25-30 gm were used and divided in three groups; in each group six albino mice were kept. Group-A received are control i.e. 1% CMC, Group-B received standard (aspirin 10 mg/kg), Group-C treated with ethanolic extract (200 mg/kg) respectively. Suspensions of plant extracts and aspirin were prepared by using Sodium CMC suspension. The animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The test and standard drugs were given orally. After 30 minutes, the animals were placed on the hot plate and the observations were recorded and at the time interval of 0.5, 1, 1.5, 2, 2.5 hours. The result of Hot plate method in mice was tabulated in Table-1.

2.4 Formalin method

The anti-inflammatory activity of the extracts is measured by formalin method [12]. Swiss albino mice weighing between 25-30 gm were used for evaluation of anti-inflammatory activity; in each group six albino mice were kept. Group-A received are control i.e. 1% CMC, Group-B received standard (aspirin 10 mg/kg), Group-C treated with ethanolic extract (200 mg/kg) respectively. Suspensions of plant extracts and aspirin were prepared by using Sodium CMC suspension. Six male Swiss albino mice were taken and marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The test and standard drugs were given orally. After 30 minutes, the animals are injected with 0.1 ml of 1 % formalin on the dorsal surface of the right hind paw and the scores were recorded as formalin test ratings for the time interval of 90 minutes. Pain intensity was rated according to the following numerical scale:

1. Both forepaws are placed on the floor and weight is evenly distributed.
2. The injected paw rests lightly on the floor or on another part of the animal's body and little or no weight is placed upon it.
3. The injected paw is elevated and not in contact with any surface. The uninjected paw is placed firmly on the floor.
4. The injected paw is licked, bitten or shaken, while the uninjected paw is not.

The mouse was observed for 60 min after the formalin injection and the amount of time (sec) spent in each scale (0, 1, 2, and 3) was recorded.

Ratings were averaged over 3 min blocks. Numerical ratings are calculated from the following formula:

$$\text{Pain rating} = \frac{T1 + 2T2 + 3T3}{180}$$

Where T1, T2 and T3 are the durations (in sec) spent in categories 1, 2 or 3, respectively during each 3 min block. The pain score has been designated as Formalin test rating (FTR).

During the study, the basal reaction time has been measured initially (before dose administration) and another set of measure was observed at 30 min after the dose administration for 1h after injecting formalin into the dorsal portion of the right hind paw of the albino mice. These reaction times were then expressed as formalin test scores. The result of formalin method in mice was tabulated in Table-2

2.5 Actophotometer method

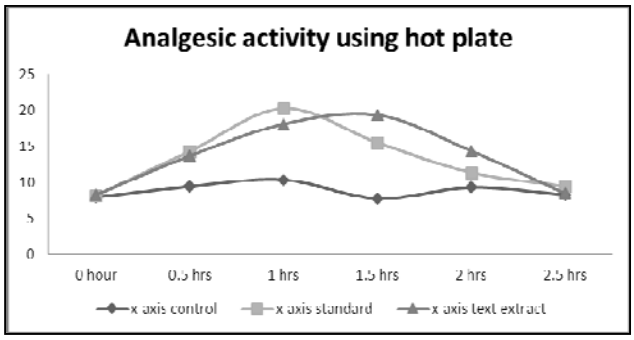
The CNS depressant activity of the extracts is measured by Actophotometer test [13]. Swiss albino mice weighing between 25-30g were used for evaluation of CNS depressant activity in each group six albino mices were kept. Group-A received are control i.e. 1% CMC, Group-B received standard (chlorpromazine 4 mg/kg), Group-C treated with ethanolic extract (200 mg/kg) respectively. A suspension of chlorpromazine was prepared in normal distilled water with 1% sodium CMC. Suspensions of plant extracts were prepared by using Sodium CMC suspension. Male Swiss albino mice were divided into three different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 30 minutes, the animals are placed in to the Actophotometer for 10 mins and the observations/counts were recorded and at the time interval of 0.5, 1, 1.5, 2, 2.5 hours. The result of depressant activity in mice was tabulated in Table-3.

