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Analgesic and antipyretic activity of ethanolic extract of leaves of *Catharanthus Roseus*

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ABSTRACT

The present study establishes the analgesic and anti-pyretic activities of the ethanolic extract of leaves of Catharanthus roseus in the models used. Since antipyretic and analgesic activities are commonly mentioned as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis, the yeast induced hyperthermia in rat model was, therefore, employed to investigate the antipyretic activity of this plant. It was found that the ethanolic extract at the dose of 100, 200 and 400 mg/kg b.w. showed a significant decrease in rectal temperature similar to that shown by the standard drug, paracetamol. This result seems to support the view that the extract has an inhibitory effect on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature. The analgesic activity of ethanolic extract was evaluated using the hot plate method and acetic acid induced writhing test in mice. The hot plate method is useful in detecting centrally acting analgesics, whereas writhing method is useful to detect peripheral analgesic effects. Acetic acid, which is used as an inducer for writhing syndrome, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings. The fact that ethanolic extract of Catharanthus roseus showed analgesic activity in both the models studied, indicate that this effect could be due to the presence of two components; one acting centrally and the other via peripheral route. As the phytochemical screening has shown the presence of alkaloids and flavonoids, its potent activity may be attributed to the presence of these phytoconstituents.

Key words: *Catharanthus roseus*, analgesic, antipyretic

INTRODUCTION

Catharanthus roseus (family: Apocynaceae) is an evergreen sub shrub or herbaceous plant growing to 1 m tall. The leaves are oval to oblong, 2.5–9 cm long and 1–3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole 1–1.8 cm long; they are arranged in opposite pairs. The species has long been cultivated for herbal medicine and as an ornamental plant. The species has long been cultivated for herbal medicine and as an ornamental plant. In traditional Chinese medicine, extracts from it have been used to treat numerous diseases, including diabetes, malaria, and Hodgkin's disease [1]. *Catharanthus roseus* was formerly known as *Vinca rosea* and main source of vinca alkaloids, now sometimes called catharanthus alkaloids. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia[2]. Traditionally it is used to relieve pain, depression, mouth ulcers, hypertension etc.[3, 4]. Various pharmacological activities of this plant have been reported like antimicrobial, anthelmintic, anti-diabetic, antioxidant, hypolipidemic, wound healing and cytotoxic activities [5]. Researchers are making efforts to discover agents that can reduce pain and fever and therefore may reduce the cost of hospitalization and save the patient from severe complications. The need for safer and effective analgesic and antipyretic agents and the lack of enough scientific data to support the traditional claims made in literature prompted the present study.

MATERIALS AND METHODS

Experimental Animals
Healthy albino Wistar rats of both sexes weighing between 200-250g were used. Also albino mice of both sexes weighing between 20 – 25g were used. Institutional Animal Ethics Committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). (Approval No. 711/02/a/CPCSEA).

Plant Material
The leaves of Catharanthus roseus were collected from the local area of Meerut district and identified and authenticated by Dr. Anjula Pandey, Taxonomist, National Herbarium of Cultivated Plants, New Delhi. Voucher specimens have been kept in National Herbarium of Cultivated Plants, New Delhi and Department of Pharmaceutical Technology, MIET for future reference.

Extraction
The leaves were dried under shade, reduced to moderately coarse powder, loaded into soxhlet extractor and were subjected to extraction with Petroleum ether for defatting and ethanol.

Preliminary Phytochemical Studies
The ethanolic extract was then subjected to qualitative phytochemical screening for the identification of different phytoconstituents. Ethanolic extract showed positive tests for the presence of alkaloids and flavonoids. As traditionally, the plant is used to cure pain and fever, the analgesic and anti-pyretic activities of the ethanolic extract of the plant in different dose levels (100 mg/kg, 200 mg/kg, 400 mg/kg) [6] is being reported here.

Antipyretic Testing
Hyperthermia was induced in rats following the method of Teotino et al., 1963. Initial rectal temperatures of rats were recorded using a six channel tele-thermometer for 1 min. Rats were made hyperthermic by subcutaneous injection of 20% yeast suspension at a dose of 1 ml/100 gm body weight. When the temperature was at peak (18 hours after yeast injection) the rectal temperature were again recorded. Those animals that showed a rise in rectal temperature of more than 1.2° C were used [7] Different doses of ethanolic extract of *Catharanthus roseus* were given orally as a suspension prepared in 2% Tween 80 solution. Animals were divided into five groups of six animals each. First group received 1 ml of 2% Tween 80 solution orally and served as control. Second, third, fourth and fifth groups received standard antipyretic agent i.e. paracetamol suspension (100 mg/kg) [8], ethanolic extract (100 mg/kg, 200 mg/kg, 400 mg/kg), respectively. The rectal temperatures of animals were recorded at 30 minutes intervals for 4 hours following the administration of Tween 80, standard drug and plant extracts. [8]

