

Evaluation of Analgesic and Antipyretic activity of ethanolic leaf extract of *Catharanthus roseus* (Nayantara) in experimental animals

ABSTRACT

Catharanthus roseus has traditionally been used for relieving pain and inflammation. Few studies have been done to scientifically evaluate its analgesic and anti-pyretic activity. Hence our aim was to evaluate the analgesic and anti-pyretic activity of leaves of *Catharanthus roseus* in experimental animals.

Objective: To evaluate the analgesic and antipyretic activity of *Catharanthus roseus* leaf extract in animal models.

Study design: Experimental study of analgesic and antipyretic activity in animal models.

Place and Duration of Study: Department of Pharmacology, Assam Medical College & Hospital, Dibrugarh, Assam, India.

Methodology: We prepared ethanolic extract of the powdered leaves of *Catharanthus roseus* (CREE). 30 healthy albino mice (20-35 g) of either sex were assigned to five groups of six animals each and administered the vehicle or drug as follows - Group I or normal control (gum acacia 10 ml/kg), Group II (CREE 100 mg/kg), Group III (CREE 250 mg/kg), Group IV (CREE 500 MG/KG) and Group V (Aspirin 100mg/kg). We assessed analgesic activity by writhing following 0.6 % glacial acetic acid i.p. injection and recording reaction time in Eddy's Hot Plate method. Thereafter, five groups of six Wistar albino rats each were treated with the above doses in Groups I-IV and Paracetamol 50 mg/kg in Group V following s.c. injection of 20% aqueous suspension of dried yeast at 20 ml/kg to induce fever. Rectal temperature (Celsius) for anti-pyretic activity was recorded. Quantitative variables were expressed as Mean \pm SD and one way ANOVA followed by Tukey's multiple comparison test were used for statistical analysis with $P < 0.05$ at 95% confidence level.

Results: Writhing response was significantly decreased ($P < 0.05$) in test groups compared to control with dose dependent effect. Reaction time of mice to thermal pain stimulus in test groups was significantly increased ($P < 0.05$) over time in hot plate method. Significant temperature reduction was not observed in test groups compared to control. ($P > 0.05$)

Conclusion: Ethanolic extract of leaves of *Catharanthus roseus* possesses significant analgesic but not anti-pyretic activity.

Keywords: [*Catharanthus roseus*, Nayantara, Analgesic, Antipyretic, Wistar Rats, Swiss Mice]

1. INTRODUCTION

Catharanthus roseus (L.) G. Don. also known as *Vinca rosea* or *Lochnera rosea* is commonly known as Madagascar Periwinkle, belonging to the family *Apocyanaceae*.^[1] This perennial flowering plant with dark green and glossy leaves is native to the island of Madagascar in the Indian ocean, but is commercially grown in Spain, United States, China, Africa, Australia, India and Southern Europe. Its folk names in India are Sadaabahaar, Nayantara, Baramassi, Ainskati, Ushamanjairi. It finds mention in complementary medicine literature as a traditional therapy with anti-diabetic, hypolipidemic, anti-inflammatory,

analgesic, antimicrobial, anthelmintic, antioxidant, nootropic, antispasmodic, digestive, emetic, anti-ulcer and wound healing properties to name a few. Its alkaloids Vincristine, Vinblastine, Vindesine, Vinorelbine are potent anticancer drugs in modern Medicine as well.^[1-18]

Pain, inflammation and fever are commonly managed with the help of steroidal or non-steroidal anti-inflammatory drugs. Both these classes of drugs are notorious for their adverse effects, especially when used over prolonged periods for chronic inflammatory diseases. Moreover, centrally acting analgesics used for severe pain have their own list of adverse effects including addiction liability. Hence, herbal alternatives with analgesic, anti-inflammatory and anti-pyretic properties may provide a safer therapeutic solution. Few studies have scientifically evaluated these properties of *Catharanthus roseus*. It has been reported that Vinca drug components accumulate exclusively in its leaf exudates.^[3] So we planned this study to evaluate the analgesic and anti-pyretic potential of the leaves of the plant.

