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Effect of bitter leaf (*Vernonia amygdalina*) aqueous extract on pancreatic α-amylase and intestinal α-glucosidase repressive potentials of Acarbose

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ABSTRACT: There is a continuous search for antidiabetic drugs. The available synthetic drugs, including Acarbose could only provide temporary relief with some negative effects. This has resulted in the co-administration of Acarbose with plants possessing antidiabetic potential such as bitter leaf. In Nigeria, co-administration of bitter leaf (BL) vegetable with acarbose (a synthetic inhibitor of α-amylase and α-glucosidase) in the management of Type-2 diabetes mellitus is on the increase, basically to potentiate the therapeutic value and amelioration of side effects of Acarbose. However, there is no information on the influence of BL on the effectiveness of Acarbose. This study determined the influence of BL on the α-amylase and α-glucosidase inhibitory properties of Acarbose. BL extract, acarbose and their combinations (75:25, 50:50, and 25:75) were prepared and their effect on α-amylase and α-glucosidase, and the production of Fe^{2+}-induced thiobabituric reactive acid species (TBARS) in pancreas homogenate were determined. Results revealed that all the combinations exhibited the antagonistic effect except 50:50 combined ratios, which exhibited additive effects. All the samples inhibited TBARS production except 100% Acarbose. This study shows that the use of bitter leaf with Acarbose could reduce the potency of Acarbose or the bitter leaf.

Keywords: Acarbose, α-amylase, α-glucosidase; bitter leaf; food-drug interaction

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Introduction

Diabetes mellitus is among the world's leading metabolic diseases, affecting millions of people across the globe (Maiti *et al.,* 2004). The growing incidence of diabetes and unfavourable side effects of controlling synthetic drugs has geared researchers to search for natural products with minimal side effect, and herbal materials rich in phenolic compounds have demonstrated equal if not the same potential with synthetic drugs (Adefegha *et al*., 2016; Erasto et al., 2009; Oboh *et al.,* 2015). An example of such plants

is bitter leaf (*Vernonia amygdalina* Del. Family: Astercieace), locally known as "Ewuro" by Yoruba speaking people of Nigeria. BL is widely consumed as a vegetable and for the management of Type-2 diabetes, having α-amylase and α-glucosidase inhibitory properties (Atangwho *et al.,* 2013; Leonard *et al.,* 2002; Oboh *et al.,* 2016). As a result, it is often consumed locally with Acarbose to potentiate the therapeutic value and reduce the side effects of Acarbose without considering possible food-drug interaction.

Acarbose is an effective synthetic antidiabetic drug (Ademiluyi *et al.,* 2016; Oboh *et al.,* 2016b). It acts by inhibiting the activities of α-amylase and α-glucosidase, hence, controlling the release of blood glucose level (Adefegha *et al.,* 2016). However, this drug could only provide temporary relief with attendance abdominal discomfort, flatulence among other side effects (Ademiluyi *et al.,* 2016; Adefegha *et al.,* 2016). In this study, the effects of phenolic-rich extract from bitter leaf (BLE), acarbose and their various combinations on α-amylase and α-glucosidase activities, and on the production of Fe^{2+}-induced thiobarbituric reactive acid species (TBARS) in pancreas homogenate. Phenolic constituents of bitter leaf were also determined using HPLC-DAD.

Materials and Methods

Chemical and reagent

Acarbose was procured from Glenmark Generics Limited, Middlesex, UK. Porcine α-amylase and pancreatic α-glucosidase were procured from Sigma Aldrich Co. Other chemicals and reagents were of analytical grade. The water used was glass distilled.

Collection of Bitter leaf and samples preparation

Bitter leaf was freshly harvested from a farmland, and authenticated by A. A. Sorungbe (voucher numbers FUTA/BIO/201), Department of Biology, Federal University of Technology, Akure. The leaf was air-dried and blended into powdery form using laboratory blender. Thereafter, the phenolic-rich extract and 25 mM of acarbose were prepared in accordance with the report of Adefegha *et al*., (2016). Acarbose (25 mM) and bitter leaf extract (BLE, 0.1 mg/ml) were prepared with distilled water. Thereafter, sample mixtures were prepared as follows: 100% ACA; 75% ACA: 25% BLE; 50% ACA: 50% BLE; 25% ACA: 75% BLE; 100% BLE. All samples were stored at 4°C for subsequent analysis.

