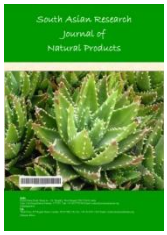

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Evaluation of Analgesic and Antipyretic Activity of Ethanolic Leaf Extract of *Catharanthus roseus* (Nayantara) in Experimental Animals

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Authors' contributions

This work was carried out in collaboration among all authors. Author KL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DK and AR performed the experimental procedures, collected the data and managed the analyses of the study. Author SD guided the experimental procedures, checked the data and reviewed the manuscript. All authors read and approved the final manuscript.

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

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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 ± 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 rosues (L.) G. Don., also known as Vinca rosea or Lochnera rosea, is a member of the Apocyanaceae family and is commonly known as Madagascar Periwinkle [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, anthelminthic, 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. *Catharanthus roseus'* qualities have only been professionally studied in a few studies. Medicinal components of *Catharanthus roseus* are reportedly only found in the leaf exudates. [3]. As a result, we designed this study to assess the potential analgesic and antipyretic activity of the leaves of the Nayantara plant.

1.1 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. MATERIALS AND METHODS

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^{o}C) during the experimental procedures.

2.2 Drugs and Chemicals

2.2.1 Plant material and preparation of *Catharanthus roseus leaf 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):

The acetic acid-induced writhing test was carried out according to the method of Koster R, et al. [23]. Pain is induced in the animals by irritant action of glacial acetic acid. The animals react with display of a characteristic stretching behaviour called writhing. The groups were given 10 ml/kg body weight of 0.6 percent glacial acetic acid intra-peritoneally to generate abdominal contractions (writhes) by chemical irritation. Abdominal muscle contractions, stretching of the rear limbs, and trunk twisting were defined as writhes. We counted the number of writhes for each group of mice starting from five minutes following the injection of acetic acid up to 20 minutes thereafter and expressed it as a percentage of protection. Subsequently, 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 (and standard) group animals.

2.3.2 Eddy's hot plate method

The hot plate test, first reported by Eddy and Leimbach (1953), is a screening model for assessing analgesic activity of drugs/substances. It is based on the principle that when rodents are placed on a hot surface, they initially show aversion to the thermal pain stimulation by licking their paws and jumping to escape the contact with the hot surface. Changes in pain threshold either enhance the delay to licking/jumping (analgesic impact) 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 ≥ 38.00 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 ± 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 and figures:

3.1 Evaluation of Analgesic Activity by Glacial Acetic acid Induced Writhing Method (Chemical Pain) in Mice

Number of writhing movements and % protection of CREE and Aspirin against chemical pain are depicted in Table 1.

Mean ± SD of writhing movements in the groups and statistical analysis by ANOVA and Tukey's test are shown in Table 2 and Fig 1.

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 = *Catharanthus roseus* leaf ethanolic extract)

Groups	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 ± SD of writhing movements in Glacial acetic induced writhing method and Results of One way ANOVA followed by Tukey's multiple comparison test in different groups (statistical significance at $P < 0.05$) (CREE = *Catharanthus roseus* leaf ethanolic extract)

Groups	Drugs and doses	Writhing (Mean ± SD)
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. Shows the Mean ± SD of reaction time (seconds) for jumping and paw licking movements by the animals and Results of One way ANOVA followed by Tukey's multiple comparison test in different groups (statistical significance at *P* < 0.05) (CREE = *Catharanthus roseus* leaf ethanolic extract)

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
Group I	10 ml/kg 3% gum acacia	6.30±0.04	6.33±0.03	6.31±0.03	6.35±0.02	6.34±0.02
Group II	100mg/ kg CREE	6.33±0.34	8.00±0.54*	13.00±0.18*	15.66±0.23*	13.00±0.45*
Group III	250mg/ kg CREE	6.49±0.28	13.00±0.22* #	17.50±0.35* #	20.16±0.39* #	19.66±0.25* #
Group IV	500mg/ kg CREE	6.42±0.21	14.00±0.34* # $	18.83±0.42* # $	26.16±0.39* # $	20.00±0.36* #
Group V	100mg/ kg Aspirin	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 (degrees Celsius) of the animals in different groups and Results of One way ANOVA followed by Tukey's multiple comparison test (statistical significance at *P* < 0.05) (CREE = *Catharanthus roseus* leaf ethanolic extract)

