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Effects of co-administration of methanol leaf extract of *Catharanthus roseus* on the hypoglycemic activity of metformin and glibenclamide in rats

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ABSTRACT

Objective: To investigate the interacting effects of co-administration of methanol leaf extract of *Catharanthus roseus (C. roseus)* on the hypoglycemic activity of metformin as well as glibenclamide using experimental rats. **Methods:** Phytochemical analysis as well as acute toxicity and lethality (LD_{50}) test were carried out on its methanol leaf extract. The alloxan model for experimental induction of diabetes in rats was employed. Six groups comprising five rats each were used. Groups Ⅱ, Ⅲ and Ⅳ received 250 mg/kg of extract, 100 mg/kg of metformin and 1 mg/kg of glibenclamide respectively, while Ⅴ and Ⅵ were administered metformin-extract and glibenclamide-extract combinations respectively at doses as above. Group Ⅰ served as negative control and received only distilled water. All administration was done once daily for seven days. Fasting blood glucose was determined at 2, 12, 24, 72 and 168 h using a glucometer. One-way ANOVA with post-hoc tests was used to assess for significant difference due to administration of drug alone and with co-administration of drug and extract. **Results:** The LD_{50} was 2 121.32 mg/kg. The phytochemical studies indicated the presence of saponins, tannins, alkaloids, phlotatannins, flavonoids, triterpenoids, reducing sugars, anthraquinones and glycosides. All medicaments significantly reduced blood glucose levels when compared with control alone ($P<0.05$) with the highest percentage reduction in blood glucose (64.86%) exhibited by metformin-extract combination. **Conclusions:** The leaf extract of *C. roseus* significantly increases the hypoglycemic effect of metformin.

1. Introduction

The concomitant use of herbs and drugs is a growing trend especially in the elderly and management of chronic ailment[1]. Most of the chronic diseases are difficult to treat successfully with orthodox drug[2,3]. Diabetes mellitus is one of these chronic aliments which has been acclaimed to be managed by traditional healer with over 400 plants reported to have anti-diabetic properties including *Vernonia amygdalina*[3], *Grongonema latifolium*, *Catharanthus roseus (C. roseus)*[4,5]. These plants extracts are sometimes prepared as polyherbal mixtures[6]. The objective of this study is to investigate the scientific basis for the folkloric use of *C. roseus* in the treatment of diabetes by traditional healers whose patients also take prescription oral hypoglycemic drugs. The English names of *C. roseus* include: cape periwinkle, rose periwinkle and "Old-maid"[5]. The use of *C. roseus* in cancer chemotherapy is perhaps, the most popular of all its uses[7]. In Congo, the stem and root of *C. roseus* are used against diarrhea while in Brazil, an infusion of the leaf is used against internal bleeding and scurvy, as mouthwash against toothache, for cleansing and healing of chronic wounds[8,9]. The fresh juice from the flowers of *C. roseus* has been reported to exert antimicrobial effect[10]. *C. roseus* has been shown to contain various constituents which are implicated for its numerous pharmacological activities. Four indole alkaloids found to inhibit cell growth have been discovered namely vincristine, vinleurosin, vinblastine and vinposidin[7].

2. Materials and methods

2.1. Plant material

Fresh stalks of *C. roseus* were collected from the botanical garden and environs of the University of calabar in the month of October 2009 and authenticated by Dr. Owolabi of the department of botany, university of calabar. The fresh leaves of *C. roseus* were plucked from the stem and

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air-dried at room temperature (26 ℃). The dried leaf was pulverized into powder using an electric blender. The powdered leaf (150 g) was extracted with methanol by cold maceration for 48 h[11], and filtered to obtain the methanol extract. Using a rotary evaporator at reduced pressure, the extract was concentrated and further dried in the oven, yielding a value of 11.37 g (7.58% w/w). The extract was subjected to phytochemical analysis using standard methods[11,12].

2.2. Animals

Fifteen (15) mice (18-32 g) and thirty five (35) albino rats of both sexes (150-220 g) bred in the Laboratory Animals facility of the Department of pharmacology and Toxicology, Madonna University, Elele, were used in the studies. The animals were maintained under standard laboratory situations and had free access to standard pellets (vital feeds, plc, Nigeria) and clean water. Prior to experimental uses, the animals were transferred to work area and allowed for two weeks of acclimatization.

2.3. Acute toxicity and lethality (LD_{50}) test

The acute toxicity and lethality tests (LD_{50}) of the methanol extract (ME) was determined, in mice, adopting the method described by Lorke[13].

2.4. Alloxan-induced diabetes test

The fasting blood glucose (FBG) of the albino rats was determined before induction of diabetes. Animals model Type 1 diabetes was then induced in the overnight-fasted animals by a single i.p. injection of 110 mg/kg alloxan monohydrate using distilled water as vehicle[14]. The FBG was then determined 48 h later to ensure induction of diabetes. Animals with blood glucose level of > 150 mg/dL were considered diabetic[9].

