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Comparative Evaluation of Antihyperglycaemic and Hypoglycaemic Activity of Various Parts of *Catharanthus roseus* Linn.

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Abstract: Hydroalcoholic extracts of flowers, leaves, stems and roots of *Catharanthus roseus* Linn. (Apocynaceae) were tested for antihyperglycaemic and hypoglycaemic activities. Antihyperglycaemic activity was tested in glucose over loaded hyperglycaemic rats and hypoglycaemic activity in fasted normal rats at two dose levels, 100 and 200 mg kg⁻¹, respectively. Glimperide 0.1 mg kg⁻¹ was used as the reference drug for both the activities. Results showed that the hydroalcoholic extracts of every part tested, exhibited significant antihyperglycaemic and hypoglycaemic activity. Comparatively the hydroalcoholic extract of leaves exhibited better activity, next to this stems and flowers were equally effective followed by roots. This study gives an indication to traditional healers those who use different parts of this plant to use the active part that has the ability to manage the complications of diabetes.

Key words: Antihyperglycaemic, *Catharanthus roseus*, diabetes, hypoglycaemic, glimepride, glucose

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic with a worldwide incidence of 5% in the general population. More than 100 million of the world's population has already reached the diabetic mark. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS (Anonymous, 2006). Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades (Shastri, 1980). Overt diabetes affects 2-3% of the total world population. In conventional therapy, Type 1 diabetes is treated with exogenous insulin and Type 2 with oral hypoglycaemic agents (sulphonylureas, biguanides etc.) (Pepato *et al.*, 2005). Though different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes, there is an increase demand by patients to use the natural products with antidiabetic activity (Venkatesh *et al.*, 2003). Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India a number of plants are mentioned in ancient literatures (Ayurveda) for the management of diabetic complications. *Catharanthus roseus* Linn. (Rosy Periwinkle or Madagascar Periwinkle) is a species of the genus *Catharanthus* in the family Apocynaceae that is commonly used in most of the herbal preparations for diabetes. It has been reported to possess anticancer activity (Jean *et al.*, 1999), antidiabetic activity (flowers and leaves) (Sumana and Suryawanshi, 2001), hypolipidemic activity (Antia and Okokon, 2005), antioxidant activity (Jaleel *et al.*, 2007) wound healing activity, has vasodilating, blood thinning,

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memory-enhancing actions and also used in alzheimer's disease (Nayak *et al.*, 2007). The main active constituents present in this plant are alkaloids (Jean *et al.*, 1999), flavonoids (Gilles *et al.*, 1999) and steroids (Akirai, 1999). As mentioned earlier it has been used effectively in various traditional systems of medicines for the treatment of diabetes. Few practitioners may use the leaves alone and others may use whole plant. In this direction our efforts were directed to identify the most effective antidiabetic part of *C. roseus*.

MATERIALS AND METHODS

Plant Material

Leaves, flowers, stems and roots of *C. roseus* were collected from Mandsaur in the month of July and authenticated by Dr. H.S. Chattree, Department of Botany, Government PG College Mandsaur (MP). A voucher specimen (BRNCP/V/001/2006) was deposited in the herbarium of Department of Pharmacognosy, BRNCP, Mandsaur (MP) for future reference.

Extraction

The collected plant parts (Flower, Leaves, Stems and Roots) were dried and powdered. The powdered material (500 g) was firstly defatted with petroleum ether and extracted with hydroalcohol (50:50) for 72 h in soxhlet apparatus. The extract was evaporated under reduced pressure to obtain solid mass. Percent yield and phytoconstituents (Brain and Turner, 1975; Khandelwal, 2005) present in the extracts are given in Table 1.

Animals

After getting approval from the Institutional Animal Ethical committee (registration number 918/ac/05/CPCSEA), Wistar strain rats (weighing between 100-150 g) procured from the animal house of B.R. Nahata College of Pharmacy, Mandsaur were used for investigation. The animals were housed in standard environmental conditions of temperature ($21\pm 2^{\circ}\text{C}$), humidity ($55\pm 10\%$) and a 12 h light-dark cycle. Rats were supplied with standard pellet diet and water *ad libitum*.

Acute Toxicity Studies

The acute toxicity test (LD_{50}) of the extract was determined according to the OECD guidelines No. 420 (Organization for Economic Co-operation and development). Female albino rats (100-150 g) were used in this study. After the sighting study, starting dose of 2000 mg kg^{-1} (P.O.) of the extracts were given to 5 animals in each groups. The treated animals were monitored for 14 days for mortality and general behavior. No death was observed till the end of the study. The extract was found to be safe up to the dose of 2000 mg kg^{-1} and from the results two doses of 100 and 200 mg kg^{-1} were chosen for further experimentation.