Groups	Drugs & doses	Rectal temperature (degrees Celsius) recording by digital thermometer				
		Baseline	18h (after yeast induced fever)	(After drug administration)		
				60 mins	120 mins	180 min
Group I	10 ml/kg (3% gum acacia)	37.27±0.03	38.25±0.06	38.22±0.08	38.21±0.35	38.21±0.06
Group II	100mg/ kg CREE	37.36±0.02	38.14±0.10	37.98±0.26	37.96±0.17	37.97±0.04
Group III	250mg/ kg CREE	37.61±0.06	38.19±0.15	37.93±0.10	37.91±0.11	37.95±0.11
Group IV	500mg/ kg CREE	37.47±0.05	38.26±0.04	37.77±0.09	37.71±0.14	37.78±0.11
Group V	50mg/kg Paracetamol	37.37±0.04	38.31±0.05	37.75±0.10	37.61±0.07	37.72±0.05

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

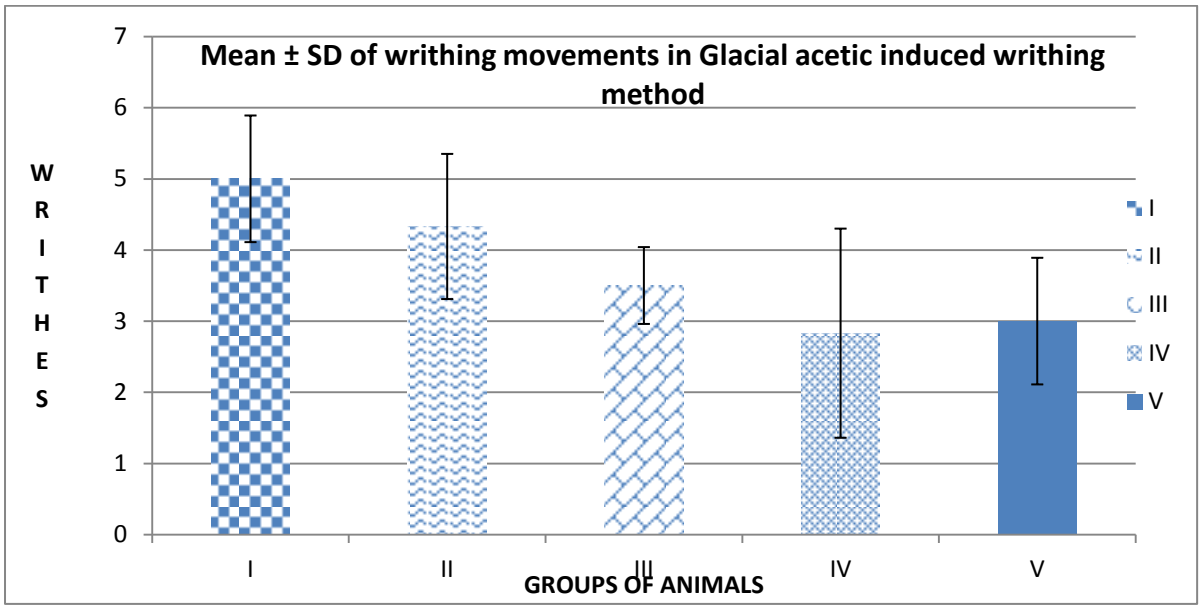

Fig. 1: Shows the Mean ± SD of writhing movements in Glacial acetic induced writhing method in different groups (I = 3% gum acacia 10 ml/kg; II = CREE 100mg/kg; III = CREE 250 mg/kg; IV = CREE 500 mg/kg; V = Aspirin 100 mg/kg)
(CREE = *Catharanthus roseus* leaf ethanolic extract)

