



***Catharanthus roseus* Combination Therapy with Orthodox Oral Hypoglycemic Drugs: A Novel Approach to Diabetes Mellitus Treatment**

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Article Information

Received 19 July 2017

Received in revised form 15 Oct 2017

Accepted 16 October 2017

Keywords:

Catharanthus roseus,
oral hypoglycemic drugs,
diabetes mellitus,
combination therapy,
novel approach

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Abstract

Most of the chronic diseases including diabetes mellitus are difficult to treat successfully with orthodox drugs. Investigation in complementary and alternative medicines is now being intensified to proffer lasting solution. Natural products and their derivatives may be considered as a potential source of novel compounds which can combine with orthodox drugs to enhance their hypoglycemic effect. To investigate the complementary and synergistic effect of *Catharanthus roseus* (*C. roseus*) leaves extract with oral hypoglycemic synthetic drugs sulphonylurea (glibenclamide) and biguanide (metformin). Experimental induction of diabetes in rats using alloxan model was employed. Five rats each of six groups were used. Group I received distilled water only. Groups II, III and IV received 250 mg/kg of methanol extract, 100 mg/kg of biguanide (metformin) and 1 mg/kg of sulphonylurea (glibenclamide), respectively. Groups V and VI received extract-metformin and extract-glibenclamide combination at doses as above, respectively. Administration of the six groups was carried out once daily for seven days. At 2, 12, 24, 72 and 168 h, fasting blood glucose was determined using a glucometer. When compared with control alone, all medicaments significantly ($p < 0.05$) lowered blood glucose levels. The highest percentage reduction in blood glucose (64.9%) occurred with extract-biguanide (metformin) combination. The leaves extract of *C. roseus* – biguanide (metformin) combination offered a promising novel approach to diabetes mellitus treatment.

1 Introduction

The concomitant use of medicinal plants and drugs especially in Africa is a growing tendency because of the difficulty in successfully treating some chronic diseases such as diabetes mellitus with orthodox drug^{1,2}. It is estimated that Nigeria, South Africa, Kenya, and Cameroun top the list of countries with the prevalence of DM in each sub-region of Africa affecting 3.2, 2, over 0.7, and over 0.5 million people respectively³. Notable synthetic hypoglycemic agents such as sulphonylureas (glibenclamide), biguanides (metformin) and glucosidase inhibitors (Acarbose) possess inherent adverse effects such as gastrointestinal (GIT) disturbances, weight increase, hypoglycemia, among others⁴. Plants with hypoglycemic activity are employed for their therapeutic potentials in folkloric setting.⁵

This is mainly because herbal remedies are more efficient and have little or no adverse effects and could as well be due to the fact that they form a vital component of the health care delivery system in most African nations⁶. Over 400 plants have been reported to have anti-diabetic properties, including *Gongronema latifolium*⁷, *Vernonia amygdalina*⁸, *Catharanthus roseu*⁹, *Phyllanthus emblica* and *Annona squamosa*¹⁰. The therapeutic activity of plants have been reported to serve as a potential source of novel compounds but despite their pronounced folkloric activity, they have not been commercially formulated as modern medicines¹¹⁻¹³. *C. roseus* belonged to the family Apocynaceae, and the common names include: cape periwinkle, rose periwinkle and "old-maid"⁹. Documented phytochemical studies of *C. roseus* indicated the presence of alkaloids, saponins, tannins, phlotatannins, flavonoids,

triterpenoids, anthraquinones, glycosides, and reducing sugars. Vincristine, Vinorelbine, Vinorelbine, Vinorelbine and Vinorelbine are four indole alkaloids isolated from *C. roseus*². In addition to the anti-diabetic activity^{2, 9}, *C. roseus* has other folkloric applications notably in cancer chemotherapy¹³; the root and stem are employed for the treatment of diarrhea in Congo, the leaf infusion employed for cases of scurvy and internal bleeding, mouthwash against toothache, and healing of chronic wound in Brazil¹⁵. The aim of this study was to investigate a possible novel approach for treatment of diabetes mellitus based on *C. roseus*-orthodox oral hypoglycemic combination therapy.

2 Materials and Method

2.1 Plant material preparation and extraction

The fresh stalks of *C. roseus* were collected from Calabar (Nigeria) in October 2015 and authenticated in the Department of Pharmacognosy, Madonna University, Elele, Nigeria, where a voucher specimen (M/PC.65/15) has been deposited in the Herbarium. The fresh leaves of *C. roseus* were detached from the stem and air-dried at 26°C (room temperature). The dried leaf was pulverized into powder using an electric blender. The powdered leaves (300 g) were extracted with methanol (Sigma Aldrich, Germany) by cold maceration for 48 h. The mixture was filtered to obtain the methanol extract. Using a rotary evaporator (RV 05 basic IB, IKA Staufen, Germany) at a reduced pressure, the extract was concentrated and further over dried and stored in a refrigerator.

2.2 Animals

Thirty albino rats of both sexes (160 – 220 g) obtained in the Laboratory Animals facility of the Department of Pharmacology and Toxicology, Madonna University, Elele were used in the studies. Under standard laboratory situations, the animals were maintained having free access to standard pellets (Vital Feeds Plc, Nigeria) and clean water. The animals were transferred to work area and allowed for two weeks of acclimatization.

2.3 Experimental protocols

The Alloxan-induced diabetes test, animal grouping, and determination of blood glucose were performed as previously described². In brief, animals model Type 1 diabetes was induced (but not before the fasting blood glucose [FBG] of the albino rats was determined) in the overnight-fasted animals by a single interperitoneal injection of 110 mg/kg alloxan monohydrate using distilled water as a vehicle. The FBG was then determined 48 h later to ensure induction of diabetes. Animal were considered diabetic when a blood-glucose level is greater than 150 mg/dL.