Antihyperglycemic Activity

Antihyperglycaemic activity was studied in glucose overloaded hyperglycemic rats (Gupta *et al.*, 2005; Babu *et al.*, 2002). Animals were divided in to 10 groups ($n = 5$), group 1 was kept as vehicle control, treated only with vehicle, 1% Tween 80. The positive control group animals, group 2, received

Table 1: Percentage yield and phytochemical screening of hydroalcoholic extracts of *Catharanthus roseus*

Plant materials	Yield (%) (w/w)	Phytoconstituents
Flower	13.29	Alkaloids, carbohydrates, saponins, flavonoids, tannins, steroids
Leaves	8.72	Alkaloids, carbohydrates, saponins, flavonoids, tannins, steroids, triterpenoids
Stem	11.65	Alkaloids, carbohydrates, flavonoids, tannins, steroids
Roots	10.92	Alkaloids, carbohydrates, saponins, flavonoids, tannins, steroids, triterpenoids

0.1 mg kg⁻¹ of Glimepride, remaining 8 groups were treated with extracts of different parts of plants suspended in 1% Tween 80 at two dose levels, 100 and 200 mg kg⁻¹ respectively. In zero hour blood sugar level was determined from overnight fasted animals. After 30 min of the drug treatment animals were fed with glucose (1.5 g kg⁻¹) and blood glucose was determined after 1/2, 1, 2 and 3 h of the glucose load. Blood glucose concentration was estimated by the glucose oxidase enzymatic method using a commercial glucometer and test-strips (Accu-chek Active™ Test meter).

Hypoglycaemic Activity

Animals were classified in to 10 groups (n = 5). Group 1 was kept as control, received a single dose of 0.5 mL/100 g of the vehicle, group 2 was treated with 0.1 mg kg⁻¹ of Glimepride as hypoglycemic reference drug. Groups 3 to 10 were treated with various plant extracts at two dose levels (100 and 200 mg kg⁻¹) as mentioned in Table 3. Blood samples were collected from the tail tip at 0 (before oral administration), 1/2, 1, 2, 3, 4 and 5 h after vehicle, extract and drug administration (Ekrem *et al.*, 2005). The blood sugar level was measured using Accu-chek Active™ Test strips in Accu-chek Active™ Test meter.

Statistical Analysis

The values are expressed as mean±SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test.

RESULTS

Results of the phytochemical investigations carried out for various parts are given in Table 1. Alkaloids, carbohydrates, flavonoids, tannins and steroids are found in all the extracts. Saponins are present in the flower, leaves and roots and triterpenoids were identified only in leaves and roots (Table 1). The acute toxicity studies of hydroalcoholic extracts were determined using OECD guidelines No. 420. At a starting dose of 2000 mg kg⁻¹ no mortality was observed for any extracts. So 1/10th of this dose was selected for all the *in vivo* studies as a maximal dose and the activity was studied using two dose levels 100 and 200 mg kg⁻¹, respectively.

In order to identify the active antihyperglycaemic part, the hydroalcoholic extracts of various parts of *C. roseus* were studied for their antihyperglycaemic activity using glucose induced hyperglycaemic model. The different hydroalcoholic extracts of the plant parts of *Cathranthus* were studied at two different doses (100 and 200 mg kg⁻¹ b.wt.) on glucose fed hyperglycaemic animals. The results of the experiment indicated that all the extract of different parts prevented the rise of blood glucose level significantly compared to negative control group (Table 2). Also the extracts of flowers,

Table 2: Effect of hydroalcoholic extracts of various parts of *C. roseus* Linn. on glucose loaded hyperglycemic rats

Groups	Dose (mg kg ⁻¹)	Blood glucose level (mg dL ⁻¹)				
		0	1/2	1	2	3
Negative control	-	106.42±5.20	180.30±9.10	147.81±4.91	127.85±5.23	102.28±5.09
Glimepride	0.1	95.12±2.13	120.29±2.63 ^a	36.80±3.86 ^a	50.28±3.68 ^a	60.26±5.89 ^a
Flower	100	97.43±4.92	148.62±6.92	71.23±3.92 ^b	88.67±4.04 ^b	97.69±4.92
	200	89.29±3.22	143.40±6.86	75.84±3.58 ^b	82.56±4.70 ^b	96.34±5.31
Leaves	100	99.40±4.80	141.23±6.02	47.88±3.92 ^a	53.80±4.58 ^a	72.60±4.28 ^b
	200	103.45±4.92	130.28±6.39	41.67±3.70 ^a	54.83±4.65 ^a	73.56±4.70 ^b
Stems	100	104.34±4.91	157.26±6.21	73.86±3.50 ^b	93.81±3.37 ^b	97.56±4.58
	200	92.60±4.96	160.22±6.70	70.44±3.50 ^b	90.23±4.12 ^b	93.67±3.66
Roots	100	105.23±4.84	165.78±6.96	79.23±3.20 ^b	96.34±2.88 ^b	98.84±3.70
	200	107.45±3.01	170.62±6.03	89.84±3.07 ^b	94.24±3.58 ^b	99.57±4.70

Values are expressed in Mean±SEM, (n = 5); ^a: p<0.001, ^b: p<0.01 vs control (Dunnet's test)

Table 3: Effect of hydroalcoholic extracts of various parts of *C. roseus* on fasted normal rats