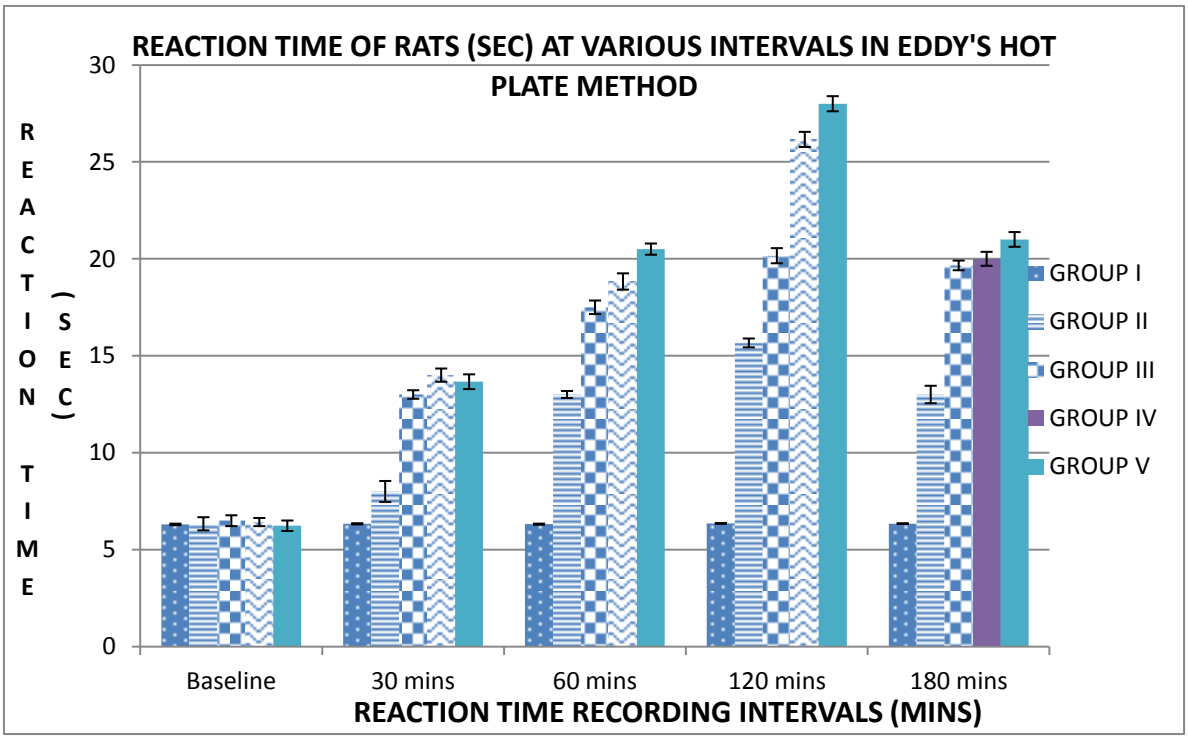

Fig. 2: Shows the Mean ± SD of reaction time for jumping and/or paw licking movements by the animals in Eddy's Hot Plate method in different groups. (I = 3% gum acacia 10 ml/kg; II = CREE 100mg/kg; III = CREE 250 mg/kg; IV = CREE 500 mg/kg; V = Aspirin 100 mg/kg)
(CREE = *Catharanthus roseus* leaf ethanolic extract)