α-Amylase and α-glucosidase activities assays

The effects of the samples on α-amylase and α-glucosidase activities were carried out using the methods described in the study of Ademiluyi *et al*., (2016). The enzyme inhibitory effects of the samples were expressed as percentage inhibition.

Handling of experimental animals

Wistar strain male albino rats (weighing between 180 and 210g) were purchased from the Animal Breeding Colony of Animal Health and Production (APH) Department, Federal University of Technology, Akure for this study, and their handling were in accordance to the regulation published by the National Institutes of Health (NIH, 2011) for the care and Use of Laboratory Animals, and Ethics Committee of the Federal University of Technology, Akure, Nigeria. They had access to the feed and water ad libitum for 14 days of acclimatization period.

Preparation of pancreas homogenate and lipid peroxidation assay

The rats' pancreas tissue was isolated and placed on ice, weighed and rinsed in cold 0.9% normal saline (1:3, w/v), subsequently homogenized in phosphate buffer (pH 7.4). The homogenate was centrifuged at 5,000 × g. The clear supernatant obtained was used for thiobarbituric acid reactive species (TBARS) assay according to the method described by Shodehinde et al. (2017). The TBARs produced was measured at 532 nm and subsequently calculated.

Determination of reducing power, Fe^{2+} chelating and hydroxyl radical scavenging abilities of the samples

The reducing power of the samples was determined by assessing the ability of the samples to reduce $FeCl_3$ solution as described by Pulido et al., (2000), and was subsequently calculated and expressed as ascorbic acid equivalent (AAE). The ability of the samples to chelate Fe^{2+} was carried out using the method described by Shodehinde *et al*. (2017). The method of Halliwell and Gutteridge, (1981) was used to determine the scavenging ability of the hydroxyl (OH) radical produced from Fe^{2+}/H_2O_2 induced decomposition of deoxyribose.

High Performance Liquid Chromatography Analysis (HPLC-DAD)

The quantification of phenolic compounds in the extract was carried out according to the method described by Ademosun *et al*. (2015) using high performance liquid chromatography coupled with Diode-Array Detector (HPLC-DAD). The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). Chromatography operations were carried out in triplicates under ambient condition. Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Ademosun *et al*. (2017).

Data Analysis

The results of triplicate experiments were pooled and expressed as mean ± standard deviation (SD). Means were compared by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Significant difference was accepted at $p \leq .05$. GraphPad Prism version 5.00 for Windows was used for statistical analysis.

Results and Discussion

Regulation of blood glucose level is one of the therapeutic points in the management of T2DM. Acarbose, an α-amylase and α-glucosidase inhibitor is being used to control blood glucose level (Ademiluyi *et al.,* 2016; Adefegha *et al.,* 2016). Although, about 2% of acarbose could be absorbed, after oral dosing (Oboh *et al.,* 2016b), however, according to Boath et al. (2012), co-administration with some berries exhibited synergistic effect on α-glucosidase activities *in vitro,* and possibly reduced its side effects. In this study, Acarbose, BLE and their various combinations inhibited α-amylase and α-glucosidase activities (Fig. 1). The various combinations exhibited reduced α-amylase inhibitory effect compared to 100% ACA, but higher than that of 100% BLE (Fig. 1A). The combinations also exhibited lower α-glucosidase inhibitory effect when compared to 100% ACA (Fig. 1B). Interestingly, 100% BLE had high α-glucosidase (71.76 ± 1.20%) inhibition than α-amylase (48.95 ± 2.01%). The effect of the BLE on α-amylase and α-glucosidase agree with earlier reports on the mild inhibition of α-amylase activity, but stronger α-glucosidase inhibitory properties of plant materials (Ademiluyi *et al.,* 2016), which could be an added advantage over acarbose that has stronger α-amylase inhibitory properties (Adefegha *et al.,* 2015). Chlorogenic and caffeic acids, rutin and quercetin, which were among the phenolic identified in bitter leaf, have been reported to inhibit α-amylase and α-glucosidase activities (Adefegha *et al.,* 2015). Therefore, we could affirm that the enzymes inhibitory effect of the extract could be linked with the phenolic. However, contrary to the reports of Oboh et al. (2016b), Adefegha et al. (2016) and Boath et al. (2012), that phenolic content of the plant materials may potentiate the therapeutic value of Acarbose in the management of T2D, this study shows that there could be a conflicting effect when bitter leaf is consumed with acarbose