2.6 Statistical analysis

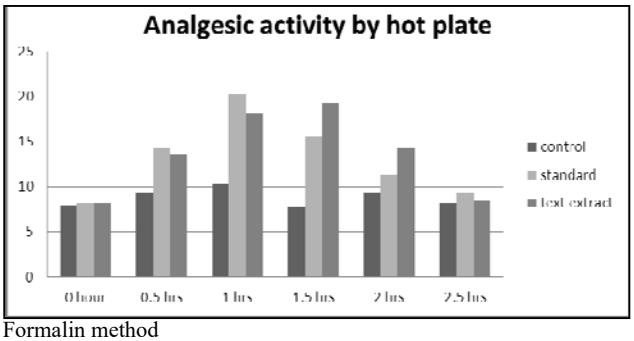
The result were expressed as the mean ± SEM and subjected to one way analysis of variance (ANOVA) followed by Dunnet's test for comparison between groups. In all the cases $P < 0.05$ was considered as statistically significant. Data were computed for statistical analysis by using graph PAD prism.

Table 1: Analgesic Activity of *Allmania Longipedunculata* (Trimen) Gamble by Using Hot Plate Method:

		Reaction time in seconds at different hrs.					
group treatment	**dose (mg/kg)**	**0 hour**	**0.5 hrs**	**1 hrs**	**1.5 hrs**	**2 hrs**	**2.5 hrs**
Control	0	7.91±0.2386	9.41±0.1394	10.33±0.0833	7.75±0.255	9.28±0.0674	8.26±0.066
Standard	10	8.16±0.1666	14.30±0.0916	20.25±0.0912	15.51±0.1434	11.34±0.0506	9.43±0.044
test extract	200	8.25±0.1707	13.66±0.2297	18070±0.1842	19.35±0.0523	14.35±0.0707	8.45±0.0316



Graph 1

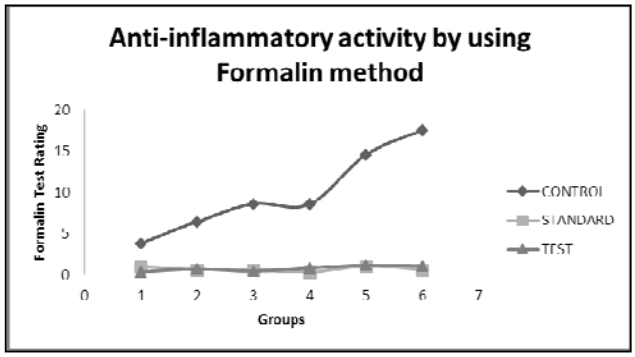


Formalin method

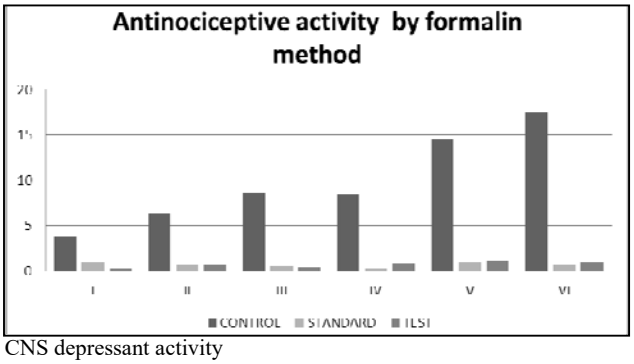
Graph 2

Table 2: Anti-Inflammatory Activity of *Allmania Longipedunculata* (Trimen) Gamble by Formalin Method.

Groups	**I**	**II**	**III**	**IV**	**V**	**VI**
Control	3.816±0.0103	6.4145±0.017	8.5725±0.0085	8.5251±0.0060	14.5295±0.0079	17.5158±0.0033
Standard	0.9371±0.0043	0.621±0.0085	0.58±0.0088	0.3275±0.0098	1.019±0.0008	0.6371±0.0089
Test	0.355±0.0126	0.647±0.0045	0.4291±0.0037	0.782±0.0037	1.135±0.0362	1.038±0.0142