Aims and Objectives:

- i. To evaluate the analgesic activity of ethanolic extract of the leaves of *Catharanthus roseus* in swiss albino mice
- ii. To evaluate the anti-pyretic activity of ethanolic extract of the leaves of *Catharanthus roseus* in Wistar albino rats

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Experimental animals: After getting permission by the Institutional animal ethics committee (Registration No. 634/02/a/CPCSEA), we obtained 30 healthy adult Swiss albino mice (20-35 g) and 30 Wistar albino rats (150-250 g) of either sex from the central animal house of our institute. They were acclimatized for a period of seven days before starting the experiments. Animals were maintained as per the principles of laboratory animal care prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).^[19] They were administered respective diets and water *ad libitum* on a 12 hour light / dark cycle in a temperature regulated room (20-25°C) during the experimental procedures.

2.2 Drugs and chemicals:

2.2.1 Plant material and preparation of *Catharanthus roseus* ethanolic extract (CREE):

Catharanthus roseus plant and its leaves were identified by Ms. Belinda Lahon, PhD in Botany from North Bengal University. One kg of the fresh leaves of *Catharanthus roseus* were collected locally and washed thoroughly with cold water. They were then air dried in shade at room temperature. The dried leaves were then crushed to a fine powder. We packed 100 g of this air-dried powder of the leaves in a percolator and used 70% ethanol as the solvent for extraction as per standard methods.^[20]

Aspirin was obtained from Zydus Cadila pharmaceutical Co. and Paracetamol from JB Chemicals and Pharmaceuticals Ltd. (Mumbai, India) Gum acacia was obtained from Neelkanth Finechem Co. All drugs and chemicals were of pharmaceutical/analytical grade.

2.3 Grouping and treatment schedule of animals for analgesic activity:

The mice were randomly allotted to five groups of six animals each. They were administered single dose of the following drugs by intra-peritoneal route:

Gr I: Control, 3% w/v gum acacia 10ml/kg

Gr II: CREE, 100 mg/kg

Gr III: CREE 250 mg/kg

Gr IV: CREE 500 mg/kg

Gr V: Standard, Aspirin 100mg/kg

Doses were selected based on acute toxicity study of the ethanolic leaf extract which produced median lethal dose at 4500 mg/kg to no effect even at 5000 mg/kg.^[21,22]

The following models were then used for evaluation of the analgesic activity:

2.3.1 Chemical pain (Writhing method):

Acetic acid-induced writhing test was performed as per the method of Koster R, *et al.*^[23] Here, 0.6% glacial acetic acid, 10 ml/kg body weight was administered intra-peritoneally to the different groups to induce abdominal contractions (writhes) by chemical irritation. Writhes consisted of abdominal muscle contraction, stretching of the hind limbs and trunk twisting of the body. We counted the number of writhes for each group of mice starting from 5 minutes after the injection of acetic acid up to 20 minutes and expressed it as per cent protection.

Thereafter, drugs were administered to the different groups and after some time, writhes were counted. The percentage protection of extract and standard against acetic acid was calculated using the following formula:

$$\% \text{ Protection} = N_c - N_t / N_c \times 100$$

Where N_c is number of writhes in control, and N_t is the number of writhes in test group animals

2.3.2 Eddy's hot plate method:

The hot plate test first described by Eddy and Leimbach (1953) is a screening model which is used for estimating the analgesic activity of drugs/substances. It is based on the principle that when rodents are placed on a hot surface, they initially demonstrate aversion to the thermal pain stimulus by licking their paws and through attempts to escape the environment by jumping. Substances that alter pain threshold either increase the latency to licking/jumping (analgesic effect) or decrease it (hyperalgesic effect). The temperature of the hot plate was maintained at 55°C for our experiment. Paw-licking and jumping are the two parameters measured in this test. The rats were removed as soon as they demonstrated the pain response and the time to elicit this response was recorded as the reaction time. A cut off time of 30 seconds was fixed to protect the rats from serious thermal injury.^[24,25]

2.4 Grouping and treatment schedule of animals (Wistar albino rats) for anti-pyretic activity:

The rats were randomly allotted to five groups of six animals each. The normal body temperature of each rat was measured group-wise with a digital thermometer rectally and recorded.

