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Concomitant Administration of *Catharanthus roseus* Extract Improves Efficacy and Safety by Nullifying Diarrhea-Related Toxicity of Acarbose and Metformin in Wistar Rats

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ABSTRACT

The study conceptualized the sites and mechanisms of action as well as explored the interacting benefits of *Catharanthus roseus (C. roseus)* against the diarrhea-like toxicity associated with the hypoglycemic activity of acarbose and metformin using experimental rats. The diabetes was induced with alloxan. Diarrhoea was induced with castor oil (1 mL per rat) after 30 min of determining blood glucose levels of the different groups including additional Group VIII which received loperamide (3 mg/kg) serving as positive control. The sugar level was determined using a glucometer; while inhibition percent of faecal count and faecal weight were employed to determine antidiarrheal activity. All medicaments showed significant lowering of blood glucose levels when compared with control alone ($P < 0.05$) having the combination of extract-metformin-acabose extremely significant ($P < 0.01$), followed by extract-metformin. Also, significant antidiarrheal activity was shown in all groups treated with *C. roseus*, with highest percentage inhibition in fecal count 86.72% and fecal weight 89.78% exhibited by extract-metformin-acarbose combination compared to loperamide (86.74% and 81.17%, respectively). The extract of *C. roseus* enhances hypoglycemic efficacy and safety by significantly nullifying diarrheal-like adverse effects of acarbose and metformin.

Keywords: *Catharanthus roseus*, Acarbose, Metformin, Diarrhea-related toxicity, Concomitant administration.

Introduction

Therapeutic effectiveness alone is not the basis for the choice of drugs used in therapy.[1] There are measurable biological characteristics referred to as biomarkers that can differentiate response to a therapeutic agent.[2] One of them is Fasting Serum C-peptide-a biomarker of insulin production as well as its resistance.[3] Incidence and prevalence of diabetes has gone from 108 million to 422 million in 1980 and 2014, respectively.[4] International Diabetes Federation (IDF) highlighted that worldwide, estimated rise has continuously prevailed yearly, 415 and 451 million in 2015[5] and 2017,[6] respectively and diabetes accounting for 8-12% of mortality globally as at 2017.[7, 6] Safety considerations also should form part of the indices for choice of drug therapy.

This explains why thalidomide - a potent and efficacious sedative and antianxiety drug first marketed in West Germany in 1957 was withdrawn from European market in 1961 due to its teratogenicity.[8] Some other agents are not withdrawn but are not used adequately. Biguanides fall into this category as they are known to cause lactic acidosis[9] especially phenformin; alpha-glucosidase inhibitors fall victim of this due to flatulence and diarrhoea associated with their usage.[10]

Lactic acidosis manifests with over production or underutilization of lactic acid causing the patient's liver and kidneys the problem of eliminating excess acid from the body thereby leading to pH level imbalance, which normally always should be slightly alkaline not acidic.[11] And among others, abdominal discomfort and diarrhoea remain the prominent symptoms of lactic acidosis;[12] thereby making diarrhoea a common denominator of both alpha-glucosidase inhibitors and biguanides in terms of adverse effect. *C. roseus* is known by many common names prominent among them is Madagascar periwinkle. Other English names of *C. roseus* include: bright eyes, old maid, rose periwinkle, cape periwinkle, pink periwinkle.[14] Apart from its use in cancer treatment, *C. roseus* has been reported to exert numerous pharmacological activities including wound healing,[16] antimicrobial,[17] hypoglycemic,[18] and antidiarrhoea[19] activities. Various phytoconstituents contained in *C. roseus* including four indole alkaloids (vincristine, vinblastine, vinposidin, and vinleurosin) have been found to inhibit cell proliferation and implicated in its many pharmacological activities.[15,19]

Materials and Methods

Plant material: Preparation and extraction

Mature *C. roseus* leaves were collected from the environs of the University of Calabar, Nigeria in February 2020. A botanist from the Department of Botany, University of Calabar, Nigeria authenticated the plant sample (Herbarium number DB-CAL-022-20). The fresh

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leaves of *C. roseus* were air-dried at room temperature and pulverized using an electric blender into powder. By cold maceration, 300 g of the powdered leaf was extracted with 1 L of methanol (Sigma Aldrich, Hamburg, Germany) for 48 h. The mixture was subjected to filtration to obtain the methanol extract. At reduced pressure, a rotary evaporator (RV 05 Basic 1B, 1KA Staufen, Germany) was employed to concentrate the extract which was further oven-dried and stored in a refrigerator.

