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Hypoglycaemic Effects of Methanolic Leaf Extract of *Catharanthus Roseus* (LINN.) G. DON (Apocynaceae) in Normal and Diabetic Mice

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Summary. **In Southern Africa, as in many other parts of Africa, *Catharanthus roseus* (Linn.) G. Don is traditionally used in forklore medicine to treat diabetes and a number of other human ailments. Consequently, the hypoglycaemic effects of methanolic leaf extract of *C. roseus* have been investigated in streptozotocin (STZ)-induced diabetic (hyperglycaemic) mice as well as in normal (normoglycaemic), STZ-untreated mice. Tolbutamide has been used as reference hypoglycaemic agent. While tolbutamide produced highly significant reductions ($P<0.01$-0.001) in the plasma blood glucose levels of normal (normoglycaemic) mice, the leaf extract of *C. roseus* produced only a relatively slight hypoglycaemic effect in the normal (normoglycaemic) animals. Data obtained in this study further showed that, following acute and/or sub-acute administrations, the methanolic leaf extract of *C. roseus* (MLCR, 500 mgkg^{-1} p.o.), like tolbutamide (500 mgkg^{-1} p.o.), produced highly significant hypoglycaemic effects ($P<$ 0.01-0.001) in STZ-induced diabetic mice. The hypoglycaemic effect of the plant extract was found to be more pronounced in diabetic (hyperglycaemic) mice than in normal (normoglycaemic) animals. Although the exact mechanism of the hypoglycaemic action of the plant extract remains speculative and will have to await further studies, the results of the present investigation tend to support the folkloric use of *C. roseus* in diabetic conditions.**

Key words—*Catharanthus roseus*, methanolic leaf extract (MLCR), folklore medicine, hypoglycaemic effects, diabetic conditions.

INTRODUCTION

Although the use of medicinal plants for the treatment of diabetes mellitus has largely disappeared in Europe and Americas since the introduction of insulin, ethnobotanical and floristic surverys in various parts of Africa have shown that a large number of African medicinal plants are still being used in folklore medicine to treat diabetes on that continent. *Catharanthus roseus* (Linn.) G. Don (Family: Apocynaceae) is one of such plants widely used by Traditional Medical Practitioners (Herbalists, N'angas, Sangomas, and so forth) to treat diabetes on the African Continent. Although *C. roseus* is native to Madagascar (and hence, the common English name: "Madagascar Rose Periwinkle"), the plant is now dispersed in all tropical and sub-tropical countries of the world. In Southern Africa, as in the Eastern and Western Parts of the continent, the roots, and more commonly the leaves, of this popular and attractive biennial/ perennial herbaceous garden plant (of about 50 cm in height), are frequently used in folklore medicine to treat diabetes and rheumatism (Watt and Breyer-Brandwijk, 1962). The principal aim of the present study was to evaluate the hypoglycaemic effects of *C. roseus* methanolic leaf extract in normal (normoglycaemic) and diabetic (hyperglycaemic) mice, following acute and sub-acute administrations of the plant extract.

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MATERIALS AND METHODS

Plant material

One kilogramme (1 kg.) of sun-dried leaves of *C. roseus* were cut into smaller pieces and pulverised. The powdered leaves were subjected to 60% methanol extraction procedure (as described in detail earlier by Odebiyi and Sofowora (1978)). The resulting methanolic plant extract was concentrated *in vacuo* and freeze-dried to produce a brownish-dark residue. Aliquot portions of this residue were dissolved in distilled water, and used in this study. Fresh solutions and/or dilutions of the plant extract (in distilled water) were made on each day of our experiment.

Animal material

Balb C. albino mice (*Mus domesticus*) of both sexes (weighing 25-30 g) were used. The animals were randomly divided into groups of 20 mice for test and control groups.

Induction of diabetes

Diabetes was induced in each of the test mice by intraperitoneal injections of streptozotocin (STZ, 100 $mgkg^{-1}$). Control mice were treated with intraperitoneal injections of 0.5 ml of distilled water. The control and test animals were kept and maintained under laboratory conditions of temperature, humidity, and light and given free access to food (standard pellet diet) and water. The mice were left to rest for two weeks after STZ and distilled water treatments to enable diabetes to develop and reach a steady state in the STZ-treated animals. Mice with plasma blood glucose levels $\geq$ 300 mg/100 ml were considered to be diabetic, and used in this study.

