Assessing Therapeutic Potentials of *Catharanthus roseus* (L.) G. Don Focusing on Anti-Diabetic, Analgesic and Anti-Diarrheal Activities

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Abstract

*Catharanthus roseus*, or 'Nayantara' in Bengali, is a perennial flowering herb. This study aimed to evaluate the pharmacological effectiveness of the plant focusing on its anti-diabetic, peripheral and central analgesic and anti-diarrheal effects. Methanol soluble partition 200 (MSP-200) and 400 (MSP-400) mg/kg body weight were administered for glucose tolerance test (GTT), heat and chemical-induced response tests, sodium thiosulphate induced test, and castor oil induced test, evaluating anti-diabetic, peripheral and central analgesic, and anti-diarrheal effects via in-vivo assay in Swiss-Albino mice model. Methanol soluble partition (MSP) at 200 and 400 mg/kg doses significantly reduced the blood glucose levels of mice by 31.00% and 32.97%, respectively (standard 46.24%). In peripheral analgesic activity, MSP-400 (62.82 ± 4.62%) outperformed MSP-200 (50 ± 3.62%) and MSP-200 (p = 0.0001) exhibited statistically significant central analgesic activity. MSP-400 demonstrated potent anti-diarrheal activity (p = 0.0002). The outcomes from this study put forward a cogent argument that *C. roseus* plant is capable of producing numerous bioactive compounds such as flavonoids, alkaloids, terpenoids, tannins, etc. which requires systematic phytochemical and pharmacological investigations.

Key words: *Catharanthus roseus*, anti-diabetic, central analgesic, peripheral analgesic, anti-diarrheal.

Introduction

Plants, considered Earth's earliest life forms, play a vital role in sustaining all living creatures through photosynthesis, converting sunlight into carbohydrates, the basis of our food (Peretó et al., 2009). Plants have been integral to human well-being since our earliest existence as remedies for ailments and injuries (Petrovska 2012). The amount of pharmaceutical chemicals humans consume in food, beverages, medicines, and different home and industrial items has dramatically expanded. Because of this, depending on their characteristics, these compounds may cause mild to severe acute or chronic poisoning (Mimi et al., 2024). Medicinal plants, defined as those containing therapeutic secondary metabolites or drug precursors, according to the World Health Organization, have been a cornerstone of traditional healing methods (Rasool Hassan et al., 2012). This profound relationship between humans and plants underscores their enduring significance in our lives as sources of sustenance and healing.

*Catharanthus roseus*, also called 'Nayantara' in Bengali and rosy periwinkle in English, belongs to the Apocynaceae family. It is a perennial or annual herbaceous flowering plant (Barkat et al., 2017).
C. roseus, commonly called Madagascar periwinkle, is primarily found in Madagascar and thrives in various regions, including America, continental Africa, Asia, Southern Europe, Australia, and several Pacific Ocean islands (Mujib et al., 2012). This plant has a long tradition of traditional medicinal applications across multiple countries. The dried leaves or the entire plant are boiled in various regions, and the resulting extract is consumed to address specific health issues. For instance, it treats diabetes in Northeast India and several other countries (Pham et al., 2020). Kenyans explore it as a complementary therapy for cancer types such as throat, stomach, and esophageal cancers (Ochwang’i et al., 2014). The dried and ground C. roseus root is decocted to combat urogenital infections in South Africa’s Venda region (Fernandes et al., 2008) and gonorrhea in Limpopo Province (Semenya et al., 2013).

Two of the most economically and pharmacologically important cytotoxic dimeric alkaloids of C. roseus, vinblastine and vincristine, have been extensively utilized in chemotherapy for cancer treatment (Jacobs et al., 2004). Polyphenols, alkaloids, steroids, flavonoids, glycosides, anthocyanins, and iridoid glucosides are just some of the chemical groups that have been found in different parts of C. roseus (Mustafa et al., 2007). This literature review and traditional uses corroborated the choose this plant for comprehensive investigation by following In-vivo assays. The goal of this research is to comprehensively assess the therapeutic potential of C. roseus extracts through in vivo pharmacological tests. The objective is to offer guidance for the identification of crucial compounds that have the potential to be developed into pharmaceutical drugs.