The antioxidative properties of the samples were also determined, via four antioxidant assays method: inhibition of Fe^{2+}-induced TBARs production, ferric reducing antioxidant power (FRAP), Fe^{2+} chelation and OH radical scavenging abilities. The incubation of rat's pancreatic homogenate with 250

mM $FeSO_4$ leads to significant ($p < 0.05$) increase in TBARs (173.2 ± 3.17%) content relative to the basal (100%) (Fig 2A). But, the addition of the samples reversed the TBARs levels while the combinations and 100% BLE had a stronger inhibitory effect on TBARs production compared to 100% ACA. The result of the reducing power (FRAP) of the samples shows that the 100% BLE had the highest ($p < 0.05$) reducing property compared to the combinations, while 100% ACA had the least (Fig 2B). Figure 2C revealed that the combinations and 100% BLE had higher Fe^{2+} chelating abilities compared to 100% ACA. The result of the hydroxyl radical scavenging ability (Fig.2D) revealed that the samples scavenged OH radical, and 100% BLE had the highest, while 100% ACA had the least.

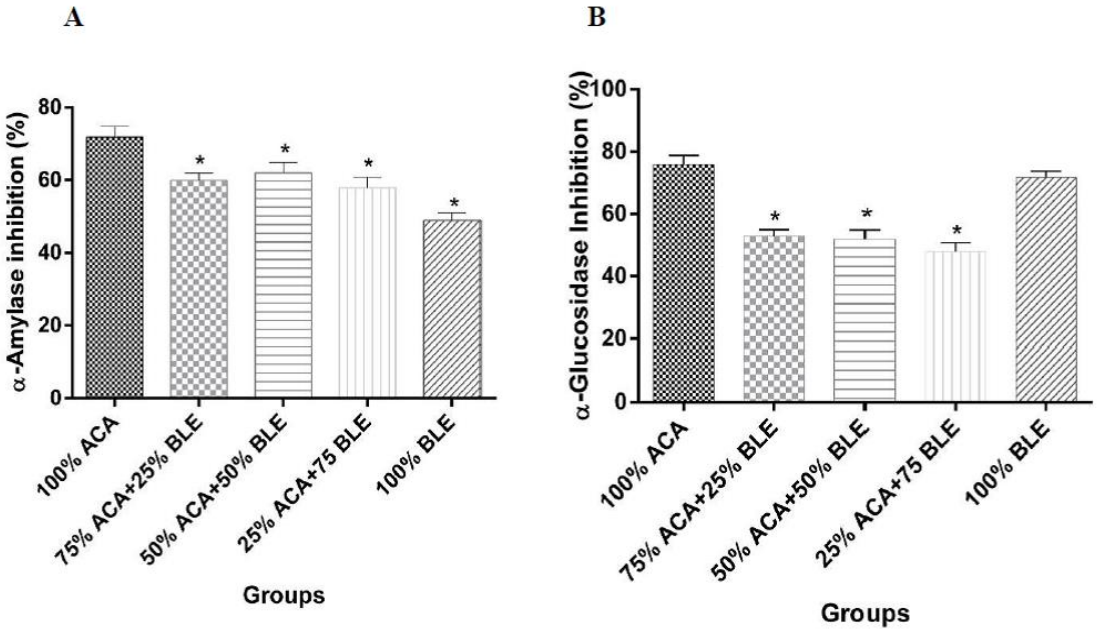

Figure 1: α-Amylase (A) and α-glucosidase (B) inhibitory abilities of various combinations of acarbose (ACA) and bitter leaf extract (BLE) (*$p < 0.05$ vs. 100% ACA).