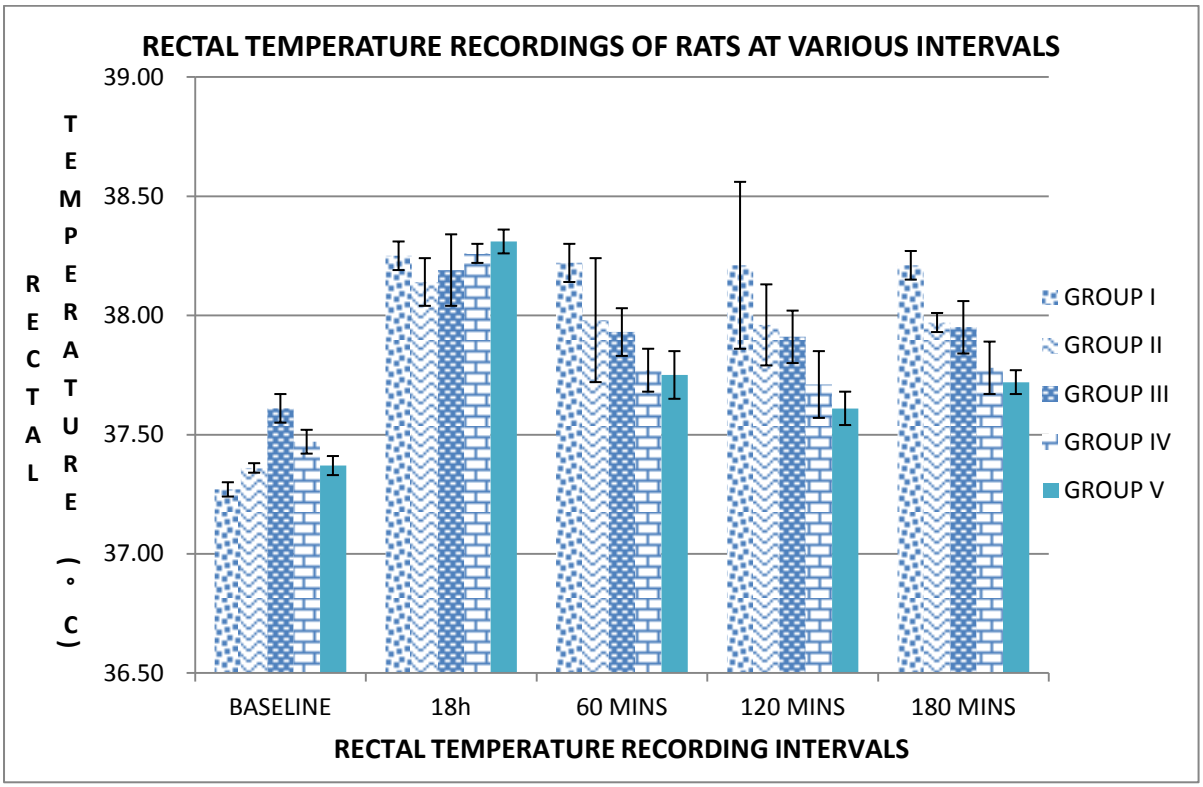

Fig. 3. Shows the Mean ± SD of rectal temperature recordings of the animals in different groups in Baker's Yeast induced Fever method (I = 3% gum acacia 10 ml/kg; II = CREE 100mg/kg; III = CREE 250 mg/kg; IV = CREE 500 mg/kg; V = Aspirin 100 mg/kg) (CREE = *Catharanthus roseus* leaf ethanolic extract)

3.2 Evaluation of Analgesic Activity by Eddy's Hot Plate Method (Thermal Pain) in Rats

Mean ± SD of reaction times for jumping/paw licking (in seconds) among different groups at baseline (prior to drug administration), 30, 60, 120 and 180 minutes following drug administration and statistical analysis by One Way ANOVA and Tukey's multiple comparison test ($P < 0.05$) are shown in Table 3 and Fig 2.

3.3 Evaluation of Anti-pyretic Activity by Baker's Yeast-induced Fever in Rats

Mean ± SD readings of rectal temeratures (in degrees Celsius) recorded by digital rectal thermometer at baseline (prior to drug administration), 18h after induction of experimental fever by dried Baker's yeast and 60, 120 and 180 minutes therafter and statistical analysis by One Way ANOVA and Tukey's multiple comparison test ($P < 0.05$) are shown in Table 4 and Fig 3.