Materials and Methods

Collection, authentication and extract preparation: C. roseus, locally known as Nayantara, was sourced from Savar, Bangladesh, and taxonomically verified by Professor Dr. Zasim Uddin, Department of Botany, University of Dhaka. A voucher specimen (DACB 65132) resides at the Bangladesh National Herbarium. The whole plant was gathered, purified, and dehydrated at ambient temperature, then subjected to oven drying at a temperature below 40°C. It was stored airtight after grinding into a fine powder (1 kg) using a Cyclotec grinder (200 mesh). This powder, approximately 850 g, was soaked in methanol for two weeks in a flat-bottomed flask, occasionally agitated. Post-soaking, the methanolic extract underwent filtration with cotton and filter paper. The filter was subsequently removed by evaporation using a Stuart rotary evaporator from the United Kingdom, while reducing the pressure. This cold extraction method yielded about 32 g of extract, subsequently partitioned into various solvents like hexane, chloroform, dichloromethane and ethyl acetate via liquid-liquid extraction (Rodriguez et al., 2021).

Chemicals and reagents: The investigation utilized solvents and reagents bought from Merck (Germany) and BDH (England), all of which were of high quality and suitable for analytical or laboratory purposes. Before using the commercial grade, solvents (hexane, dichloromethane, ethyl acetate, and methanol) were distilled. Standard drugs such as morphine, streptokinase (sk), glibenclamide, acetyl salicylic acid (ASA), G-thiopental (Thiopental sodium), and loperamide were obtained from Gonohasilshthaya Pharmaceuticals Ltd, Beximco Pharmaceuticals Ltd. and Square Pharmaceuticals Ltd., respectively.

Animals: A group of Swiss albino mice, aged four to five weeks and of both genders, were housed in the Department of Pharmacy at the State University of Bangladesh to conduct pharmacological studies. Before commencing the study, the Ethics Review Committee of the State University of Bangladesh granted the ethical clearance. The mice were kept in standard thermoset plastic cages in a controlled environment with a temperature of 24 ± 2°C, humidity ranging from 60-70%, and a 12-hour light-dark cycle. The subjects were given unlimited access to food and water and were allowed to adjust to the laboratory setting for a period of 3-4 days.
before the testing began. This was done to reduce the influence of environmental changes on their physiological reactions. The standard laboratory diet provided to the mice consisted of the following components: 55.7% rice polish and ground maize (weight/weight), 30.9% ground whole grain wheat (weight/weight), 5.4% dried fish meal (weight/weight), 3.2% soybean oil (volume/weight), 2.4% salt (NaCl) (weight/weight), 0.4% vitamin mixture (Vitamin B12 and Vitamin C) (weight/weight), and 2.0% distilled water for mixing (Sarwar et al., 2022). Prior to the start of the study, mice were given a period of 3-4 days to adapt to the laboratory environment in order to reduce the influence of environmental changes on their physiological responses. The animals were provided with care and treated in accordance with the guidelines set forth by the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (Amee et al., 2024). In addition, the Ethical Review Committee at the Department of Pharmacy, State University of Bangladesh (SUB) thoroughly examined and approved all laboratory protocols and procedures.

Study design: Male Swiss albino mice, with a weight range of 25-35 gm and an age range of 4-5 weeks, were acquired from the State University of Bangladesh (SUB). The subjects were accommodated in the university's animal facility and supplied with a standard rodent diet while being subjected to meticulously regulated environmental conditions. The mice were given a period of 4 days to acclimate to these conditions before any experimental procedures commenced. The study adhered to the guidelines set forth by the Federation of European Laboratory Animal Science Associations (FELASA) in order to minimize the mice's pain and distress during experimentation.