The following model was then used for evaluation of the anti-pyretic activity:

2.4.1 Baker's Yeast-induced fever in Rats:

Sub-cutaneous administration (in the back just below the nape of the neck) of 20% aqueous suspension of 20 ml of dried baker's yeast in Wistar rats induced fever in the animals, as per the method described in Vogel, *et al.*^[25] The site below the nape of the neck was massaged to spread the yeast suspension beneath the skin.

After 18 hours, the rise in rectal temperatures of the rats was recorded by digital thermometer. We verified that all the animals had a body temperature of ≥ 100.40 degrees Celsius and then included them in the test.

Thereafter, we administered single dose of the following drugs by oral gavage to the rats as follows:

Gr I: Control, 3% w/v gum acacia 10ml/kg

Gr II: CREE, 100 mg/kg

Gr III: CREE 250 mg/kg

Gr IV: CREE 500 mg/kg

Gr V: Standard, Paracetamol 50mg/kg

Rectal temperatures were recorded again at 60, 120 and 180 min post dosing.

2.5 Statistical analysis:

GraphPad QuickCalcs statistical software was used for data analysis. Numerical values were expressed as Mean \pm SD. Differences between the groups were analysed using one-way ANOVA followed by Tukey's Multiple comparison test taking $P < 0.05$ as statistically significant at 95% confidence level.

3. RESULTS

Results are shown in the following tables:

TABLES

Table [1]: Shows number of writhing movements and % protection conferred by test (CREE) and standard drug in 0.6% Glacial acetic induced writhing in different groups (CREE = *Cathanthus roseus* ethanolic extract)

Group	Drugs and doses	Number of writhing movements	% protection of drugs
Group I	3% gum acacia w/v 10 ml/kg	30	0
Group II	CREE 100 mg/kg	25	16.66
Group III	CREE 250 mg/kg	21	30
Group IV	CREE 500 mg/kg	17	43.33
Group V	Aspirin 100 mg/kg	18	40

Table [2]: Shows Mean \pm SD of writhing movements in Glacial acetic induced writhing method in different groups. Results of One way ANOVA followed by Tukey's multiple comparison test to determine the significant differences ($P < 0.05$) is shown.

Groups	Drugs and doses	Writhing (Mean \pm SD)
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Group I	3% gum acacia w/v 10 ml/kg	5.00 ± 0.89
Group II	CREE 100 mg/kg	4.33 ± 1.02
Group III	CREE 250 mg/kg	3.50 ± 0.54
Group IV	CREE 500 mg/kg	2.83 ± 1.47*
Group V	Aspirin 100 mg/kg	3.00 ± 0.89*

Group I vs. all other groups significance denoted by *; no other significant inter-group differences observed.

Table [3]: Mean ± SD of reaction time for jumping and paw licking movements by the animals in different groups. Results of One way ANOVA followed by Tukey's multiple comparison test (with significance at $P < 0.05$) is also shown.

GROUPS	DRUGS & DOSES	Reaction time for Paw licking or Jumping responses in rats (seconds)				
		(Baseline)	After drug administration			
			30 min	60 min	120 min	180 min
GR I (3% gum acacia)	10 ml/kg	6.30 ± 0.04	6.33 ± 0.03	6.31 ± 0.03	6.35 ± 0.02	6.34 ± 0.02
GR II (CREE)	100mg/ kg	6.33 ± 0.34	8.00 ± 0.54*	13.00 ± 0.18*	15.66 ± 0.23*	13.00 ± 0.45*
GR III (CREE)	250mg/ kg	6.49 ± 0.28	13.00 ± 0.22* #	17.50 ± 0.35* #	20.16 ± 0.39* #	19.66 ± 0.25* #
GR IV	500mg/	6.42 ±	14.00 ±	18.83 ±	26.16 ±	20.00 ±

(CREE)	kg	0.21	0.34* # \$	0.42* # \$	0.39 # \$	0.36 #
GR V (Aspirin)	100mg/ kg	6.23 ± 0.27	13.66 ± 0.38* # \$	20.50 ± 0.29* # \$ £	28.00 ±0.39* # \$ £	21.00 ± 0.38* # \$ £

Groups II to V compared to control Group I depicted by *, Group II Vs. III, IV, V by #, Group III vs. IV, V by \$ and Group IV Vs. V by £

Table [4]: Shows the Mean ± SD of rectal temperature recordings of the animals in different groups. Results of One way ANOVA and Tukey's multiple comparison test for significant differences ($P < 0.05$) is also shown.