Animals

Forty (40) Wistar rats (160 – 220 g) of both sexes were used for the studies. The rodents were bred in the Laboratory Animal facility of the Department of Pharmacology, University of Calabar, Nigeria; and maintained with free access to standard pellets (Vital feeds, Plc, Lagos, Nigeria) and clean water. Ethical approval was sort and approved (IACUC/UNICAL /019/20) and the animals were handled in accordance to the Guide for the Care and Use of Laboratory Animals.[20] The rats were transferred to the work area, prior to experimental use and allowed for 14 days acclimatization.

Alloxan-induced diabetes test

Before induction of diabetes, the fasting blood glucose (FBG) of the animal was determined. Diabetes was induced by single intraperitoneal (ip) administration of 110 mg/kg alloxan monohydrate in distilled water (vehicle) to, overnight-fasted Wistar rats.[21] To ensure induction of diabetes, the FBG was then determined (48 h) after alloxan injection in which blood level >150 mg/dL were considered diabetic rats.[22]

Experimental protocol and animal grouping

The diabetic rats were assigned into seven groups of 5 rats each.
Group I: received orally 0.2 mL of distilled water once daily and served as control group.
Group II: received orally 250 mg/kg of extract daily for 7 days.
Group III: received orally 100 mg/kg of metformin daily for 7 days.
Group IV: received orally 5 mg/kg of acarbose daily for 7 days
Group V: received orally 100 mg/kg of metformin and 250 mg/kg of extract daily for 7 days.
Group VI: received orally 5 mg/kg of acarbose and 250 mg/kg of extract daily for 7 days.
Group VII: treated orally with 5 mg/kg acarbose + 100 mg/kg + 250 mg/kg extract daily for 7 days.

Determination of blood glucose

The blood glucose levels of the rats was determined with a glucometer. From the cut tail-tip of conscious rat, blood samples were obtained. The glucose test-strip was soaked with the blood and allowed to dry for 60 s before insertion to be read by the glucometer. Pre-induction (basal) and 48 h post-induction blood glucose levels were noted and recorded. After that, the distilled water, extract, Metformin, Acarbose, metformin-extract acarbose-extract, or metformin-acarbose-extract combinations were administered daily for 7 d. The level of blood glucose were measured and recorded at 2 h, 12 h, 24 h, 72 h, and 168 h.

Determination of anti-diarrhoeal activity

Diabetic rats pretreated with distilled water (negative control), extract, metformin, acarbose, tetformin-extract, acarbose-extract, and acarbose-metformin-extract combinations were all administered castor oil orally to induce diarrhoeal. Another group (the 8th) was given loperamide, positive control group.
Thirty (30) min after determining blood glucose of the different groups, pure castor oil (Oil Worth Enterprise Pvt Ltd. Comp., Punjab) was administered orally to all 8 groups of rats using oral gavage (1 mL per rat). Stool was collected, 1 h after the castor oil was given and the subsequent collections were done at a one-hour interval for 3 h. Each filter paper was previously weighed and put on the floor of separate cages and were changed hourly for the whole duration.

The degree of diarrhoea was assessed by courting the total number of stool; calculating, the total weight of stool on each filter paper hourly for the duration of 3 h. The percentage of inhibition of diarrhea was determined using the following formulae:

$$\text{Percentage inhibition (Fecal count)} = \frac{stool\ count\ (control) - stool\ count\ (treatment)}{Stool\ count\ (control)} \times 100$$

$$\text{Percentage inhibition (stool weight)} = \frac{stool\ weight\ (control) - stool\ weight\ (treatment)}{Stool\ weight\ (control)} \times 100$$

Statistical analysis

Data were analyzed using the SPSS version 20 (IBM SPSS Corp. Armonk, NY, USA), One-way ANOVA at $P < 0.05$ level of significance.