During the in vivo bioassays, the mice were categorized into four groups (Groups I, II, III, and IV), with each group containing 5 individuals. Group I and Group II were used as the negative and positive controls, respectively. Groups III and IV received oral doses of 200 and 400 mg/kg body weight of a crude extract of C. roseus, using the method outlined by Kayser et al. (2019).

The purpose of this study was to examine the pharmacological effects of a methanolic extract derived from C. roseus. Swiss albino mice were used as the experimental model. The assessment of anti-diabetic activity involved a Glucose Tolerance Test (GTT), where mice were categorized into control, standard (Glibenclamide-treated), and two experimental groups receiving different doses of C. roseus extract (200 mg/kg and 400 mg/kg). The blood glucose levels were periodically observed after administration to assess the extract's effect on glucose tolerance compared to the standard medication. Simultaneously, the writhing test caused by acetic acid evaluated the peripheral analgesic activity. The mice were divided into control, standard (treated with Diclofenac), and experimental (administered with varying doses of C. roseus extract). The writhing responses were quantified to evaluate the extract's effectiveness in reducing pain sensation compared to the standard medication. Statistical analyses were used to identify significant distinctions between groups, offering crucial understanding into the potential therapeutic uses of C. roseus in regulating blood sugar levels and managing pain.

Anti-diabetic activity: Glucose level (GL) in blood was measured by the glucose tolerance test (GTT) (Govindarajan et al., 2008) to assess the anti-diabetic activity of the plant extract of C. roseus. The extract doses (400 mg/kg and 200 mg/kg body weight) for mice were prepared by triturating the extracts with Tween-80 and normal saline, resulting in a 3.0 mL suspension. Similarly, a 3.0 mL suspension was prepared for a 10 mg/kg body weight dose of Glibenclamide, which was used as a standard. Blood samples were drawn from the tail vein at 30-, 90-, and 150 min post-administration of the test samples. Blood glucose levels were determined using a glucometer by following the equation.

\[
\% \text{ Reduction of GL in Blood} = \left(\frac{GL_c - GL_t}{GL_c}\right) \times 100\%
\]

Where, \(GL_c\) = glucose level in control group

\(GL_t\) = glucose level in test group
Peripheral analgesic activity: Chemically induced muscle strain exhibited peripheral writhing (Whittle et al., 1964). The number of writhing was accounted to assess the peripheral analgesic activity of *C. roseus* extract. Diclofenac-Na was used as the standard. The following equation was applied to estimate percentage of writhing due to the dosage form of *C. roseus* plant extract,

\[
\% \text{ Inhibition of writhing} = \frac{\text{Avg. control writhing} - \text{Avg. sample writhing}}{\text{Avg. control writhing}} \times 100\%
\]

Central analgesic activity: Thermal induced reflexive effect was employed in tail immersion method for assessing central analgesic activity (Islam et al., 2019). Greater elongation indicated stronger central analgesic effects. Test sample activity was compared to that of Diclofenac-Na.

\[
\% \text{ of time elongation} = \frac{T_c - T_e}{T_c} \times 100\%
\]

Here, \(T_c\) represents the test group and standards whereas \(T_e\) represents the negative control.

Anti-diarrheal activity: Diarrhea triggered on by castor oil in rats according to the procedures described by (Zhu et al., 2022), was conducted. The mice were categorized into four groups, with each group comprising five mice (\(n = 5\)). Prior to the test, the animals underwent a period of fasting for 18 hours. As a control, Group I received normal saline (2 ml/kg), while Group II received loperamide (5 mg/kg). Methanol extracts (200 and 400 mg/kg) were given to groups III and IV. Oral administration was used for all dosages. All groups were given 1 ml of castor oil orally after 1 hour. Percent inhibition (%) of diarrhea was used to measure the activity of each group.