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. Decrease in writhing movements with the extract implies that it shows analgesic activity, as per the methods described in Vogel, *et al.*[25] Thus, the *Catharanthus roseus* leaf 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 and Fig. 1. 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 and 2 and Fig. 1. Thus the analgesic effect of *Catharanthus roseus* leaf 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 and Fig. 2.

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 and Fig. 2.

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 and Fig. 2.

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 and Fig. 2.

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 and Fig. 2.

Catharanthus roseus leaf ethanolic extract thus demonstrated significant increase in reaction times at different doses which is indicative of its analgesic activity, as per the methods described in Vogel, *et al.*[25]. 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 activity against hyperalgesia associated with inflammation and analgesic property. The inflammatory process causes peripheral sensitization of pain receptors. This leads to increases in the inputs and transmission of nociceptive stimuli by these receptors [27]. Increased afferent activity causes long-term increases in excitability of spinal cord neurons, which contributes to inflammatory pain hypersensitivity. Vinpocetine may cause analgesia by inhibiting nuclear factor kappa B signalling and causing the release of pro-

inflammatory cytokines IL-1 and TNF- near the dorsal root ganglion. Vinpocetine also inhibits neuronal reactive oxygen species (ROS) production thus exhibiting anti-oxidant effect. TNF-α and IL-1β, 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 [28-35].

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. Mean ± SD of rectal temperature recordings (degrees Celsius) of the animals in different groups was calculated and statistical analysis was done by One way ANOVA followed by Tukey's multiple comparison test (statistical significance at $P < 0.05$), as shown in Table 4 and Fig 3.

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 and Fig. 3.

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], who had observed significant decrease in rectal temperature with *Catharanthus roseus* leaf extract, thus demonstrating its antipyretic activity. We could not find any other experimental study on antipyretic effect of leaves of *Catharanthus roseus* (Nayantara) for comparison with our findings.

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.

DISCLAIMER

The products used for this research are derived from commonly and predominantly available natural garden plants in our area of research and country. Also, the research was funded by personal efforts of the authors without any other source of funding.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Khare CP. Indian Medicinal plants An illustrated dictionary. 1st ed. New York: Springer Science+Business Media; 2007.
2. Nejat N, Valdiani A, Cahill D, Tan YH, Maziah M, Abiri R. Ornamental exterior versus therapeutic interior of Madagascar periwinkle (*Catharanthus roseus*): The two faces of a versatile herb. Scientific World Journal. 2015;2015:982412.

DOI: 10.1155/2015/982412 Epub 2015 Jan 15. PMID: 25667940; PMCID: PMC4312627

3. Roepke J, Salim V, Wu M, Thamm AM, Murata J, Ploss K, et al. Vinca drug components accumulate exclusively in leaf exudates of Madagascar periwinkle. Proc Natl Acad Sci U S A. 2010 Aug 24;107(34):15287-92.

   DOI: 10.1073/pnas.0911451107 Epub 2010 Aug 9. PMID: 20696903; PMCID: PMC2930567.

4. Pham HNT, Vuong QV, Bowyer MC, Scarlett CJ. Phytochemicals derived from *Catharanthus roseus* and their health benefits. Technologies. 2020; 8(4):80.

   DOI: 10.3390/technologies8040080

5. Vega-Ávila E, Cano-Velasco JL, Alarcón-Aguilar FJ, Fajardo Ortíz Mdel C, Almanza-Pérez JC, Román-Ramos R. Hypoglycemic Activity of Aqueous Extracts from *Catharanthus roseus*. Evid Based Complement Alternat Med. 2012; 2012:934258.

   DOI: 10.1155/2012/934258 Epub 2012 Sep 27. PMID: 23056144; PMCID: PMC3463976.

6. Nammi S, Boini MK, Lodagala SD, Behara RB. The juice of fresh leaves of *Catharanthus roseus* Linn. reduces blood glucose in normal and alloxan diabetic rabbits. BMC Complement Altern Med. 2003 Sep 2;3:4.

   DOI: 10.1186/1472-6882-3-4 Epub 2003 Sep 2. PMID: 12950994; PMCID: PMC194756.

7. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity— A review of ten years of herbal medicine research (1990–2000). Int J Diabetes Metab. 2006:14(1):1-25

8. Omar F, Tareq AM, Alqahtani AM, Dhama, K, Sayeed MA, Emran, TB, et al. Plant-Based Indole Alkaloids: A Comprehensive Overview from a Pharmacological Perspective. Molecules 2021;26:2297.

   Available:https://doi.org/10.3390/molecules26082297

9. Chauhan K, Sharma S, Rohatgi K, Chauhan B. Antihyperlipidemic and antioxidative efficacy of *Catharanthus roseus* Linn [Sadabahar] in streptozotocin induced diabetic rats. Asian Journal of Pharmaceutical and Health Sciences. 2011;2(1):235–43.

10. Naz S, Haq R, Aslam F, Ilyas S. Evaluation of antimicrobial activity of extracts of in vivo and in vitro grown Vinca rosea L. (*Catharanthus roseus*) against pathogens. Pak J Pharm Sci. 2015;28(3):849-53. PMID: 26004716.

11. Singh N, Pandey BR, Verma P. An overview of phytotherapeutic approach in prevention and treatment of Alzheimer's Syndrome & Dementia. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(3):162–72

12. Agarwal S, Chettri N, Bisoyi S, A. Tazeen A, AB, Vedamurthy AB, V Krishna V, et al. Evaluation of *In-vitro* anthelminthic activity of *Catharanthus roseus* extract. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(3):211-3.

13. Pham HNT, Sakoff JA, Vuong QV, Bowyer MC, Scarlett CJ. Phytochemical, antioxidant, anti-proliferative and antimicrobial properties of *Catharanthus roseus* root extract, saponin-enriched and aqueous fractions. Mol Biol Rep. 2019 Jun;46(3):3265-73.

    DOI: 10.1007/s11033-019-04786-8 Epub 2019 Apr 3. PMID: 30945069.

14. Pereira DM, F. Ferreres F, Oliveira JMA, Gaspar L, Faria J, Valentao P, et al. Pharmacological effects of *Catharanthus roseus* root alkaloids in acetylcholinesterase inhibition and cholinergic neurotransmission. Phytomedicine. 2010;17(8-9):646–52.

15. Nayak BS, Pinto Pereira LM. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. BMC Complement Altern Med. 2006 Dec 21;6:41.

    DOI: 10.1186/1472-6882-6-41 PMID: 17184528; PMCID: PMC1764761.

16. Hassan KA, Brenda AT, Patrick V, Patrick OE. *In vivo* antidiarrheal activity of the ethanolic leaf extract of *Catharanthus roseus* linn. (Apocyanaceae) in wistar rats. Afr J Pharm Pharmacol. 2011;5(15);1797–1800.

17. Johnson IS, Armstrong JG, Gorman M, Burnett JP. The vinca alkaloids: A new

class of oncolytic agents. Cancer Res. 1963;23:1390-427.

18. Cragg GM, Newman DJ. Plants as a source of anticancer agents. J Ethnopharmacol. 2005;100(1-2):72–9.

19. Committee for the purpose of control and supervision of experimental animals. Compendium of CPCSEA. New Delhi: Ministry of Environment, Forests and Climate Change, Animal Welfare Division, Govt. of India. 2018;202.

Available from: http://cpcsea.nic.in/WriteReadData/userfiles/file/Compendium%20of%20CPCSEA.pdf

Accessed 11th June 2021

20. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength, and limitation. Med Aromat Plants. 2015;4:196.

21. Chattopadhyay RR, Banerjee RN, Sarkar SK, Ganguly S, Basu TK. Antiinflammatory and acute toxicity studies with the leaves of *Vinca rosea* Linn in experimental animals. Ind J Physiol. Pharmacol.1992;36(4):291-2.

