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Original Article

Influence of gallic and tannic acid on therapeutic properties of acarbose *in vitro* and *in vivo* in *Drosophila melanogaster*

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ABSTRACT

Background: In this study, gallic acid (GA) and its polymeric form-tannic acid (TA) which are two phenolic acids found abundantly distributed in plant food sources were investigated for their influence on therapeutic properties of acarbose (AC) *in vitro* and *in vivo* in *Drosophila melanogaster*.

Methods: Combinations of AC and GA or TA were assessed for their alpha-glucosidase and alpha-amylase inhibitory effects as markers of anti-hyperglycemic properties, as well as their free radicals scavenging, Fe^{2+} chelating and malondialdehyde (MDA) inhibitory effects (*in vitro*). Furthermore, wild type *D. melanogaster* cultures were raised on diets containing AC, GA, TA and their various combinations for seven days. Thereafter, flies were homogenized and glucose concentrations, alpha-glucosidase and alpha-amylase activities, as well as reactive oxygen species (ROS) and total thiol levels were determined.

Results: The results showed that GA and TA up to 5 mg/ml significantly ($p < 0.05$) increased the enzymes' inhibitory effects and antioxidant properties of AC *in vitro*. Also, there was significant reduction in glucose concentration, enzyme activities and ROS level in *D. melanogaster* fed diets supplemented with phenolic acids and acarbose.

Conclusions: These bioactive compounds–drug interactions provide useful information on improving the therapeutic properties of acarbose especially in its use as an antidiabetic drug.

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At a glance commentary

Scientific background on the subject

Control of postprandial hyperglycemia remains one of the major therapeutic intervention for type-2 diabetes mellitus. This has been achieved through drugs such as acarbose that reduce activities of carbohydrate-metabolizing enzymes, but its use has limiting side effects. Plant polyphenols have been reported to be promising for the management of diabetes.

What this study adds to the field

Gallic acid and tannic acid, as well as diets rich in these phenolic acids could present as promising antioxidant and anti-hyperglycemic candidates on their own, as well as confer additional antioxidant benefits on the use of acarbose as anti-diabetic drug.

Type 2 diabetes mellitus (T2DM) has become a major health challenge globally. According to the World Health Organization fact sheet on diabetes [1], the number of people living with diabetes globally has risen by 74.4% between 1980 and 2014. In addition, the global prevalence of diabetes for adults (20–79 years) was estimated to be 6.4% in 2010 and projected to be 7.7% in 2030 [2]. The scourge of T2DM is still believed to be more prevalent in middle and low-income countries, with a projection of 69% increase in adult diabetic patients between 2010 and 2030 in developing counties [2]. Hence, T2DM is still a major health challenge requiring more attention.

In managing T2DM, therapeutic drugs such as acarbose and metformin are often used in clinical practices, but their uses are limited by their attendant side effects majorly gastrointestinal complications such as diarrhea, flatulence and abdominal distension [3]. Acarbose is an oral alpha-glucosidase and alpha-amylase (carbohydrate hydrolyzing enzymes) inhibitor used in the management of T2DM [4,5]. The enzyme alpha-amylase degrades complex dietary saccharides to oligosaccharides and disaccharides that are ultimately converted into monosaccharide by alpha-glucosidase [6]. Hence, inhibition of these enzymes is one of the therapeutic approaches for managing postprandial hyperglycemia [7].

In lieu of the several side effects of antidiabetic drugs such as acarbose, dietary interventions are often encouraged as long term complementary/alternative therapeutic measure for T2DM. Dietary polyphenols have been widely researched to possess antidiabetic and antioxidant properties essential for the management of T2DM. Gallic acid (GA) and its polymeric form-tannic acid (TA) are two polyphenols found abundantly in different plant food sources such as vegetables, fruits, green tea and legumes [8,9]. Previous studies [9–12] have shown that GA and TA exhibit antioxidant, antihyperglycemic, anticarcinogenic and antimicrobial properties. Furthermore, the concept of food–drug interactions in the management of chronic diseases is gaining popularity; earlier reports from our laboratory showed that GA synergizes the therapeutic properties of acarbose *in vitro* [9]. Similarly, clinical management of T2DM often involves a multidimensional approach in which patients could be placed on antidiabetic drugs alongside regimented antidiabetic diets [13] which could contain one or more bioactive constituents with antioxidant properties and blood glucose lowering effects.

The use of non-vertebrate models for various biological research is gaining more attention with the fruit fly *(Drosophila melanogaster)* attracting most attention recently. The use of *D. melanogaster* to study metabolic diseases including T2DM is gaining popularity. The isolation and characterization of alpha-amylase and alpha-glucosidase which have both been linked to maintenance of glucose homeostasis in the flies, as well as the presence of eight drosophila insulin-like peptides (DILP1-8) that share functional similarities with the mammalian insulin/IGF pathway have made *D. melanogaster* a useful model for studying glucose homeostasis, as well as pathogenesis and progression of diabetes [14–16]. In addition, the relative ease of culturing, short generation time, small genome, absence of ethical constraints and ease of duplicability of experiments have made these flies quite useful for biomedical research works [17]. Therefore, in this study, we sought to investigate the effects of GA and TA on the antidiabetic properties of acarbose *in vitro* and *in vivo* in *D. melanogaster*. This is aimed to evaluate if these food–drug interactions could possibly offer improved therapeutic management strategy for T2DM.