Statistical analysis: The statistical analysis of in vivo data obtained from experiments with *C. roseus* was conducted using GraphPad Prism (version 8.4). The mean values, along with their corresponding standard errors of the mean (SEM), were presented as mean ± SEM to summarize the findings of in vivo evaluations. The researchers used a one-way ANOVA to compare multiple groups, and then conducted Dunnett’s multiple comparison test to compare the treated groups with the control (vehicle) group (Amee et al., 2024). The results were deemed statistically significant with a p-value of less than 0.05.

Results and Discussion

Antidiabetic activity: The group bar chart in Figure 1 depicted the effect of changes of glucose level in blood of Swiss-Albino mice due to administration of different dosages of methanolic extract of *C. roseus* and standard drug. MSP-400 dosage form showed lowest blood glucose level (6.82±1.34 mmol/l) in mice at 180 minutes time period compared to the blood glucose level (7.02 ± 1.18 mmol/l) of standard drug. The dosage form MSP-200 and 400 ensured the reduction percent age of blood glucose level 31.0% and 32.97% respectively compared to glibenclamide (46.24%). The percentage of reduction of glucose level in blood after GTT with *C. roseus* extract implies that it has potential lead compound to trigger insulin and inhibiting the enzyme’s action in stomach that disrupts the carbohydrate.

The statistical analysis reveals that the methanolic extract of *C. roseus* does not exhibit a significant reduction in blood glucose levels at doses of either 200 mg/kg or 400 mg/kg body weight. The standard treatment yielded a t-test value of 0.2906, accompanied by a standard deviation (SD) of 3.4864, a standard error of the mean (SEM) of 1.5592, and a p-value of 0.7788. The dose of 200 mg/kg (MSP-200) and the dose of 400 mg/kg (MSP-400) showed t-test values of 0.3340 and 0.1977, standard deviations of 4.4352 and 3.7232, standard errors of the mean of 1.9835 and 1.6651, and p-values of 0.7470 and 0.8482, respectively. None of these p-values demonstrate statistical significance.
Peripheral analgesic activity: Two dosages form (MSP-200 and MSP-400) of methanolic soluble extract were used to evaluate peripheral analgesic activity of *C. roseus* plant and the results obtained are shown by simple bar chart in Figure 2. MSP-400 dosage form (62.82±4.62%) exhibited greater % of writhing inhibition than MSP-200 (50±3.62%) compare the activity with standard. The plant extracts significantly reduced the occurrence of abdominal writhes in mice. These findings endings imply that the methanol soluble partitions of *C. roseus* likely contain bioactive substances that, when administered, can effectively diminish pain sensation.

![Figure 1. Plasma level of glucose of different groups of mice at different times. Values are expressed as mean ± SD (n = 4); ***p< 0.001 are very statistically significant, **p< 0.05 statistically significant and *p> 0.05 compared to control followed by Dunnet test (GraphPad Prism 8.4). MSP = Methanol Soluble Partition.](image1)

![Figure 2. Peripheral analgesic activity of the methanolic extract of *C. roseus*. Values are expressed as mean ± SD (n = 4); ****p< 0.0001 are extremely statistically significant, ***p< 0.001 are very statistically significant and **p< 0.05 statistically significant compared to control followed by Dunnet test (GraphPad Prism 8.4). (MSP) = Methanol Soluble Partition.](image2)
The statistical analysis of the data confirmed that the crude methanol extract of *C. roseus* demonstrated a substantial analgesic effect at 200 mg/kg and 400 mg/kg doses. The standard treatment (STD) produced a t-test result of 8.4971, with 6 degrees of freedom (df) and a p-value of 0.0001. This indicates that the results were statistically highly significant. The dose of 200 mg/kg (MSP-200) and the dose of 400 mg/kg (MSP-400) had t-test values of 5.5841 and 5.2272, degrees of freedom (df) of 6, and p-values of 0.0014 and 0.0020, respectively. Both of these values denote statistical significance. These findings indicate that *C. roseus* should be further examined for its potential as a source of new lead compounds.