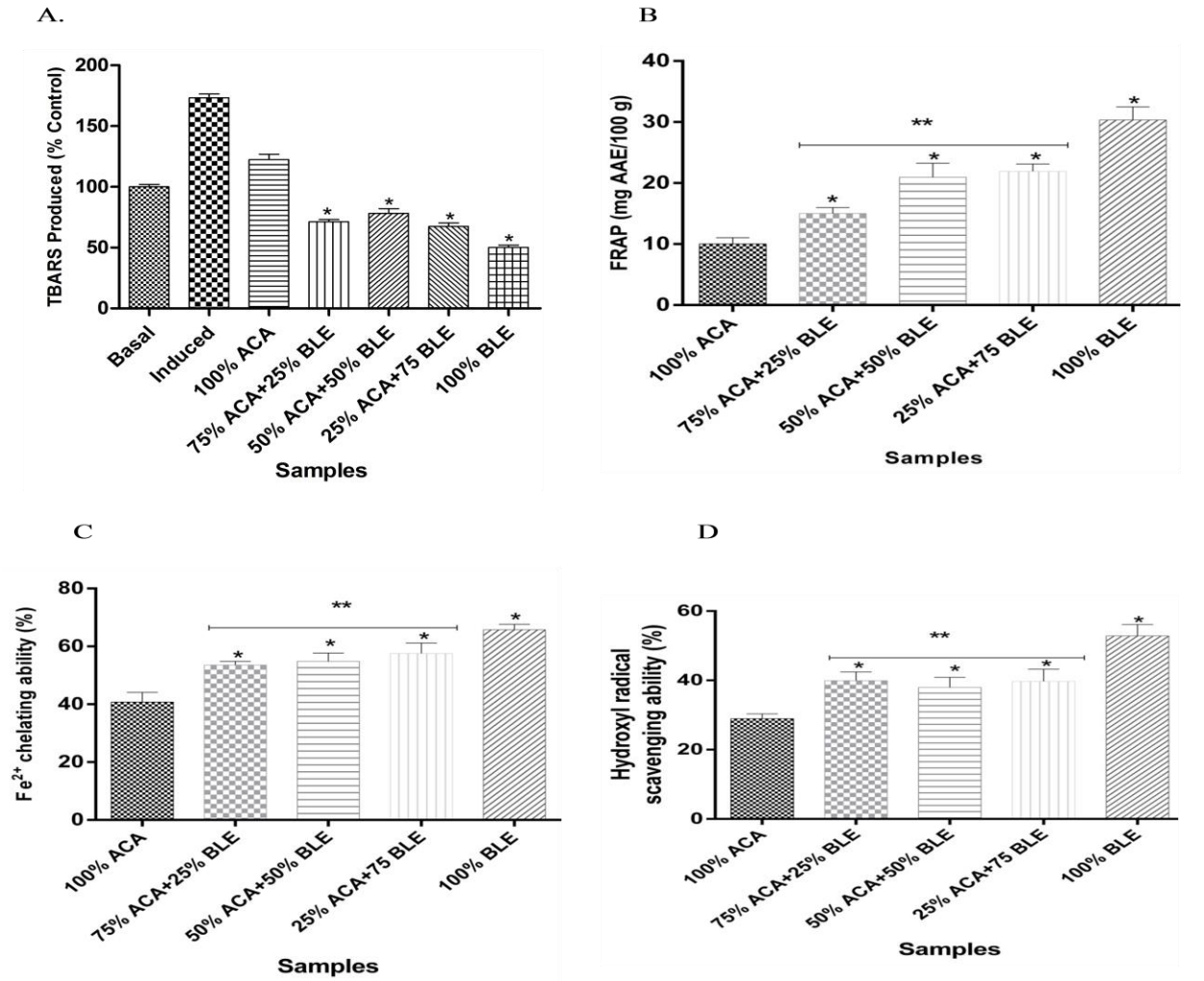

Figure 2: A-Inhibition of TBARS, B-Ferric reducing antioxidant power (FRAP), C-Fe 2+ chelating and D-hydroxyl radical scavenging abilities of various combinations of acarbose (ACA) and bitter leaf extract (BLE) (*$p < 0.05$ vs. 100% ACA, **$p < 0.05$ vs. 100% BLE).

Report has shown that pancreatic β-cells are prone to oxidative damage, probably due to limited antioxidant defense systems and attack from reactive species (Ademiluyi *et al.,* 2016). Although Fe^{2+} is very important and its quantity is required in a small amount for proper metabolic function of the biological system, however, its free form in the circulation can initiate production of reactive species and ultimately oxidative stress; a major culprit in the pathophysiology DM (Swaminathan *et al.,* 2007). This is confirmed by this study as incubation of 250 μM Fe^{2+} solution with the pancreas homogenate caused a significant ($p < .05$) increase in TBARS level. However, the addition of the samples caused reduction of TBARS level, which could either be as a result of ferric-to-ferrous reducing ability of the samples (Fig 2B) or their Fe^{2+} chelating and hydroxyl radical scavenging abilities; an indication of their corresponding concentration of electron donating antioxidants molecules of the samples (Halvorsen *et al.,* 2002). Furthermore, the high antioxidant activity of 100% BLE compared to 100% ACA and the combinations could be due to its phenolic composition (Table 1).