22. Ajuru MG, Ajuru G, Nmom FW, Worlu CW, Igoma PG. Acute toxicity study and determination of median lethal dose of *Catharanthus roseus* in Wistar albino rats. Journal of Applied Sciences 2019;19:217-22.

23. Koster R, Anderson M and De Beer J. Acetic acid for analgesic screening. Proc Soc Exp Biol Med.1959;18:412–417

24. Castagné V, Hernier, AM, Porsolt RD. CNS safety pharmacology. Reference Module in Biomedical Sciences 2014. Elsevier Inc; 2014.

Available:https://doi.org/10.1016/B978-0-12-801238-3.04931-X Accessed 27th June 2021

25. In Vogel, HG (Ed.). Drug discovery and evaluation pharmacological assays. 2nd ed. Springer-Verlag Berlin Heidelberg. 2002;716,772-4.

26. Garg VK, Saini D. Analgesic and antipyretic activity of ethanolic extract of leaves of *Catharanthus roseus*. Der Pharmacia Lettre. 2016;8(18): 48-52.

27. Ruiz-Miyazawa KW, Zarpelon AC, Pinho-Ribeiro FA, Pavao-de-souza GF, Casagrande R, Verri WA. Vinpocetine reduces carrageenan-induced inflammatory hyperalgesia in mice by inhibiting oxidative stress, cytokine production and NF-κB activation in the paw and spinal cord. PLoS One 2015 Mr 30;10(3):e0118942.

DOI: 10.1371/journal.pone.0118942 PMID: 25822523; PMCID: PMC4379066.

28. Lourenco-Gonzalez Y, Fattori V, Domiciano TP, Rossaneis AC, Borghi SM, Zaninelli TH, et al. Hindawi Mediators of Inflammation. 2019;Article ID 6481812

Available from: https://doi.org/10.1155/2019/6481812

Accessed 14th June 2021

29. Ruiz-Miyazawa KW, Pinho-Ribeiro FA, Zarpelon AC, Staurengo-Ferrari L, Silva RL, Alves-Filho JC, et al. Vinpocetine reduces lipopolysaccharide-induced inflammatory pain and neutrophil recruitment in mice by targeting oxidative stress, cytokines and NF-κB. Chem Biol Interact. 2015 Jul 25;237: 9-17.

DOI: 10.1016/j.cbi.2015.05.007 Epub 2015 May 14. PMID: 25980587.

30. Jeon KI, Xu X, Aizawa T, Lim JH, Jono H, Kwon DS, et al. Vinpocetine inhibits NF-κB-dependent inflammation via an IKK-dependent but PDE-independent mechanism. Proc Natl Acad Sci USA. 2010;107(21):9795–800.

DOI: 10.1073/pnas.0914414107 PMID: 20448200 PMCID: PMC2906898

31. Medina AE. Vinpocetine as a potent antiinflammatory agent. Proc Natl Acad Sci U S A. 2010 Jun 1;107(22):9921-2.

DOI: 10.1073/pnas.1005138107 Epub 2010 May 21. PMID: 20495091; PMCID: PMC2890434.

32. Abdel Salam OM. Vinpocetine and piracetam exert antinociceptive effect in visceral pain model in mice. Pharmacol Rep. 2006;58(5):680–91.

33. Csillik B, Mihaly A, Krisztin-Pe´va B, Farkas I, Knyiha´r-Csillik E. Mitigation of nociception via transganglionic degenerative atrophy: Possible mechanism of vinpocetine-induced blockade of retrograde axoplasmic transport. Ann Anat. 2008;190:140-5.

34. Csillik B, Mihály A, Knyihár-Csillik E. Antinociceptive effect of vinpocetine--a comprehensive survey. Ideggyogyaszati Szemle. 2010;63(5-6):185-92.

35. Zhang YS, Li JD, Yan C. An update on vinpocetine: New discoveries and clinical implications. Eur J Pharmacol. 2018 Jan 15;819:30-4. DOI: 10.1016/j.ejphar.2017.11.041 Epub 2017 Nov 26. PMID: 29183836; PMCID: PMC5766389.

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