Central analgesic activity: According to Figure 3, after oral administration of doses and standard in different group the time of tail flicking in hot water bath was increased with period of time. The analysis and statistical evaluation of the data leads to the conclusion that the methanol extract of *C. roseus* showed significant central analgesic activity at both doses of 200 mg/kg and 400 mg/kg body weight after 60 and 90 minutes of administration. So, they can be further investigated for the development of central analgesic drug.

![Figure 3](image.png)

Figure 3. Percent time elongation of tail immersion of methanolic extract of *C. roseus*. Values are expressed as mean ± SD (n = 4); ****p< 0.0001 are extremely statistically significant ***p< 0.001 are very statistically significant and **p< 0.05 statistically significant compared to control followed by Dunnet test (GraphPad Prism 8.4). (MSP) = Methanol Soluble Partition.

The statistical analysis of the data collected after a 30-minute period showed that the raw methanol extract of *C. roseus* displayed notable central analgesic activity. The standard group exhibited a t-test value of 17.0022, having a degree of freedom (df) of 4 and a p-value of 0.0000, indicating a high level of statistical significance. The dose of 400 mg/kg (MSP-400) exhibited a t-test value of 8.5086, with a degree of freedom (df) of 4, and a p-value of 0.0010, indicating a highly significant statistical difference. In the same way, the 200 mg/kg dose (MSP-200) had a t-test value of 6.7679, 4 degrees of freedom (df), and a p-value of 0.0025, all of which are very strong statistical results. The results indicate that *C. roseus* has strong pain-relieving effects in the central nervous system, as demonstrated by the two doses tested.

Anti-diarrheal activity: The anti-diarrheal activity in terms of decreasing the diarrheal feces due to two different MSP-200 and MSP-400 treatments of *C. roseus* plant and standard is depicted in Figure 4. The methanolic extract of *C. roseus* at 400 mg/kg
body weight showed highly significant antidiarrheal activity. This can be related to the plant’s tradition use in GIT problems. It is widely known that tannins and flavonoids have antidiarrheal qualities because they can inhibit gastrointestinal movement, show antibacterial qualities, and reduce fluid activity (Ali et al., 2012). So, this plant extract can be further searched for potential tannin or flavonoid lead compounds.

![Graph showing anti-diarrheal effect of methanolic extract of C. roseus at different doses.](image)

Figure 4. Anti-diarrheal effect of methanolic extract of C. roseus at different doses. ***p< 0.001 are very statistically significant, **p< 0.05 statistically significant and ^p> 0.05 compared to control followed by Dunnet test (GraphPad Prism 8.4), MSP = Methanol soluble partition.

Different levels of significance were found in the statistical analysis of how the methanolic extract of C. roseus affected the diarrhea that mice got from castor oil. The STD group showed a t-test value of 13.9076 with 6 degrees of freedom (df) and a p-value of 0.0000, indicating a highly significant statistical difference. The dose of 200 mg/kg (MSP-200) had a t-test value of 1.1188, degrees of freedom (df) of 6, and a p-value of 0.3060, indicating that it was not statistically significant. In contrast, the dose of 400 mg/kg (MSP-400) exhibited a t-test value of 8.2219, with a degree of freedom (df) of 6 and a p-value of 0.0002, indicating a high level of statistical significance. These findings indicate that the higher dosage of the methanolic extract exhibits notable anti-diarrheal effects.

**Conclusions**

The methanol soluble fraction of C. roseus plants contains a variety of secondary metabolites with diverse pharmacological properties. C. roseus also exhibited anti-diabetic, peripheral and central analgesic and anti-diarrheal, properties. The results demonstrate that the methanolic extracts from C. roseus exhibit statistically significant central & peripheral analgesic activity, and anti-diarrheal property. Thus, the findings trigger that the isolation of lead compounds from C. roseus plant should be a fruitful initiation.

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**Conflict of interest**

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Data availability statement

The primary data utilized to substantiate this investigation's conclusions can be obtained from the corresponding author upon inquiry.

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