The animals were grouped in six, and each contain 5 rats according to similar weights were employed. Group 1 served as control and received orally 0.2 mL of distilled water once daily. Group II received orally 250 mg/kg of extract daily. Group III

received orally metformin 100 mg/kg daily. Group IV received orally 1 mg/kg of glibenclamide, daily. Group V received orally a combination of 100 mg/kg metformin and 250 mg/kg extract, daily, while Group VI received orally a combination of 1 mg/kg glibenclamide and 250 mg/kg extract, daily. All administration was done for seven days.

In the determination of blood glucose, glucometer (Prestige smart systems) was used. Blood samples were collected from the cut tail-tip of conscious rat and the glucose test-strip soaked with the blood and allowed to dry for 60 seconds and then inserted to be read by the glucometer. Basal and 48 hours post-induction blood glucose levels were recorded. Thereafter, the drug, extract or drug-extract combination was administered daily for 7 days. At 2 h, 12 h, 24 h, 72 h, and 168 h, blood glucose concentrations were measured and recorded.

2.4 Statistical analysis

The generated data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA, followed by Tukey-Kramer multiple comparisons test and Student-Newman-Keuls multiple comparisons test and the values were considered statistically significant when p value is less than 0.05 (p<0.05)

3 Results

The control group throughout the experimental period did not show any significant (p>0.05) reduction in the blood glucose level (Table 1). The blood sugar level of the extract-drug combinations and extract alone at 2 h showed significant (p<0.01 and p<0.05, respectively) difference in comparison with the control. The standard drug alone did not show significant (p>0.05) difference. Greater significant (p<0.01) difference was shown by metformin as well as extract-metformin combination. At 168 h, all treatment groups showed significant variations compared with control. Interestingly, extract-metformin combination showed extremely significant (p<0.01) difference when compared with metformin alone, which showed the highest percentage reduction (64.9%) in blood glucose by the extract-metformin combination at 72 h.

4 Discussions

The results obtained in this study revealed that methanol extracts of *C. roseus* concomitantly given with metformin showed great percentage in reduction of blood glucose as well as synergy. However, glibenclamide-metformin combination was less pronounced in lowering the blood glucose effect than the extract of *C. roseus*. This may not be unconnected with the presence of flavonoids, alkaloids, tannins, anthraquinones, glycosides, reducing sugars and saponins in *C. roseus* since hypoglycemic activity has been supported by evidences from previous studies of plant extracts possessing alkaloids, flavonoids and saponins⁸. Both glibenclamide and metformin

are known to reduce the concentration of glucose in the blood. Glibenclamide achieves this primarily by stimulating a first-phase release of insulin from functioning pancreatic beta cells in

response to endogenous insulin thereby reducing insulin resistance, while metformin stimulates tissues uptake of glucose and increase insulin receptor binding^{16, 17}.

Table 1: Fasting plasma glucose of alloxan-induced diabetic rats at intervals during daily oral administration of glibenclamide, metformin, and methanol extract of *C. roseus* (mg/dL)

Gp	Treatment	Pre-induction FBG	Pre-induction FBG	Fasting plasma glucose during treatment				
				2 h	12 h	24 h	72 h	168 h
I	Distilled H ₂ O (1ml/kg)	47.5±3.0	311.6±37.1	412.2±47.9	414.2±64.8	506.6±30.5	505.4±31.9	542.8±16.8
II	Extract (250mg/kg)	46.8±5.3	205.7±70.5	97.0±33.6**	314.7±157.9	477.7±37.9	272.0±49.4**	128.7±11.8**
III	Metformin (100mg/kg)	44.7±4.4	360.4±59.1	224.0±81.8	226.6±59.7	316.6±55.1	211.1±48.8**	372.0±46.1**
IV	Glibenclamide(1mg/kg)	42.8±3.9	306.8±56.2	248.8±44.3	459.4±63.0	500.8±26.4	365.2±49.3*	265.6±32.6**
V	Extract +metformin	59.5±9.9	265.6±52.3	135.4±26.0*	236.8±38.0	206.4±69.0**	99.0±28.6**	131.4±45.6**Δ
VI	Extract +Glibenclamide	48.0±3.2	336.0±88.8	182.4±64.5*	432.0±89.4	515.0±9.0	318.8±55.9*	148.4±57.3**

*P<0.05; **P<0.01 Significant level when compared with control.; ΔP<0.01 Significant level when compared with metformin (standard drug)

Reduction of plasma glucagon levels, glucose entry, and inhibitory effect on glucose absorption reduced hepatic glyconeogenesis, direct stimulation of glycolysis in peripheral tissues, enhanced response to glucose obligatory tissues (nervous tissue, brain etc) are some of the mechanisms of action of *C. roseus* extract which can be emphasized to act in similar way to glibenclamide^{18, 14}.

In addition to mechanisms of action of herb and drugs (pharmacodynamic factor) pharmacokinetics considerations of interaction such as enzyme inhibition may have contributed in delaying the appearance of significant difference between metformin monotherapy and extract-metformin combination therapy until the 7th day. Alteration of absorption (pharmacokinetics factor) due to binding has been reported with co-administration of some herbs²⁰, resulting in no significant changes in hypoglycemic activity of the orthodox drugs.

5 Conclusion

The leaves extracts of *C. roseus*-metformin combination therapy was synergistic and showed higher hypoglycemic effect than any of the two monotherapy. This finding may provide a novel approach to diabetes mellitus treatment.

6 Conflict of interest

We declare that we have no conflict of interest.

7 Source of support:

Nil

8 Author contributions

OSC and MHU participated in collection of data and literature review and approved the final manuscript.

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