



Original Research Article

Screening for anti-inflammatory and peripheral analgesic activity of Coleus amboinicus leaves using wistar albino rats

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ABSTRACT

Context: We have abundant plant resources with many health benefits. Use of plant material for health benefits will help in reduce the cost of drugs and also provide a substitute drug that is less expensive. Coleus amboinicus is plant largely distributed in Dakshina kannada and is used for its medicinal properties as household cure.

Aim: We planned to find out the anti-inflammatory and peripheral analgesic activity of Aqueous Extract of leaves of Coleus amboinicus.

Settings and Design: Wistar albino rats were randomly selected and tests were performed by dividing the animals into three groups- control, standard, test.

Materials and Methods: Aqueous extract of leaves of Coleus amboinicus was prepared using soxhlet apparatus. The dose of leaf extract used was 500mg/kg.

Statistical Analysis: Data was analysed by applying one way ANOVA followed by Dunnett's multiple comparison test using Graphpad Instat.

Results: The results obtained were statistically significant. In Carragenan induced inflammation model significant inhibition of inflammation was shown by the test drug at the end of 3 hrs and in Acetic acid induced writhing test significant inhibition of occurrence of writhes was noted by the test drug.

Conclusion: This study validates the folkloric use of aqueous extract of leaves of Coleus amboinicus (AECA) at the dose of 500mg/kg for its anti-inflammatory and peripheral analgesic activity.

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1. Introduction

Majority of Indians use traditional medicine for their common health needs. In recent years, there has been a prominent rise in the global use of herbal medicine. The increase in use of herbal medicines can probably be credited to their natural source of origin, faith for these medicines because of their long history of use, cost effectiveness and most importantly minimal side effects.¹ Active principles of the plant sources when used as medicine for variety of illnesses are often viewed to replace the pharmacological efficacy of allopathic drugs.¹

As per estimate made by WHO regarding the use of herbal medicines, about 80% of world population primarily in the developing countries use Herbal medicines for primary health care. Over 50% of the modern clinical drugs are of plant origin.² However, the main concern of clinicians is endorsement, validation of efficacy and safety standardisation of an ethno-medicine. Keeping this fact in mind screening herbal drugs for their potential use would be appreciable as they are being used since decades but have no authentic scientific data. If a proper scientific data is established on the clinical use of herbal drug, it would help clinicians prescribe herbal drugs confidently.

Coleus amboinicus is an aromatic perennial herb with thick juicy leaves that has been used for cooking purposes and in folk medicine for ailments like abdominal pain,

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duodenitis, diarrhoea due to cholera,³ malarial fever, epilepsy, renal and bladder stones.⁴ The Previous studies have explored following potential properties of this herb i.e anticlastogenic and radioprotective,⁵ antioxidant,^{5,6} Mast cell stabilization property,⁷ Antimicrobial and anthelmintic,⁸ Anti Urolithiatic and anti hyperlipidemic activity^{9,10} and Hepatoprotective.¹¹

We aimed to explore the aqueous extract of *Coleus amboinicus* for its anti-inflammatory property using carrageenan induced paw edema and peripheral analgesic property using acetic acid induced pain model in wistar albino rats.

2. Materials and Methods

The study was performed in the ethnopharmacology lab, Department of Pharmacology, Yenepoya Medical College, Mangaluru after obtaining permission from the Institutional Animal Ethics Committee (IAEC).

The plant was cultivated in Dakshina Kannada district and was authenticated by plant taxonomist. The aqueous extract of leaves of *Coleus amboinicus* (AECA) was prepared by using the below mentioned method. At first the green leaves were collected. It was cleaned and shade dried. The dried leaves were powdered and extracted using distilled water (1000ml) as solvent in Soxhlet apparatus. It was extracted till a clear fluid was noticed. The yield obtained was 23.7% w/w.

In order to fix the dose, previous articles on the toxicity studies done on the leaf extract of this plant, following the OECD guidelines was reviewed. The LD₅₀ level determined was more than 5000mg/kg.^{10,12} Hence we fixed to the 1/10th of the above dose i.e 500mg/kg after the pilot study. The required amounts of test drug were dissolved in distilled water and were administered to the animals orally according to the body weight of animal, for ten days.

3. Experimental animals

Wistar albino rats of 6-8 months of age and weighing 150-200g of either sex were used for the study. The rats were kept under standard housing conditions as per CPCSEA guidelines with free access to standard pellet diet and water. The experiment was performed following CPCSEA guidelines. Wistar albino rats were randomly allocated to different groups. The animals were acclimatized to the laboratory conditions for one week prior to the experiment. The animals were grouped into 3 groups as below:

Groups	Drug/ dose/ route of administration
I – Control (n-6)	Normal saline -10ml/kg, oral
II – Standard (n-6)	Indomethacin-10mg/kg ¹³ , oral
III- Test (n-6)	AECA -500mg/kg, oral

On the 10th day at the same time of the day, 30 min after the oral administration of drugs experiments were carried out on all the 3 groups.

3.1. Anti-inflammatory study: Carrageenan induced paw edema in rats:^{13,14}

1% solution of carrageenan was prepared and 0.1ml of this carrageenan solution was injected on the plantar surface of the left hind paw of the rats. The left paw was marked with ink at the level of lateral malleolus. The paw till the marking made was immersed in the water column of digital plethysmometer for measuring paw volume. The paw volume was measured immediately after carrageenan injection and then at 3hrs. The peak effect of the carrageenan usually occurs at the 3 hr after the injection.

