



SCREENING OF CENTRAL ANALGESIC ACTIVITY OF CALOTROPIS GIGANTEA FLOWER USING RATS

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1. Introduction

Analgesics are a class of drugs used to relieve pain. The pain relief induced by analgesics occurs either by blocking pain signals pathway to the brain or by interfering with the brain's interpretation of the signals, without producing anesthesia or loss of consciousness. A substance that, when swallowed, inhaled, or injected, induces stupor, sleep, and insensibility. Narcotic analgesics induce a state of reversible depression on the central nervous system and are administered to relieve pain. However, the use of these drugs carries a high risk of psychological and physiological dependence, and side-effects include dose-related respiratory depression. There are two types of narcotic analgesics such as opiates and opioids. Opiates drugs derived from opium; the dried juice of the Oriental poppy seed. The pharmacologically active substances, which constitute approximately 25% of the extract, are the alkaloids morphine, codeine, and papaverine. Opioids are the derivatives of opiates and has a morphine-like action in the body. The main use is for pain relief. These agents work by binding to opioid receptors, which are found principally in the central nervous system and the gastrointestinal tract. Non-narcotic analgesics, which relieves pain, but does not have narcotic effects such as acetaminophen and aspirin.

The μ -receptors are thought to be responsible for most of the analgesic effects of opioids, and for some major unwanted effects like respiratory depression, euphoria, sedation and dependence. Most of the analgesic opioids are μ -receptor agonists. The δ -receptors are probably more important in the periphery but may also contribute to analgesia. The κ -receptors contribute to analgesia at the spinal level and may elicit sedation and dysphoria; they produce relatively very few unwanted effects and do not contribute to dependence. Some analgesics are relatively κ -selective. The σ -receptors are not true opioid receptor but are the site of action of certain psychotomimetic drugs, to which some opioids also bind. All opioid receptors are linked through G-Proteins to inhibition of adenylate cyclase. They also facilitate opening of potassium channels causing hyperpolarization and inhibit opening of calcium channels which inhibiting transmitter release. These membrane effects are not linked to the decrease in cAMP formation.

Pentazocine is a mixed agonist-antagonist. In low dose, its potency and effects are similar to morphine, but increasing the dose does not cause a corresponding increase in the effects produced. Therefore, at high dose pentazocine causes only slight respiratory depression and causes marked dysphoria, with nightmares and hallucination, rather than euphoria. Binding studies show that it has a higher affinity for κ -receptor than μ -receptors, and also acts on non-opioid σ -receptors, this spectrum being somewhat different from that of conventional opioid drugs. Pentazocin is effective in reducing acute and chronic pain. Apart from raising pain threshold, it reduces the emotional reaction to pain. This eliminates the distress caused by pain and helps the patients to tolerate pain better. The analgesic action of Pentazocin is primarily due to its effect on indigenous opioid receptors in supraspinal pain processing sites and on midbrain and brain stem (nucleus raphe magnus) areas. Inhibitory impulse from these areas to the dorsal

horn constitute the 'gating' system. In addition, pentazocin also acts directly on the dorsal horn, where it inhibits the release of substance. The emotional component of Pentazocin action is due to its effect on limbic system and sensory cortex *Calotropis gigantea*

2. Materials and Methods

Plant material

The flowers of the plant *Calotropis gigantea* (Family: Asclepiadaceae) (as shown in Fig. 1) were collected from Thanjavur district of Tamil Nadu, India. The plant material was taxonomically identified by division of Botany, Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University. A voucher specimen has been preserved in laboratory for future reference. The flower collected was washed with distilled water to remove impurities. The fresh flower was extracted with methanol in a Soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained (Methanolic *Calotropis Gigantea*-, MCG yield 12.4%). The MCG at the different doses of 200 and 400 mg/kg was suspended in 1% acacia and pentozocin at the dose of 6 mg/kg in saline were used for pharmacological studies. The dried extract was preserved in dessicator for future use.

Chemicals and reagents

Pentozocin (Sigma, UK) were used as the standard drug for analgesic activity. Acacia (S. D. Fine Chemicals Limited, Bombay) was used as suspending agent.

Selection of animal's species:

Healthy young adult male Albino Wistar rats (150-200 gm) were kept separately in individual polypropylene cages (38 cm \times 23 cm \times 10 cm) with stainless steel hopper. At the commencement of the study, the weight variations of the animals were minimal and not exceed \pm 20 % of the mean weight. The test animals should be characterized by species, strain, source, sex, weight and/or age.

Housing and feeding conditions

The temperature in the experimental animal room was $22 \pm 3^\circ\text{C}$. Although the relative humidity was 30 % and preferably not exceeding 70 % other than during room cleaning, the aim was 50-60 %. Lighting used artificially, the sequence being 12 hours light and 12 hours dark. The animals were housed individually. For feeding, conventional laboratory diets was used with an unlimited supply of drinking water.