GROUPS	DRUGS & DOSES	Rectal temperature (degrees Celsius) recording by digital thermometer				
		Baseline	18h (after yeast induced fever)	60 mins	120 mins	180 min
GR I (3% gum acacia)	10 ml/kg	37.27 ± 0.03	38.25 ± 0.06	38.22 ± 0.08	38.21 ± 0.35	38.21 ± 0.06
GR II (CREE)	100mg/ kg	37.36 ± 0.02	38.14 ± 0.10	37.98 ± 0.26	37.96 ± 0.17	37.97 ± 0.04
GR III (CREE)	250mg/ kg	37.61 ± 0.06	38.19 ± 0.15	37.93 ± 0.1	37.91 ± 0.11	37.95 ± 0.11
GR IV (CREE)	500mg/ kg	37.47 ± 0.05	38.26 ± 0.04	37.77 ± 0.09	37.71 ±	37.78 ±

					0.14	0.11
GR V (Paraceta mol)	50mg/kg	37.37 ± 0.04	38.31 ± 0.05	37.75 ± 0.1	37.61 ± 0.07	37.72 ± 0.05

No significant difference between groups at baseline, 30 minutes, 120 minutes and 180 minutes post-dosing

4. DISCUSSION

Our aim was to evaluate the analgesic and antipyretic activity of ethanolic extract of the leaves of *Catharanthus roseus*. Ethanolic extract of *Catharanthus roseus* leaves (CREE) was prepared by percolation method.

4.1 Analgesic activity of *Catharanthus roseus* ethanolic leaf extract

For testing analgesic activity, 30 Swiss albino mice were divided into five groups of six animals each with administration of vehicle (10 ml/kg of 3% gum acacia), CREE 100 mg/kg, CREE 250 mg/kg, CREE 500 mg/kg and Aspirin 100 mg/kg in the different groups.

4.1.1 Evaluation of Analgesic activity by Glacial acetic acid induced Writhing method (Chemical pain) in mice:

Writhing test with 0.6% glacial acetic acid was done to evaluate analgesic activity of the extract. Number of writhing movements of the mice was counted over a period of 20 minutes and % protection by the drugs was calculated. Number of writhing movements decreased and % protection of extract increased with the use of extract (16.66 % at 100 mg/kg, 30% at 250 mg/kg and 43% at 500 mg/kg) and standard drug (40%), with higher dose associated with higher protection against chemical pain. Thus, the *Catharanthus roseus* ethanolic extract demonstrated dose dependent analgesic activity, as shown in Table [1]. Mean ± SD of writhing movements in the groups were calculated and statistical analysis by ANOVA and Tukey's test revealed that extract at highest dose (CREE 500 mg/kg) showed significant analgesic effect and protection against pain compared to control, as shown in Table [2]. However, there was no significant difference between the extract at any dose compared to the standard drug Aspirin 100 mg/kg, as shown in the Tables [1], [2]. Thus the analgesic effect of *Catharanthus roseus* ethanolic extract was comparable to Aspirin at the given doses.

4.1.2 Evaluation of Analgesic activity by Eddy's Hot plate method (Thermal pain) in rats:

The animals were then subjected to thermal pain in Eddy's hot plate method, keeping a temperature of 55 degrees (cut-off 30 seconds). Reaction time of the rats to avoidance measures like jumping, paw licking were recorded. Mean ± SD of reaction times in different groups were calculated and statistical analysis was done by One Way ANOVA and Tukey's multiple comparison test ($P < 0.05$), as shown in Table [3].

Difference between groups was not significant prior to drug administration. ($P > 0.01$)

At 30 minutes after drug administration, there was highly significant difference ($P < 0.01$) between all groups compared to control.

Significant difference was observed between groups II to III, IV, V and between III to IV, V. However there was no significant difference between highest dose of extract and standard, as shown in Table [3].