Results and Discussion

At 168 h, all treatment groups manifested significant variations when compared with the control group. The triple combination of extract-metformin-acarbose showed extremely significant different ($P < 0.01$) when combined with metformin or acarbose alone; followed by extract-metformin combination. The control group did not have manifest significant decrease in the blood glucose level ($P < 0.05$). However, the blood sugar level of the extract and various extract-drug(s) combinations started showing significant difference at 2 h (Table 1). The results showed that extract of *C. roseus* improves hypoglycemic efficacy and safety of acarbose and metformin. Acarbose and other alpha-glucosidase inhibitors (miglitol and voglibose) used as third-line drug[10] because flatulence and diarrhoea are major limitations despite the facts that they significantly reduce post prandial glucose, do not cause weight gain but improve glycemic control.[25] The short term goal of AGIs therapy is to reduce current level of blood glucose, the long term target is to diminish HbAIc (glycated haemoglobin) level.[10] Metformin and other biguanides such as phenformin on the other hand, precipitate lactic acidosis[9, 11, 12] and lactose intolerance-caused diarrhoea,[26] which of course, call for concern despite being effective even in the absence of functional pancreatic beta cells. The extract of *C. roseus* in the study showed antidiarrhoeal activity in the presence of both acarbose and metformin. This may not be unconnected with the numerous phytochemicals,[18,19,27] and corroborate documented studies which have highlighted that plant extract containing flavonoids, saponins and alkaloids do possess hypoglycemic[28, 29] and antidiarrhoeal activities.[23,24] These secondary metabolites occur as complexes of structurally related compounds.[30].Diarrhoea is defined as loose, watery bowel movements that occur frequently, and may present a danger of dehydration due to fluid loss.[26] Acarbose cause diarrhea based on the understanding that alpha-glucosidase is a membrane bound enzyme in the small intestines, known to hydrolyze and reduce carbohydrates into glucose molecules. The mechanism of action of AGIs is by inhibition of the enzyme thereby preventing the digestion of complex carbohydrate to glucose (monosaccharide) molecules hence less glucose will be available for absorption; while carbohydrate remains in the ileum and later delivered to the large intestine (colon) where bacteria act upon the complex carbohydrates for the purpose of digestion leading to diarrhoea, flatulence and other gastrointestinal adverse effects[31] referred to as colonic starch fermentation.[32] Increasing the influx of glucose into skeletal muscles, stimulation of glycolysis in tissues, and increased insulin-receptor bindings are the main mechanisms of action of biguanides and found to be effective even independent of functional beta cells yet are less widely used than sulphonylurea because of the tendency to cause lactic acidosis.[31-33]
The possible mechanism of action of *C. roseus* extract can be said to mimic glibenclamide but may not rule out enhanced response to glucose through glucose obligatory tissues such as red blood cells, brain and nervous tissues among others.[15] Both the mechanisms of action of drugs and herb (pharmacodynamics factor) and

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pharmacokinetic attributes of interaction including enzyme inhibition[34,35] may have been involved in delayed manifestation of significant difference between the combination therapies and monotherapy until 168 h (7th day) (Table I). The highest percent inhibition of stool count was 86.7% observed in loperamide serving as control group and combined group of extract-metformin-acarbose (86.72%) while the lowest was from extract-acarbose group (Table 2). The differences in mean of the treatments ranged from 0.02 to 18.93%. The mean percentage inhibition of stool weight was highest in Group VII - acarbose-metformin-extract (89.78%) ($P < 0.01$) and the lowest was 53.80% observed in Group VI - acarbose-extract showing significant ($P < 0.05$) compared with the positive control. On the antidiarrhoeal outcome of *C. roseus* (Table 2) in the presence of acarbose and metformin, the mechanism of action is believed to depend on its ability to enhance reabsorption of intestinal fluids. This is in consonance with reports that plant secondary metabolites in *C. roseus* improve reabsorption of intestinal fluids hence contributing to their antidiarrhoeal properties.[36, 23] Castor oil promote and stimulate diarrhoea because its active component-ricinoleic acid, is involved in cascades of events that lead to frustration of re-absorption of K^+, Na^+, and water thereby causing diarrhoea. This causes irritation of the intestinal mucosa which triggers release of endogenous nitric oxide and prostaglandins furthering GIT secretion, motility, oedema and epithelial permeability[1,36] Concisely, the antidiabetic and castor oil-caused diarrhoea in GIT. The former prevents digestion of complex carbohydrate to glucose (monosaccharides) molecules causing irritation due to action of intestinal bacteria leading to diarrhea and other gastrointestinal effects. While the latter due to cascades of events frustrate the reabsorption thereby causing diarrhoea. The cascade of events includes irritation of the intestinal mucosa. So the extract arrests irritation in the GIT whether caused by castor oil or the antidiabetics. This suggests why it reduced diarrhoea as shown in the data (Table 2).