2.5. Animal grouping and experimental protocol

The diabetic animals were assigned into six groups of 5 rats each according to similar weights.

Group Ⅰ: Served as control group and was orally administered 0.2 mL of distilled water once daily.

Group Ⅱ: Were treated orally with 250 mg/kg of extract daily for 7 days.

Group Ⅲ: Were treated orally with metformin 100 mg/kg daily for 7 days.

Group Ⅳ: Treated orally with glibenclamide 1 mg/kg daily for 7 days.

Group Ⅴ: Treated orally with 100 mg/kg metformin and 250 mg/kg extract for 7 days.

Group Ⅵ: Treated orally with 1 mg/kg glibenclamide and 250 mg/kg extract for 7 days.

2.6. Determination of blood glucose

Glucometer (prestige Smart systems) was used for the determination of the blood glucose levels of the rats. Blood samples were obtained from the cut tail-tip of conscious rat and the glucose test-strip soaked with the blood was allowed to dry for 60 s and then inserted to be read by the glucometer. Basal and 48 h post-induction blood glucose levels were recorded. Thereafter, the extract, drug or drug-extract combinations were administered daily for 7 d. Blood glucose concentrations were measured at 2 h, 12 h, 24 h, 72 h and 168 h.

2.7. Statistical analysis

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

3.1. Phytochemical constituents

The phytochemical studies showed the presence of alkaloid, tannins, phlobatannin, flavonoids, terpenoids, glycoside, reducing sugar and saponins saponins and flavonoids were most abundant (Table 1).

Table 1
Phytochemical constituents of methanol extract of *C. roseus*.

Phytochemical constituents	Extract (7.58% w/w)
Saponins	+++
Alkaloids	++
Tannins	++
Phlobatannins	+
Flavonoids	+++
Triterpenoids	++
Reducing sugars	+
Anthraquinones	+
Glycosides	+

Value in parenthesis is the extractive yield. +++ = heavy presence; ++ = medium presence; + = slight presence.

3.2. Acute toxicity and lethal tests

The acute toxicity test (LD_{50}) of methanol extract was calculated to be 2 121.32 mg/kg.

3.3. Hypoglycemic activity

The blood sugar level of the extract and extract-drug combinations showed significant difference at 2 h ($P<0.05$ and $P<0.01$, respectively), when compared to the control. The standard drugs alone showed no significant difference ($P>0.05$). Glibenclamide however, started manifesting significant activity at 72 h ($P<0.05$). Greater significant difference was shown by metformin and metformin-extract combination ($P<0.01$). All treatment groups showed significant variations at 168 h when compared with control. Metformin-extract combination showed extremely significant difference ($P<0.01$) when compared with the administration of metformin alone. The highest percentage reduction in blood glucose was shown by metformin-extract combination at 72 h (64.86%). The control group did not show any significant reduction in the blood glucose level throughout the experimental period ($P>0.05$) (Table 2).

Table 2

Fasting plasma glucose levels of alloxan-induced diabetic rats at intervals during daily oral administration of methanol extract of *C. roseus* (mg/dL).

Group	Medication	Pre-induction FBG	Post-induction FBG	Fasting plasma glucose during treatment				
				2 h	12 h	24 h	72 h	168 h
Ⅰ	Distilled water (1 mL/kg)	47.50±2.99	311.60±37.07	412.20±47.91	414.20±64.82	506.60±30.48	505.40±31.94	542.80±16.83
Ⅱ	Extract (250 mg/kg)	46.75±5.25	205.67±70.48	97.00±33.55**	314.67±157.91	477.67±37.92	272.00±49.41**	128.67±11.84**
Ⅲ	Metformin (100 mg/kg)	44.70±4.42	360.40±59.06	224.00±81.78	226.60±59.71	316.60±55.11	211.10±48.83**	372.00±46.11**
Ⅳ	Glibenclamide (1 mg/kg)	42.75±3.92	306.80±56.18	248.80±44.31	459.40±62.95	500.80± 26.41	365.20±49.26*	265.60±32.63**
Ⅴ	Metformin+extract	59.50±9.91	265.60±52.31	135.40±25.98*	236.80±38.03	206.40±68.95 **	99.00±28.56**	131.40±45.60**,△
Ⅵ	Glibenclamide+extract	48.00±3.18	336.00±88.81	182.40±64.48*	432.00±89.44	515.00±9.04	318.80±55.86*	148.40±57.53**

*P<0.05; **P<0.01 significant level when compared with control. △P<0.01 when compared with standard drug *ie.* metformin.