Materials and methods

Chemicals and reagents

Chemical reagents such as 5,5′-dithio-bis-(2-nitrobenzoic acid (DTNB), 2′,7′-dichlorodihydrofluorescein diacetate, 2-diphenyl-1picrylhydrazyl (2 DPPH), thiobarbituric acid (TBA), GA, TA, porcine pancreatic alpha-amylase and 1,10-phenanthroline were procured from Sigma Aldrich Co. (St Louis, Missouri, USA). Trichloroacetic acid (TCA) was sourced from Sigma Al-drich, Chemie GmbH (Steinheim, Germany). Hydrogen peroxide, methanol, acetic acid, hydrochloric acid, aluminium chloride, potassium acetate, sodium dodecyl sulphate, iron (II) sulphate, potassium ferricyanide and ferric chloride were sourced from BDH Chemicals Ltd., (Poole, England). Ascorbic acid and starch were products of Merck (Darmstadt, Germany). Acarbose was purchased from Glenmark Generics (Europe) pharmaceutical limited. Except stated otherwise, all other chemicals and reagents were of analytical grades and the water was glass distilled.

In vitro study

Sample preparation

Aqueous solutions of Acarbose, Tannic acid and Gallic acid were prepared and sample mixtures prepared thus: AC = Acarbose (0.5 mg/ml), GA = Gallic acid (1 mg/ml), TA = Tannic acid (1 mg/ml), AC + 1PA = Acarbose + 1 mg/ml Phenolic acid

(Gallic or Tannic acid), AC + 2PA = Acarbose + 2 mg/ml Phenolic acid (Gallic or Tannic acid), AC + 3PA = Acarbose + 3 mg/ml Phenolic acid (Gallic or Tannic acid) and AC + 5PA = Acarbose + 5 mg/ml Phenolic acid (Gallic or Tannic acid).

All samples were kept in the refrigerator at 4 °C for subsequent analysis.

Alpha glucosidase activity assay

Appropriate dilution of samples (50 μl) and 100 μl of alpha-glucosidase solution (EC 3.2.1.20; 1.0 U/ml) in 0.1 M phosphate buffer (pH 6.9) was incubated at 25 °C for 10 min. Thereafter, 50 μl of 5 mM p-nitrophenyl-alpha-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25 °C for 5 min and the absorbance was read at 405 nm in the spectrophotometer. The alpha-glucosidase inhibitory activity was expressed as percentage inhibition [18].

Alpha amylase activity assay

The alpha amylase activity assay was carried out according to a previously reported method [19]. The sample dilution (500 μl) and 500 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/ml Hog pancreatic alpha-amylase (EC 3.2.1.1) were incubated at 25 °C for 10 min. Thereafter, 500 μl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each reaction mixture. The reaction mixtures were incubated at 25 °C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid (DNSA) color reagent. Thereafter, the mixture were incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance measured at 540 nm. The reference samples included all other reagents and the enzyme with the exception of the test sample. The percentage enzyme inhibitory activity of the extract was subsequently calculated.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability*

The scavenging ability of the samples against DPPH* 2, 2-diphenyl-1-picrylhydrazyl free radical was evaluated as described by Gyamfi et al. [20], with slight modifications. 1 ml of 0.4 mM DPPH* in methanol was mixed with 0.05 ml of the extract. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm in the spectrophotometer. The DPPH* free radical scavenging ability was subsequently calculated as percentage of the control.

Hydroxyl radical scavenging ability

The method of Halliwell and Gutteride [21] was used to determine the ability of the samples to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose. The extracts (0–100 μL) were added to a reaction mixture containing 120 μL of 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer, 40 μL of 500 μM of $FeSO_4$, and the volume was made up to 800 μL with distilled water. The reaction mixture was incubated at 37 °C for 30 min and the reaction was then stopped by the addition of 0.5 mL of 28% trichloroacetic acid. This was followed by the addition of 0.4 mL of 0.8% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in a spectrophotometer.

Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the samples was determined using the method of Minotti and Aust [22] as modification by Puntel et al. [23]. Freshly prepared 500 μmol/l $FeSO_4$ (150 μl) was added to a reaction mixture containing 168 μl of 0.1 mol/l Tris-HCl (pH 7.4), 218 μl saline and the extract (0–100 μl). The reaction mixture was incubated for 5 min before the addition of 13 μl of 0.25% 1, 10-phenanthroline (w/v).The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability of the extract was subsequently calculated as percentage of the control.