Graph 3

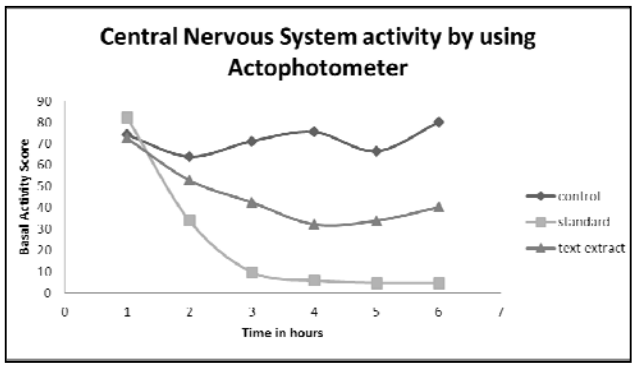


CNS depressant activity

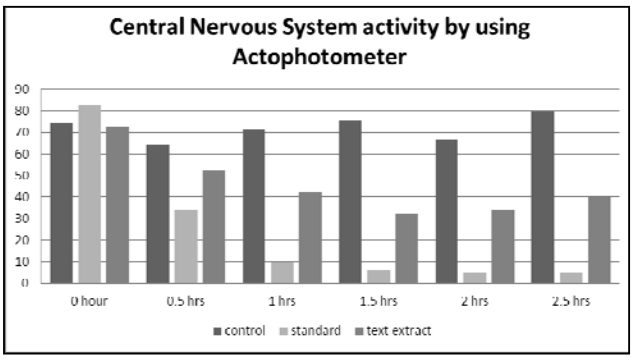
Graph: 4

Table 3: Central Nervous System Depressant Activity of *Allmania Longipedunculata* (Trimen) Gamble by Using Actophotometer

		Reaction time in seconds at different hrs.					
group treatment	**dose (mg/kg)**	**0 hour**	**0.5 hrs**	**1 hrs**	**1.5 hrs**	**2 hrs**	**2.5 hrs**
Control	0	74.166±0.6009	64±0.3651	71.166±0.3073	75.5±0.5627	66.5±0.4281	80±0.573
standard	4	82.33±0.4216	34±0.3651	9.8333±0.4772	5.833±0.3073	4.833±0.3073	4.8333±0.3073
test extract	200	72.5±0.3415	52.833±0.4772	42.51±0.4281	32.166±0.5426	33.833±0.4772	40.1666±0.6009



Graph 5



Graph 6

3. Result and discussion

The ethanol extract of whole plant of *Allmania longipedunculata* (Trimen) Gamble in a dose of 205 mg/kg showed significant analgesic activity by using hot plate method at 1.5 hrs post medication (table 1, Graph 1 and 2) anti-inflammatory activity by formalin method *Allmania longipedunculata* (Trimen) Gamble showed significant activity as that of standard (table 2, Graph 3 and 4). The extract also showed less central nervous system depressant activity (table 3, Graph 5 and 6).

In the present study the analgesic and anti-inflammatory activity of ethanolic extract of *Allmania longipedunculata* (Trimen) Gamble whole plant evaluated in different experimental model of pain like hot plate and formalin induced pain model. The results of present study clearly demonstrated that ethanolic extract of plant possessed a definite dose dependant analgesic and anti-inflammatory activity as observe by significant increase in the reaction time formalin induced pain method and hot plate method when compared to control group. The formalin induced pain test does not required sophisticated equipment and is easy and effective method, in which response of the animal, to moderate continuous pain, in this test we can observe the spontaneous nociception behaviors following injection of formalin to the right hind paw for the licking, biting or shaking, while the uninjected paw is not.

Pain produced by formalin is due to activation of TRPA1 receptors (Transient receptor potential cation channel) located on plasma membrane. The activation of receptors leads to opening of the nonselective cation channels and increasing the intracellular calcium concentration and mediating the release of neuropeptides such as substance P and calcitonin gene related peptides (CGRP). Substance P is responsible for the stimulation of mast cells to release inflammatory mediators such as histamine. CGRP relaxes arteries and also also mediates an increase cutaneous blood flow. These inflammatory process lead to pain and itch [14].

In this test, our result indicate that analgesic and anti-inflammatory property of ethanolic extract of *Allmania longipedunculata* (Trimen) Gamble is because of blocking substance P.

The plant extract of *Allmania longipedunculata* (Trimen) Gamble also possess slight CNS depressant activity, the affect is due to interaction with benzodiazepines- like compounds. The extract might have acted by potentiating GABA ergic inhibition in the CNS by membrane hyper polarization which diminish the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptors by the extract [15].

According to preliminary studies the plant is having steroids may be they form ligand with $GABA_A$ receptors in the CNS, which led to the postulation that they act as benzodiazepines [16]; hence the extract showed the CNS depressant activity.

In conclusion, this study has shown that ethanolic extract of *Allmania longipedunculata* (Trimen) Gamble has significant analgesic and anti-inflammatory activity and slight CNS depressant activity. However, further research is needed to determine the mechanism involved as well as specific chemical constituents responsible for the pharmacological action.

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