4. Discussion

The results obtained in this study showed that methanol extract of *C. roseus* possess hypoglycemic effect. The presence of flavonoids, alkaloids, tannins, anthraquinones, saponins, glycosides and reducing sugar corroborate previous studies which have shown that plant extract containing flavonoids, alkaloids and saponins do possess hypoglycemic activities[3]. Glibenclamide is known to lower glucose concentrations in the blood primarily by stimulating a first-phase release of insulin from functioning pancreatic beta cells in response to food and causes an increased sensitivity of body cells to endogenous insulin hence reducing insulin resistance. On the other hand, metformin stimulates tissue uptake of glucose and increase insulin receptor binding[15-18]. The extract of *C. roseus* can be said to act in similar fashion to glibenclamide, but enhanced response to glucose by glucose obligatory tissues such as brain, nervous tissue and red blood cells amongst others may not be ruled out as possible mechanism of action of the herbal extract[7]. The blood glucose lowering effect of *C. roseus* extract was more pronounced than metformin and glibenclamide. In addition to the pharmacodynamic factor (mechanisms of action of herb and drugs), pharmacokinetic tendencies of interaction such as enzyme inhibition may have played a role hence the delay in the appearance of significant difference between metformin-extract combination and metformin monotherapy until the 7th day[19]. Pharmacokinetic factors as alteration of absorption due to binding that has been reported with co-administration of some herbs[20], did not result in significant changes in hypoglycemic activity of the drugs. In conclusion, the leaf extract of *C. roseus* and metformin in alloxan-induced diabetic rats was synergistic whereas there was no observed interaction with glibenclamide. This gives credence to the folkloric use of *C. roseus* for the regulation of blood sugar.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Ohadoma SC, Nwosu PJC, Chilaka KC, Osuala FN, Nnatuanya I. Screening of aqueous extract of *Azadirachta indica* leaf for hypoglycemic effect in guinea pigs. *Afr J Sci* 2010; **10** (1): 2451-2458.

[2] Bailey C, Day C. Traditional plant medicines as treatment for diabetes. *Diabetes Care* 1989; **12**: 553-564.

[3] Nimenibo-Uudia R. Effect of *Vernonia amygdalina* in alloxan-induced diabetic albino rats. *J Med Lab Sci* 2003; **12**(1): 25-31.

[4] Oshumbi RA. The effect of ethanolic stem extract of *Gongronema latifolium* on blood glucose of normal and alloxan induced diabetic rabbits. *Niger J Health Biomed* 2006; **5**(2): 39-44.

[5] Srinivas N, Rabindra B. The juice of fresh leaf of *Catharanthus roseus* blood glucose of normal and alloxan-induced diabetic rabbits. *BMC Complement Altern Med* 2003; **3**: 4.

[6] Cheryl AL. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *J Ethnobiol Ethnomed* 2006; **2**: 45.

[7] Gordon SH. Alkaloids of *Vinca rosea*: a preliminary report on hypoglycemic activity. *Lloydia* 1964; **27**: 361.

[8] Nayak BS. Medicinal uses of *Catharantus roseus*. *BMC Complement Altern Med* 2003; **6**: 41.

[9] Shivananda N. Influence of ethanol extract of *Vinca rosea* on wound healing in diabetic rats. *Online J Biol Sci* 2006; **6**(2): 51-55.

[10] Sathiya S, karthikeyan B, Cheruth J. Antibiogram of *Catharanthus roseus* extracts. *Global J Mol Sci* 2008; **3**(1): 1-7.

[11] Trease GE, Evans WC. *Text book of pharmacognosy*. 15th ed. London: Bailliere Tindall; 1989, p. 315-679.

[12] Harbourne JB. *Phytochemical methods: a guide to modern techniques to plant analysis*. 2nd ed. London: Champman and Hall; 1988, p. 55-56.

[13] Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol* 1983; **54**: 272-289.

[14] Afia A, Mammir R, Washeed M. Comparison of long-term antihyperglycemic and hypolipidemic effects and between *Coccinia cordifolia* (linn) and *Catharanthus roseus* (Linn) in alloxan-induced rats. *Res J Med Sci* 2007; **2**(1): 29-34.

[15] Ohadoma SC. *Pharmacology made easy*. 1st ed. Nigeria: Reverend publishers; 2008, p. 352-361.

[16] Thirumalai T, Therasa SV, Elumalai EK, David E. Hypoglycemic effect of Brassica juncea (seeds) on streptozotocin induced diabetic male albino rat. *Asian Pac J Trop Biomed* 2011; **1**(4): 323-325.

[17] Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and in vitro antioxidant potential of Hybanthus enneaspermus (Linn) F. Muell in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed* 2011; **1**(4): 316-322.

[18] Oyedemi SO, Adewusi EA, Aiyegoro OA, Akinpelu DA. Antidiabetic and haematological effect of aqueous extract of stem bark of Afzelia africana (Smith) on streptozotocin-induced diabetic Wistar rats. *Asian Pac J Trop Biomed* 2011; **1**(5): 353-358.

[19] Blumenthal M. Interactions between herbs and conventional drugs: introductory considerations. *Herbalgram* 1998: **49**: 52-56.

[20] Fakeye T, Oladipupo T, showande O, Ogunremi Y. Effect of co-administration of extract of *Carica papaya* on activity of two hypoglycemic agents. *Trop J Pharm Res* 2007; **6**(1): 671-678.