Polyphenols are considered to be strong antioxidant molecules capable of preventing oxidative damage to pancreatic β-cells, owning to their abilities to prevent TBARS production, reduction of ferric-to-ferrous, chelate transition metals and scavenging of radicals (Oboh *et al.,* 2015: Adefegha *et al.,* 2015),

due to its redox properties of their numerous hydroxyl groups (Adefegha *et al.*, 2015). Table 1 and Fig 3 represent the HPLC-DAD phenolic analysis of BLE, which revealed 9 phenolic compounds, four of which were phenolic acids, while the remaining five were flavonoids. Quercitrin (6.37 mg/g), luteolin (4.15 mg/g), p-coumaric acid (4.11 mg/g), rutin (4.11 mg), quercetin (3.72 mg/g) caffeic (2.05 mg/g), orientin (2.01 mg/g), chlorogenic acid (1.79 mg/g) were detected (Table 1) alongside with some trace phenolics (Fig 3). Reports have shown that the correlations between the antioxidant capacities of plant extracts and their phenolic constituents are statistically significant (Dudonné *et al.*, 2009; Katalinic *et al.*, 2006). Therefore, in this study, the observed varieties of phenolic acids and flavonoids, found in the BLE extract could be responsible for its biological activities, as well as the enhanced antioxidant activities of ACA as observed in the various combinations tested.

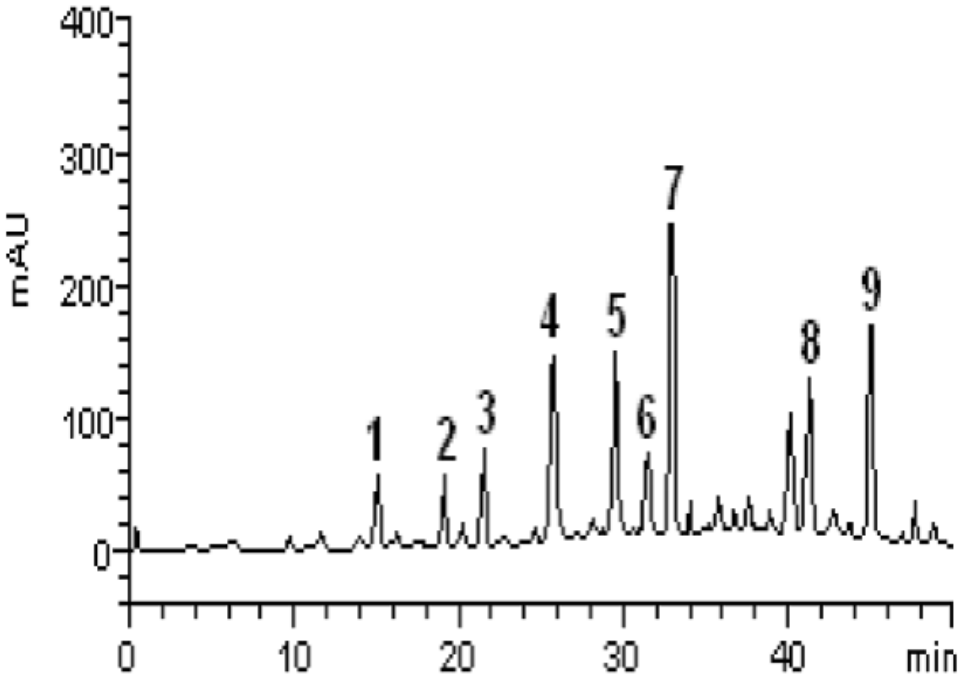

Figure 3 – Representative high performance liquid chromatography profile of bitter leaf extract. Catechin (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), p-coumaric acid (peak 4), rutin (peak 5), orientin (peak 6), quercitrin (peak 7), quercetin (peak 8) and luteolin (peak 9)

Conclusion

This study revealed that the phenolic-rich extract from bitter leaf and Acarbose, and their various combinations exhibited α-amylase and α-glucosidase inhibitory and antioxidant effects *in vitro*. The combinations exhibited reduced α-amylase and α-glucosidase inhibition compared to 100% ACA and BLE, while the extract appeared to enhance antioxidative effects of ACA. This study shows that the co-administration of bitter leaf and Acarbose could reduce the α-amylase and α-glucosidase inhibitory effect of Acarbose. However, BLE enhanced antioxidative potential of Acarbose.

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