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 ± 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 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, 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. 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^{o}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 ± 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 ± 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 ± 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]: 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 (Paracetamol)	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. 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-1β 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-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. [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.

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

[9] Chauhan K, Sharma S, Rohatgi K, and 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, A. B. 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):3265-3273. 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 p. 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 (1959) Acetic acid for analgesic screening. Federation Proceedings 18: 412–417.

[24] Castagné V, Hernier, AM, Porsolt RD. CNS Safety Pharmacology. Reference Module in Biomedical Sciences 2014. Elsevier Inc. 2014. Available from 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, p.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 (2015). 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, 14 pages 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-*κ*B-dependent inflammation via an IKK-dependent but PDE-independent mechanism. Proc Nat

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):185-192.

[35] Zhang YS, Li JD, Yan C. An update on vinpocetine: New discoveries and clinical implications. Eur J Pharmacol. 2018 Jan 15;819:30-34. doi: 10.1016/j.ejphar.2017.11.041. Epub 2017 Nov 26. PMID: 29183836; PMCID: PMC5766389.