Experimental procedure

In the acute study, a single dose of the methanolic leaf extract of *C. roseus* (MLCR, 500 $mgkg^{-1}$) or tolbutamide (500 $mgkg^{-1}$) was administered to each of the 20 diabetic test mice. Blood samples were taken from the animals at 0 hours (i.e., immediately before MLCR or tolbutamide treatment), and then 2, 4, and 8 hours after the extract or tolbutamide treatment. In the sub-acute study, each of the 20 diabetic test mice was treated with a dose of MLCR (500 $mgkg^{-1}$ p.o.) or tolbutamide (500 $mgkg^{-1}$ p.o.) per day for 9 days. Plasma blood glucose levels were estimated immediately before, and then after, MLCR (500 $mgkg^{-1}$ p.o.) or tolbutamide (500 $mgkg^{-1}$ p.o.) treatments on days 1, 3, 5, 7 and 9. In both the acute and sub-acute studies, mice in the control groups were treated with 0.5 ml of distilled water, the vehicle in which the extract and tolbutamide were dissolved and/or diluted before administration into the animals. Tolbutamide (500 $mgkg^{-1}$ p.o.) was used in this study as the standard antidiabetic agent for comparison. In all cases, blood samples were taken by orbital sinus puncture, using heparinised microhaematocrit capillary tubes. The plasma was separated by centrifugation, and plasma blood glucose concentration was determined by using a 'Analox Glucose' Analyzer.

Data analysis

Data obtained were pooled, and expressed as means. The differences between control and extract-or tolbutamide-treated means were analysed statistically by using Student's t-test. Values of $P \leq 0.05$ were taken to imply statistical significance.

RESULTS

Catharanthus roseus methanolic leaf extract (MLCR, 500 $mgkg^{-1}$ p.o.) produced a slight (10.47%) but significant fall ($P<0.05$) in the plasma blood glucose levels of normal (normoglycaemic), STZ-untreated mice. On the other hand, tolbutamide (500 $mgkg^{-1}$ p.o.) caused a profound (47.33%) and highly significant fall ($P<0.01$-0.001) in the plasma blood glucose levels of the normal (normoglycaemic), STZ-untreated mice (see Table 1). Distilled water had no significant ($P>0.05$) effect (2.52%) on the plasma blood glucose levels of the normal (normoglycaemic), STZ-untreated mice (Table 1).

Data obtrained in the acute studies showed that *C. roseus* methanolic leaf extract (MLCR, 500 $mgkg^{-1}$ p.o.), like tolbutamide (500 $mgkg^{-1}$ p.o.), significantly reduced ($P<0.05$-0.001) plasma blood glucose levels in STZ-induced diabetic (hyperglycaemic) mice at 2, 4, and 8 hours after treatment (compared with the distilled water-treated control animals). Distilled water had no significant ($P>0.05$) effect (2.13%) on the plasma glucose levels of the STZ-induced diabetic mice (see Table 2).

Sub-acute treatment of STZ-induced diabetic (hyperglycaemic) mice with the plant extract (MLCR, 500 $mgkg^{-1}$ p.o.) or tolbutamide (500 $mgkg^{-1}$ p.o.) daily 9 days also caused significant reductions ($P<0.05$-0.001)in the plasma blood glucose levels of the animals compared with the distilled water-treated con-

Table 1. Effects of *C. roseus* methanolic leaf extract (MLCR, 500 $mgkg^{-1}$ p.o.) and tolbutamide (500 $mgkg^{-1}$ p.o.) on plasma blood glucose levels (mg/100 ml) of normal (normoglycaemic), STZ-untreated mice. The figures given represent means of absolute values obtained from 17-20 mice.