Preparation of animals

The animals were uniquely identified and kept in their cages for five days prior to dosing for acclimatized to the laboratory conditions. They were kept in animal house, Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tamil Nadu, India. During acclimatization the animals were observed for ill health. Animals demonstrating signs of spontaneous disease or abnormality prior to the start of the study were eliminated from the study.

Administration of doses

The test substance was administered in a single dose by oral gavage and the standard drug (Pentazocin) was administered through intraperitoneal route. The animals were fasted prior to dosing by withholding food

overnight. Fasted body weight of rats was determined and the dose was calculated according to their body weight. Animals were divided into 4 groups having 5 animals per each group (Table 1)

Groups	No. of animals	Treatment	Drug &Dose (mg/kg, b.wt, p.o)	Sources of pain stimuli
I	5	Normal	Vehicle	Heat
II	5	STD	Pentazocin (6 mg/kg, i.p)	Heat
III	5	Test I	MCG (200 mg/kg, po)	Heat
IV	5	Test II	MCG (400 mg/kg, po)	Heat

STD - Standard

MCG – Methanolic extract of *Calotropis gigantea*

Experimental Method

The screening of central analgesic activity can be performed by using Eddy's hot plate apparatus (Turner 1965) as shown in Plate-2. This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesic. The validity of this test has been shown even in the presence of substantial impairment of motor performance (Plummer et al., 1998). The animals were placed gently on a plate maintained at $55 \pm 1^\circ\text{C}$. Reaction time was taken as the interval between the instant the animal reaches the hot plate till the moment the animal licks its forepaws or jumps out (as shown in Plate 3). Measurements were carried out 5 min before and 30, 60 and 120 minutes after oral administration of Methanolic extract of *Calotropis gigantea* flower extract suspension (200 and 400 mg/kg body weight). The control group was given 1% acacia orally while the standard reference group was treated with Pentazocin (6 mg/kg body weight) intraperitoneally.

Statistical analysis

The values of each experimental group were expressed as mean \pm SEM and compared with the control group followed by student 't' test.

3. Results and Discussion

Analgesic effect by Eddy's Hot plate test

The Methanolic extract of *Calotropis gigantea* (MCG) flower (200 & 400 mg/kg, po) was evaluated for central analgesic activity in acute experimental animal models and the result is tabulated in table 1. The Methanol extract of *Calotropis gigantea* (MCG) flower (200 & 400 mg/kg) pretreatment increased the response latency in the hot plate test. This, however, was not statistically significant. The centrally acting analgesic pentazocine also increased the response latencies at various time points

Table 1: Study protocol for Analgesic Activity

Groups	No. of animals	Treatment	Drug &Dose (mg/kg, b.wt, p.o)	Sources of pain stimuli
I	5	Normal	Vehicle	Heat
II	5	STD	Pentazocin (6 mg/kg, i.p)	Heat
III	5	Test I	MCG (200 mg/kg, po)	Heat
IV	5	Test II	MCG (400 mg/kg, po)	Heat

STD- Standard drug

MCG – Methanolic extract of *Calotropis gigantea*

Table 2: Analgesic activity of Methanolic extract of *Calotropis gigantea* (MCG) flower on Hot plate method

Treatment	Dose (mg/kg)	Route of administration	Basal reaction time (sec)	Reaction time (sec)		
				30 min	60 min	120 min
Control	-	Oral	2.76 \pm 0.17	2.68 \pm 0.35	2.80 \pm 0.24	2.69 \pm 0.16
Standard (Pentazocin)	6	Intraperitoneal	2.46 \pm 0.56	3.36 \pm 0.64	5.24 \pm 0.36	7.18 \pm 0.52
Test I (MCG)	200	Oral	3.02 \pm 0.32	3.10 \pm 0.48	3.89 \pm 0.54	5.28 \pm 0.18
Test II (MCG)	400	Oral	2.91 \pm 0.15	3.34 \pm 0.21	4.21 \pm 0.15	6.01 \pm 0.43

Values are mean \pm SEM (n = 5).

Experimental groups were compared with control $p < 0.001$



Fig 1: Calotropis Gigantea Flowers



Fig: 2, Eddy's hot plate apparatus



Fig: 3, Rat showing jumping response due to thermal stimuli

The central analgesic effect of the Methanolic extract of *Calotropis gigantea* (MCG) flower was investigated. The analgesic test used in the present study was chosen in order to test nociceptive stimuli (cutaneous thermic stimuli). MCG produced mild anti-nociceptive activity against thermal induced pain stimuli in rats at various time points post treatment when compared with pentazocin treated rats. The effect observed was, however, very mild and not statistically significant. The hot plate test is considered to be selective for opioid-like compounds, which are centrally acting analgesics in several animal species (Janssen et. al. 1963). This could be the possible explanation for its mild central analgesic activity observed in hot plate test. The present study findings indicate the MCG possess mild central analgesic activity.

4. Conclusion

Methanolic extract of *Calotropis gigantea* (MCG) flower possess mild antinociceptive activity in mice when compared with standard. Further studies have to be done for the screening for peripheral analgesic activity in animals.

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