Conclusion

Catharanthus roseus improved hypoglycemic and decreased diarrhea attributes of acarbose and metformin thereby enhancing their efficacy and safety.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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

		Fasting plasma glucose during treatment						
		Pre- Induced	Post-induction					
	Treatment	FBG	FBG	2 h	12 h	24 h	72 h	168 h
I	Distilled H_2O (1 ml/kg)	46.50 ± 2.9	310.60 ± 37.1	411.20 ± 47.9	413.20 ± 64.8	505.60 ± 30.5	504.40 ± 31.9	413.20 ± 64.8
II	Extract (250 mg/kg)	45.75 ± 5.2	204.67 ± 70.4	96.00 ± 33.5 **	313.67 ± 57.9	476.67 ± 37.9	271.00 ± 49.4**	127.67 ± 11.8 **
III	Metformin (100 mg/kg)	43.70 ± 4.4	359.40 ± 59.1	223.00 ± 81.8	225.60 ± 59.7	315.60 ± 55.1	210.10 ± 48.8**	371.00 ± 46.1**
IV	Acarbose (5 mg/kg)	41.75 ± 3.9	305.80 ± 56.2	247.80 ± 44.	458.40 ± 62.9	499.80 ± 26.4	364.20 ± 49.2*	264.60 ± 32.6**
V	Extract + Metformin	58.50 ± 9.9	264.60 ± 52.3	134.40 ± 25.9 *	235.80 ± 38.1	205.40 ± 68.9 **	98.00 ± 28.5 **	130.40 ± 45.6 **◊
VI	Extract + Acarbose	47.00 ± 3.2	335.00 ± 88.8	181.40 ± 64.4 *	431.00 ± 89.4	514.00 ± 9.0	317.80 ± 55.8 *	147.40 ± 57.3 **
VII	Extract + metformin+Acarbose	50.60 ± 4.3	360.40 ± 59.0	135.60 ± 35.1 *	230.60 ± 35.0	200.67 ± 40.6 **	99.00 ± 25.5 **	120.30 ± 50.2**◊

* $P< 0.05$; ** $P < 0.01$ significant level compared with control. ◊$P < 0.01$ when compared with standard drug

Table 2: Percent inhibition of fecal count and fecal weight during time t = 1, 2, and 3 h

		Faecal count (%)				Faecal weight (%)			
	Treatment	1h	2h	3h	Mean	1h	2h	3h	Mean
I	Distilled H_2O (1 mL/kg)	-	-	-	-	-	-	-	-
II	Extract (250 mg/kg)	65.34	65.82	81.44	70.87	35.84	37.54	89.43	52.27
III	Metformin (100 mg/kg)	-	-	-	-	-	-	-	-
IV	Acarbose (5 mg/kg)	-	-	-	-	-	-	-	-
V	Extract + Metformin	63.75	75.56	67.80	69.04	74.36	64.71	56.35	65.14
VI	Extract + Acarbose	75.66	74.50	53.28	67.81	72.96	73.24	15.19	53.80
VII	Extract + metformin+Acarbose	85.19	96.30	78.66	86.72	89.13	98.98	81.23	89.78
VIII	Loperamide (control) (3 mg/kg)	85.19	87.83	92.80	86.74	83.93	92.49	67.09	81.17

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