Analgesic Activity
Hot Plate Method
The hot plate method described by Turner (1965) was followed for the assessment of analgesic activity. Albino mice were introduced to a hot plate maintained at 55 ± 0.5°C. The reaction time to the thermal stimulus was recorded as the time interval from introduction of the animal to the plate until the first lick of the limbs or the first jump of the animals. The test groups received ethanolic extract of *Catharanthus roseus* at different dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Pentazocine (10 mg/kg) [9] and control group received only 1 ml of 2% Tween 80 solution. The reaction times were determined before and after 30 minutes, 1 hour, 2 hours and 3 hours period with reference to the control group receiving only vehicle.

Acetic Acid Induced Writhing
Acetic acid induced writhing response in mice Acetic acid solution at a dose of 10 ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed. The test groups received ethanolic extract of *Catharanthus roseus* at different dose levels prepared as suspension in 2% Tween 80 orally, the standard group received Aspirin (10 mg/kg) and control group received only 1 ml of 2% Tween 80 solution. Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated [9].

$$\% \text{ Inhibition} = \frac{WC - WT}{WC} \times 100$$

Where,
WC = Mean number of writhes in control group.
WT = Number of writhes in test group.

Statistical Analysis

All the results obtained from various activities, as described above, were analyzed statistically by using Student's t test and $p<0.05$ were considered significant [10]. The results are summarized in the tables given below.

RESULTS

Table I: Details of Qualitative Phytochemical Tests

Tests	Ethanolic Extract
1.Tests for sterols	
a. Test solution+Conc.H_2SO_4	**-ve**
b. Libermann Buchard's Test	**-ve**
c. Test solution + sulphur	**-ve**
d. Salkowski test	**-ve**
2.Tests for Glycosides	
a. Keller Killiani's Test	**-ve**
b. Balget's Test	**-ve**
c. Bromine water Test	**-ve**
d. Legal's Test	**-ve**
e. Raymond's Test	**-ve**
3. Test for Saponins	
a. Haemolytic Test	**-ve**
b. Foam Test	**-ve**
4. Test for Proteins	
a. Xanthoproteic Test	**-ve**
b. Millon's Test	**-ve**
c. Biuret Test	**-ve**
d. Ninhydrin Test	**-ve**
5. Test for Tannins	
a. Gelatin Test	**-ve**
b. Ferric Test	**-ve**
6. Test for Alkaloids	
a. Dragendroff's Test	**+ve**
b. Mayer's Test	**+ve**
c. Hager's Test	**+ve**
d. Wagner's Test	**+ve**
7.Test for Carbohydrates	
a. Barfoed's Test	**-ve**
b. Benedict's Test	**-ve**
c. Molisch's Test	**-ve**
8. Test for Flavonoids	
a. Shinoda Test	**+ve**
b. Alkaline Reagent Test	**+ve**
c. Ferric Chloride Test	**+ve**
d. Lead Acetate Test	**+ve**
e. Zn-HCl reduction Test	**+ve**

+ve indicates positive result –ve indicates negative result

Table II. Effect of different doses of Ethanolic extract of *Catharanthus roseus* and Paracetamol on yeast induced hyperthermia in rats

Groups	Rectal Temperature (°C)						
	Initial before yeast injection	18 Hrs. after Yeast injection	Time after drug administration (hrs)				
			0.5 hrs	1 hr	2 hrs	3 hrs	4 hrs
Control	36.90 ± 0.075	38.81 ± 0.099	39.01 ± 0.062	39.10 ± 0.081	39.14 ± 0.052	39.05 ± 0.043	38.72 ± 0.057
Paracetamol (100 mg/kg)	37.10 ± 0.054	38.98 ± 0.087	38.33 ± 0.094^d	37.46 ± 0.070^d	37.18 ± 0.080^d	37.93 ± 0.085^d	37.95 ± 0.069^d
Ethanolic Extract (100 mg/kg)	36.98 ± 0.101	39.02 ± 0.065	38.87 ± 0.059	38.58 ± 0.083^d	38.39 ± 0.071^d	39.01 ± 0.056	37.86 ± 0.067^d
Ethanolic Extract (200 mg/kg)	37.15 ± 0.089	39.04 ± 0.076	38.68 ± 0.067^c	37.92 ± 0.077^d	37.56 ± 0.089^d	37.95 ± 0.047^d	38.17 ± 0.051^d
Ethanolic Extract (400 mg/kg)	37.06 ± 0.068	38.92 ± 0.069	38.28 ± 0.069^d	37.61 ± 0.067^d	37.29 ± 0.077^d	37.62 ± 0.101^d	37.98 ± 0.092^d