Groups	Dose (mg kg ⁻¹)	Blood glucose level (mg dL ⁻¹)					
		0	1	2	3	4	5
Negative control	-	102.43±6.88	101.62±6.50	98.78±7.54	97.23±6.31	100.02±5.70	101.34±6.24
Glimepride	0.1	99.82±6.58	41.44±3.50 ^a	62.43±3.92 ^a	70.60±3.37 ^a	63.24±4.37 ^a	79.40±3.50 ^a
Flower	100	103.60±4.99	87.23±5.50 ^b	80.27±5.25 ^b	91.39±3.96 ^b	96.34±4.95	98.56±4.55
	200	101.20±6.56	84.34±3.30 ^b	76.30±3.25 ^a	89.25±3.50 ^b	92.56±2.50	94.54±3.50
Leaves	100	102.63±5.88	70.25±4.70 ^b	75.32±3.37 ^a	80.80±3.70 ^b	83.53±4.70 ^b	89.47±5.70 ^b
	200	105.24±6.37	65.46±4.70 ^a	69.69±4.50 ^b	78.23±3.86 ^b	81.29±4.37 ^b	85.57±4.50 ^b
Stems	100	98.43±4.92	85.49±3.25 ^b	89.23±3.56 ^b	95.34±3.56	96.23±3.55	97.46±3.45
	200	99.34±3.22	83.18±3.59 ^b	85.76±3.95 ^b	90.45±4.96	95.26±3.70	99.12±3.56
Roots	100	101.45±4.95	88.24±4.58 ^b	86.55±4.58 ^b	97.27±2.57	99.28±3.56	97.49±4.25
	200	94.63±4.55	85.85±4.58 ^b	80.72±3.58 ^b	99.34±3.99	89.34±2.35 ^b	100.26±5.56

Values are expressed in Mean±SEM, (n = 5); ^a: p<0.001, ^b: p<0.01 vs control (Dunnet's test)

stems and leaves reduced blood glucose levels below the normal values, indicating their hypoglycaemic effects. Comparatively the hydroalcoholic extract of leaves showed the maximum activity at a dose of 200 mg kg⁻¹ and the activity was found dose dependant.

The hydroalcoholic extracts of various parts of *C. roseus* were subjected to hypoglycaemic studies at two dose levels (100 and 200 mg kg⁻¹) and the results are given in Table 3. The extracts of all the parts exhibited hypoglycaemic activity at 1st, 2nd and 3rd h, but only the leaves extract was found to exhibit the hypoglycaemic activity up to 5th h. Comparatively the extract of leaves exhibited better antihyperglycaemic and hypoglycaemic activity than any other part. Next to this stems and flowers exhibited the mentioned activities and root part was found to contain less activity compared to remaining three parts.

DISCUSSION

This study was performed to identify the most effective antihyperglycaemic and hypoglycaemic part of *C. roseus*. Various traditional practitioners are using this plant in the treatment of diabetes. Diabetes is a major health problem affecting major populations worldwide. Epidemiological studies and clinical trials strongly support the notion that hyperglycemia is the principal cause of complications. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes. Thus sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications (Muniappan *et al.*, 2004). On the basis of this statement we have selected the glucose induced hyperglycaemic model to identify the active antihyperglycaemic part of this plant. Any drug that is effective in diabetes will have the ability to control the rise in glucose level by different mechanisms and the ability of the extracts to prevent hyperglycaemia could be determined by glucose loaded hyperglycaemic model.

In the glucose loaded hyperglycaemic model, all the plant parts tested for antihyperglycaemic activity exhibited significant antihyperglycaemic activity at both the dose levels 100 and 200 mg kg⁻¹. This supports the claim of Sumana and Suryawanshi (2001), who reported the antidiabetic effects of flowers and leaves. The activity of leaves and flowers was found to be more or less same in their studies. Contradictory to this, in present study the hydroalcoholic extract of leaves was found to exhibit better activity and, stems and flowers were found to be equally effective. Excessive amount of glucose in the blood induces the secretion of insulin. This secreted insulin will stimulate peripheral glucose consumption and controls the production of glucose through different mechanisms (Andrew, 2000). However from the study (negative control) it was clear that the secreted insulin requires 2-3 h to bring back the glucose level to normal. In case of the extracts and standard drug treated

groups, the glucose levels have not exceeded than that of the negative control, giving an indication regarding the supportive action of the extracts and drug in the glucose utilization. The effect of glimepride, standard drug used in this study, on glucose tolerance has been attributed to enhanced activity of beta cells of the pancreas resulting in secretion of larger amounts of insulin. So the mechanism behind this antihyperglycaemic activity of plant extracts involves an insulin-like effect, probably, through peripheral glucose consumption or enhancing the sensitivity of beta cells to glucose, resulting in increased insulin release (Muniappan *et al.*, 2004). Further the observed hypoglycaemic activity of the plant extracts supports the suggested mechanisms. In these contexts a number of other plants have also been reported to have hypoglycaemic effects (Leila *et al.*, 2007).

In conclusion, the findings described in the present study provide further evidence in support of the folkloric claim of traditional practitioners on *C. roseus* herb for the regulation of blood sugar and using the leaves alone in herbal preparations may provide better therapeutic effects to minimize the complications of diabetes. Works are in progress to isolate the active principle from the leaves responsible for antihyperglycaemic and hypoglycaemic activity.

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