At 60 minutes after drug administration, there was highly significant difference ($P < 0.01$) between all groups compared to control. Significant difference was observed between groups II to III, IV, V and between III to IV, V. Group V (standard) compared to IV (highest dose of extract) exhibited significant difference, as shown in Table [3].

At 120 minutes after drug administration, there was highly significant difference ($P < 0.001$) between all groups compared to control. Significant difference was observed between groups II to III, IV, V and between III to IV, V. Group V (standard) compared to IV (highest dose of extract) exhibited significant difference, as shown in Table [3].

At 180 minutes after drug administration, there was highly significant difference ($P < 0.01$) between all groups compared to control. Significant difference was observed between groups II to III, IV, V and between III to V. Group V (standard) compared to IV (highest dose extract) exhibited significant difference, as shown in Table [3].

Thus, *Catharanthus roseus* ethanolic leaf extract at different doses displayed significant analgesic activity and dose dependent effect. However, analgesic activity of the highest dose group (500 mg/kg) was significantly lower than standard drug Aspirin (100 mg/kg).

Analgesic activity of the ethanolic extract of *Catharanthus roseus* leaves was reported earlier.^[26] A possible mechanism of analgesia has been elaborated for one of its derivative compounds Vinpocetine which is present in its leaves. Vinpocetine has been shown to possess action against hyperalgesia of inflammation and analgesic activity. Peripheral sensitization of pain receptors by the inflammatory process leads to increases in the inputs and transmission of nociceptive stimuli by these receptors [36]. The enhanced afferent activity, in turn, induces long-lasting increases in the excitability of spinal cord neurons and contributes to inflammatory pain hypersensitivity. Probable mechanisms of analgesia by Vinpocetine include inhibition of signalling by nuclear factor kappa B and production of pro-inflammatory cytokines IL-1 β and TNF- α around the dorsal root ganglion. Vinpocetine also inhibits neuronal reactive oxygen species (ROS) production thus exhibiting anti-oxidant effect. TNF- α and IL-1 β , in conjunction with ROS, like superoxide anion radical, are important peripheral and spinal hyperalgesic mediators. Reduction of oxidative stress in the rat brain has been suggested as a mechanism for central analgesic effect. Vinpocetine also blocks the retrograde axoplasmic transport of nerve growth factor, which is probably its analgesic mechanism of action in neuropathic pain.^[27-35]

4.2 Antipyretic activity of *Catharanthus roseus* ethanolic leaf extract

For antipyretic activity, 30 Wistar albino rats were divided into five groups of six animals each. Baseline normal rectal temperatures were recorded by digital thermometer (degrees Celsius).

4.2.1: Evaluation of anti-pyretic activity by Baker's Yeast-induced fever in rats:

Fever was induced in the animals by subcutaneous injection of 20% aqueous suspension of 20 ml of dried baker's yeast. After 18 hours of induction of fever, rectal temperatures were recorded again. This was followed by administration of vehicle (10 ml/kg of 3% gum acacia), CREE 100 mg/kg, CREE 250 mg/kg, CREE 500 mg/kg and Paracetamol 50 mg/kg in the different groups. Rectal temperatures were recorded periodically at 60, 120 and 180 minutes post-dosing.

Difference in normal rectal temperatures between groups was not significant at baseline and at 18 hours after induction of fever by yeast injection. However, even at 60, 120 and 180 minutes after drug administration, we did not observe significant difference in rectal temperatures between groups, as shown in Table [4].

Thus, *Catharanthus roseus* ethanolic leaf extract did not show significant antipyretic activity when administered to Wistar rats with yeast-induced fever at doses of 100 mg/kg, 250 mg/kg and 500 mg/kg. This observation is in contrast to the findings of Garg, *et al.*^[26]

5. CONCLUSION

Catharanthus roseus ethanolic leaf extract at the doses of 100 mg/kg, 250 mg/kg and 500 mg/kg displayed significant analgesic activity and dose dependent effect, but did not show significant anti-pyretic activity.

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable, as per the guidelines for care and handling of experimental animals. All experiments have been examined and approved by the appropriate ethics committee (Institutional animal ethics committee 634/02/A/CPCSEA).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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