Mouse group	Before treatment	After treatment			Maximal reduction	%Maximal reduction
	0 hr	2 hrs	4 hrs	8 hrs		
CONTROL (distilled water)	115.52	113.48	112.61	114.55	2.91	2.52
MLCR (500 $mgkg^{-1}$ p.o.)	117.11	111.73	104.85*	108.44	12.26	10.47*
Tolbutamide (500 $mgkg^{-1}$ p.o.)	114.25	78.43*	60.18***	63.25**	54.07	47.33***

*P<0.05., **P<0.01., ***P<0.001.

Table 2. Effects of acute treatment of *C. roseus* methanolic leaf extract (MLCR, 500 $mgkg^{-1}$ p.o.) and tolbutamide (500 $mgkg^{-1}$ p.o.) on plasma blood glucose levels (mg/100 ml) of STZ-induced diabetic (hyperglycaemic) mice. The figures given represent means of absolute values obtained from 18-20 mice.

Mouse group	Before treatment	After treatment			Maximal reduction	%Maximal reduction
	0 hr	2 hrs	4 hrs	8 hrs		
CONTROL (distilled water)	465.64	461.45	455.72	463.28	9.92	2.13
MLCR (500 $mgkg^{-1}$ p.o.)	460.81	391.7	268.55**	290.36**	192.26	41.72***
Tolbutamide (500 $mgkg^{-1}$ p.o.)	466.15	385.68*	210.56**	242.37**	255.59	54.83***

*P<0.05., **P<0.01., ***P<0.001.

trols. Results obtained in the sub-acute studies were very similar to those obtained in the acute studies.

The hypoglycaemic effect of *C. roseus* leaf extract was found to be more pronounced in STZ-induced diabetic (hyperglycaemic) mice than in normal (normoglycaemic), STZ-untreated ones (41.72% versus 10.47% respectively). However, the hypoglycaemic effect of tolbutamide was found to be slightly higher also in the STZ-induced diabetic (hyperglycaemic) mice than in normal (normoglycaemic) animals (54.83% versus 47.33% respectively).

DISCUSSION AND CONCLUSION

The findings of the present study show that *C. roseus* methanolic leaf extract produced a slight but significant hypoglycaemic effect in normal (normoglycaemic) mice. This observation is in consonance with work by Farnsworth and Segelman (1971), and Marles and Fransworth (1995), who reported hypoglycaemic effects of Rose Periwinkle in normal laboratory animals, and isolated several weakly hypoglycaemic alkaloids from the plant. The present findings are, however, at variance with the observations of Swanston-Flatt et al., (1989), who reported that Periwinkle did not affect the parameters of glucose

homeostasis (basal plasma glucose and insulin, glucose tolerance, insulin-induced hypoglycaemia, glycated haemoglobin, and pancreatic insulin concentration) that they examined in normal (normoglycaemic) mice. The discrepancy in our findings and those of Swanston-Flatt et al., (1989) could be due to the different doses/concentrations of the plant extract used, as well as the different routes of administration employed in the two independent studies. *C. roseus* leaf extract produced highly significant hypoglycaemic effects following acute and/or sub-acute administrations of the plant extract into STZ-induced diabetic mice, whereas the plant extract only produced a slight (but significant) hypoglycaemic effect in normal (normoglycaemic), STZ-untreated mice. It is interesting to note, however, that the hypoglycaemic effect of the plant extract is more pronounced in diabetic (hyperglycaemic) mice than in normal (normoglycaemic) ones, and that tolbutamide produced only relatively slightly higher hypoglycaemic effects in diabetic (hyperglycaemic) mice than in normal (normoglycaemic), STZ-untreated animals. The disparity in the hypoglycaemic activity of the two compounds may be linked to their mechanisms of hypoglycaemic actions. Nevertheless, the present results suggest the presence of potentially hypoglycaemic agent/s in the methanolic extract of *C. roseus* leaves. Although the precise mechanism of the hypoglycaemic action of the plant extract still remains speculative and will have to await further studies, the results of the present study tend to support the folkloric use of *C. roseus* in diabetic conditions. Marles and Fransworth (1995) have implicated various alkaloids (such as catharanthine, leurosine and vindolinine) as possible chemical compounds of the plant that may be responsible for its hypoglycaemic effects. However, the isolation, characterization and indentification of the exact bio-active secondary metabolite/s responsible for the hypoglycaemic effects of *C. roseus* will have to await further phytochemical studies.

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