Values are expressed as mean ±S.E.M. (n=6);
significance at $p< 0.05^a$, $p< 0.02^b$, $p< 0.01^c$, $p< 0.001^d$ as compared to control.

Table III. Effect of different doses of Ethanolic extract of *Catharanthus roseus* on Hot Plate reaction time in mice

Groups	Dose (mg/kg)	Reaction Time (Seconds)				
		Initial	Time after drug administration (Hrs)			
			0.5 hrs	1 hr	2 hrs	3 hrs
Control		5.08 ± 0.0875	5.16 ± 0.0669	5.15 ± 0.0888	5.18 ± 0.0603	5.20 ± 0.0969
Pentazocine	10	5.15 ± 0.0766	7.53 ± 0.0559^{d}	8.48 ± 0.0875^{d}	9.33 ± 0.1149^{d}	9.35 ± 0.1092^{d}
Ethanolic Extract	100	5.18 ± 0.0479	5.33 ± 0.0334^{a}	5.65 ± 0.0429^{d}	6.08 ± 0.0603^{d}	6.11 ± 0.0479^{d}
Ethanolic Extract	200	5.16 ± 0.0763	5.70 ± 0.0518^{d}	6.25 ± 0.0429^{d}	6.90 ± 0.0859^{d}	6.85 ± 0.0766^{d}
Ethanolic Extract	400	5.15 ± 0.0766	5.78 ± 0.0603^{d}	6.73 ± 0.0496^{d}	7.81 ± 0.0603^{d}	7.85 ± 0.0766^{d}

Values are expressed as mean ±S.E.M. (n=6);
significance at p< 0.05^{a}, p< 0.02^{b}, p< 0.01^{c}, p< 0.001^{d} as compared to control.

Table IV. Effect of different doses of Ethanolic extract of *Catharanthus roseus* on Acetic acid induced writhing in mice

S. No.	Groups	Dose (mg/kg)	No. of Writhing (Mean ± SEM)	% Inhibition
1	Control		36.91 ± 1.4651	
2	Aspirin	10	7.1 ± 0.7624^{d}	80.76
3	Ethanolic Extract	100	22.62 ± 0.4657^{d}	38.71
4	Ethanolic Extract	200	13.10 ± 0.6579^{d}	64.50
5	Ethanolic Extract	400	8.21 ± 0.5647^{d}	77.75

Values are expressed as mean ±S.E.M. (n=6);
significance at p< 0.05^{a}, p< 0.02^{b}, p< 0.01^{c}, p< 0.001^{d} as compared to control.

DISCUSSION

The present study establishes the anti-pyretic and analgesic activities of the ethanolic extract of *Catharanthus roseus* in the models used. Since antipyretic and analgesic activities are commonly mentioned as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis [11] the yeast induced hyperthermia in rat model was, therefore, employed to investigate the antipyretic activity of this plant. It was found that the ethanolic extract at the dose of 100,200 and 400 mg/kg showed a significant decrease in rectal temperature similar to that shown by the standard drug, paracetamol. This result seems to support the view that the extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature [12].

Likewise, the analgesic activity of ethanolic extract of the plant was evaluated using the hot plate method and writhing test in mice. The hot plate method is useful in detecting centrally acting analgesics [13] whereas acetic acid induced writhing method is useful to detect peripheral analgesic effects. Acetic acid, which is used as an inducer for writhing syndrome, causes Algesia by liberation of endogenous substances, which then excite the pain nerve endings [8]. The fact that ethanolic extract of *Catharanthus roseus* showed analgesic activity in both the models studied, indicate that this effect could be due to the presence of two components; one acting centrally and the other via peripheral route [13].

From the above results, it can be deduced that ethanolic extract has shown dose dependent activity. As the phytochemical screening has shown the presence of alkaloids and flovonoids in ethanolic extract, its potent activity may be attributed to the presence of these phytoconstituents. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

CONCLUSION

From the above study, it can be concluded that the ethanolic extract of *Catharanthus roseus* leaves possesses analgesic as well as anti-pyretic activity.

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