The increase in paw volume at 3 hr was calculated and compared with the control group. The percent inhibition was calculated as:

$$\% \text{ inhibition} = (V_t - V_c) \times 100 / V_c$$

Where, V_c= paw volume of the control group

V_t= paw volume of the treated group.

3.2. Peripheral analgesic activity: Acetic acid induced writhing reflex¹⁴⁻¹⁶

0.1 ml of 0.6% acetic acid was injected intraperitoneally to each rat. Animals were observed for twisting, elongation of body and arching of the back (writhing). The number of writhes in 10 minutes time duration was recorded. The reading was compared between the three groups - control, test and standard group. The percentage inhibition of writhing was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Average writhes in the control} - \text{average writhes in the treated group}}{\text{Average writhes in the control}} \times 100$$

3.3. Statistical analysis

Data was compiled and analyzed using the statistical software, GraphPad InStat. The Results are represented as Mean of the readings \pm SEM (standard error of mean). The statistical significance between means of the groups were analysed using one way analysis of variance (ANOVA)¹⁷ followed by Dunnett's test. Values of p< 0.05 were considered as statistically significant and p<0.01 were considered statistically highly significant.

4. Results

4.1. Anti inflammatory study

In carrageenan induced inflammation model, when compared with the Control group (normal saline), AECA and indomethacin treated group showed statistically significant reduction (p < 0.01) in paw edema at the end of 3 hours.

Table 1: Showing effect of AECA (500mg/kg) on rat paw volume (ml) following inflammation induced by carrageenan (**p<0.01-very significant)

Group	Baseline paw volume (ml)	Paw volume At the end of 3 hrs (ml)	% inhibition
Control	0.610±0.06	1.255±0.039	
Test	0.820±0.03**	0.410±0.03**	36.43%
Standard	0.592±0.06**	0.265±0.02**	49.22%

Table 2: Showing effect of AECA (500mg/kg) on acetic acid induced writhing model following writhing induced by acetic acid (**p<0.01-very significant)

Drugs	Number of writhes in 10 minutes	Percent inhibition
Normal saline	15.66±0.66	
Standard drug	4.08±0.28**	73.89±1.42
Test (CA)	6.66±0.30**	56.55±2.80

4.2. Peripheral analgesic activity

In Acetic acid induced writhing model, AECA and standard drug group showed statistically significant reduction in the number of writhes as when compared with the control group.

5. Discussion

Natural products used in traditional herbal medicines can be important source for search of novel compounds. However, there is insufficient evidence at present to recommend the use of traditional complementary medicines. With this in mind and to support the already existing studies evaluation of anti-inflammatory and peripheral analgesic activity of AECA at the dose of 500mg/kg was conducted using wistar albino rats.

5.1. Anti-inflammatory activity

In Carrageenan induced paw edema model used for anti-inflammatory screening the test group showed significant reduction in inflammation evidenced by the decrease in paw volume at the end of 3 hrs, when compared to control animals (Table 1). The percentage inhibition of paw edema by aqueous extract of *Coleus amboinicus* was 36.4% and by indomethacin was 49.2% which is statistically significant. Carrageenan induced paw edema is believed to be biphasic inflammatory response model.¹⁸ The first phase (<2 hour) of inflammation is attributed to the release of histamine and serotonin. The second phase is due to the release of bradykinin, protease, prostaglandins and lysosomes.¹⁹ Since, in this study AECA is found effective in second phase of inflammation (≥ 3 hrs), it is probably having inhibitory effect on mediators like bradykinin, protease, prostaglandins and lysosomes. The effect on the first phase is not evaluated.

5.2. Acetic acid induced writhing model

This model was used to evaluate the peripheral analgesic activity of *Coleus amboinicus* leaves. In this model

intraperitoneal injection of acetic acid causes increase of chemical mediators of pain like PGE₂ and PGF_{2 α} , serotonin, histamine in the peritoneal fluid which causes writhing.⁵ *Coleus amboinicus* shows 56.5% inhibition of writhes compared to control group which is statistically significant. Indomethacin shows 73.8% inhibition of writhes. This method is associated with direct stimulation of nociceptive afferent fibers due to pH reduction¹⁹ and generation of prostanoids (PGE₂ and PGF_{2 α}) in peritoneal fluids as well as lipoxygenase products.²⁰ The possible mechanism may be the inhibition of generation of prostanoids (PGE₂ and PGF_{2 α}) and lipoxygenase products in peritoneal fluids.

Therefore aqueous extract of *Coleus amboinicus* leaves shows significant inhibition of peripheral component of pain.

Preliminary phytochemical evaluation done in previous study showed the presence of alkaloids, tannins, terpenoids, phytosterols, flavonoids, carbohydrates and protein.²¹ Alkaloids, Flavonoids and tannins have antioxidant and anti-inflammatory properties. The above described activity of this plant can be attributed to the presence of these components.²²

The mechanism of action for its anti-inflammatory activity may be probably due to inhibition of the late phase of inflammatory mediators and analgesic action due to inhibition of Prostaglandins (PGE₂ and PGF_{2 α}) and lipoxygenase. Inflammation is always associated with pain and reduction of inflammation helps in reducing pain. The secondary metabolites present in the plant responsible for the above activities needs to be studied in detail and in depth.

Hence the traditional use of this compound as anti-inflammatory and analgesic was validated with the results from this study, and we aim to perform studies to affirm the exact active principle and mechanism of action of the above mentioned activity.

6. Source of Funding

None.

7. Conflict of Interest

None.

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