Inhibition of lipid peroxidation and thiobarbituric acid reactions

Albino rats were immobilized by cervical dislocation and the pancreas was rapidly isolated and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10-up-and –down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3000×*g* to yield a pellet that was discarded, and the low-speed supernatant (S1) was kept for lipid peroxidation assay [24]. The lipid peroxidation assay was carried out using the modified method of Ohkawa et al., [25]. Briefly 100 μl S1 fraction was mixed with a reaction mixture containing 30 μl of 0.1M pH 7.4 Tris – HCl buffer, sample (0–100 μl) and 30 μl of 250 μM freshly prepared $FeSO_4$. The volume was made up to 300 μl by water before incubation at 37 °C for 1hr. The color reaction was developed by adding 300 μl 8.1% SDS (Sodium dodecyl sulphate) to the reaction mixture containing S1, this was subsequently followed by the addition of 600 μl of acetic acid/HCl (pH 3.4) mixture and 600 μl 0.8% TBA (Thiobarbituric acid). This mixture was incubated at 100 °C for 1 h. Thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm and expressed using MDA (Malondialdehyde) equivalent.

In vivo study

D. melanogaster stock culture

D. melanogaster (Harwich strain) stock culture was obtained from Department of Biochemistry, University of Ibadan, Oyo State, Nigeria. The flies were maintained and reared on basal diet made up of corn meal medium containing 1% w/v brewer's yeast, 2% w/v sucrose, 1% w/v powdered milk, 1% w/v agar, and 0.08% v/w nipagin at constant temperature 23 ± 1 °C; and humidity (60% relative humidity) under 12 h dark/light cycle conditions. All the experiments were carried out with the same *D. melanogaster* strain. All experiments were carried

out in accordance with ethical standards of the Instruction for laboratory research.

Survival study

A study was conducted to assess the survival of the flies to acarbose, TA and GA. Flies (both gender, 3–5 days old) were divided into different groups containing 40 flies each. Each group was exposed to different doses of acarbose (0, 0.00088, 0.0018, 0.0035 and 0.007 mg/g of diet), TA (0, 0.2, 2.0 mg/g of diet) and GA (0, 0.2, 2.0 mg/g of diet). The flies were observed daily for the incidence of mortality and the survival rate was determined by counting the number of dead flies, for seven days. The data were subsequently analyzed and plotted as cumulative mortality and percentage of live flies after the treatment period [15,26].

Experimental design

In the first set of experiment, flies (both gender, 3–5 days old) were divided into 5 groups containing 40 flies each. Group A was placed on basal diet alone, while group B – E, were placed on normal diets containing TA and GA at 0.2 mg/g and 2.0 mg/g of diet each.

In the second set of experiment, flies (both gender, 3–5 days old) were divided into 6 groups containing 40 flies each. Group I was placed on basal diet alone, while group II – VI were placed on normal diet containing the following [Table 1];

Table 1 Experimental design.

Groups	
I	Basal diet
II	Basal Diet + Acarbose (0.0018 mg/g)
III	Basal Diet + Acarbose + 0.2 mg/g TA
IV	Basal Diet + Acarbose + 2 mg/g TA
V	Basal Diet + Acarbose + 0.2 mg/g GA
VI	Basal Diet + Acarbose + 2 mg/g GA

The dose of acarbose was selected based on survival study (data not shown) showing that 0.0018 mg/g of acarbose was considered tolerable to the flies.

The flies were exposed to these treatments for 7 days and the vials containing flies were maintained at room temperature before being homogenized in cold 0.1 M phosphate buffer (pH 7.4), and used for different assays.

Biochemical analysis

Glucose concentration of fly homogenate was carried out according to the manufacturer's procedure of the commercially available kit (Randox Laboratories UK). Alpha-amylase and alpha-glucosidase activities of fly homogenate were carried out as described [18,19]. Total thiol level was determined according to the method of Ellman et al., [27], while total reactive oxygen/nitrogen species (RONS) generated was determine using the 2′,7′-dichlorodihydrofluorescein diacetate assay [28]. The total protein content of fly homogenates was measured by the Coomassie blue method according to Bradford [29] using serum albumin as standard.

Data analysis

The results of triplicate experiments were pooled and expressed as mean ± standard deviation (S.D). One-way Analysis of Variance (ANOVA) will be used to analyze the results followed by Duncan's Multiple Range post-hoc test [30]. Statistical Package for Social Science (SPSS 17.0) for windows was used for the analysis. The IC_{50} was calculated using linear regression analysis.

Results

[Fig. 1] showed the effect of GA and TA on alpha-glucosidase and alpha-amylase inhibitory properties of acarbose. Both GA and TA exhibited significantly higher ($p < 0.05$) alpha-glucosidase inhibitory effects than acarbose. Considering the various combinations, apart from the combination of acarbose with 2 mg/ml of GA, the other combinations of each phenolic acids synergistically increased the alpha-glucosidase inhibitory effect of acarbose. Specifically, at the highest combination ratios, acarbose combined with 5 mg/ml of TA and GA produced 80.91 ± 0.1% and 77.27 ± 0.6% inhibition of

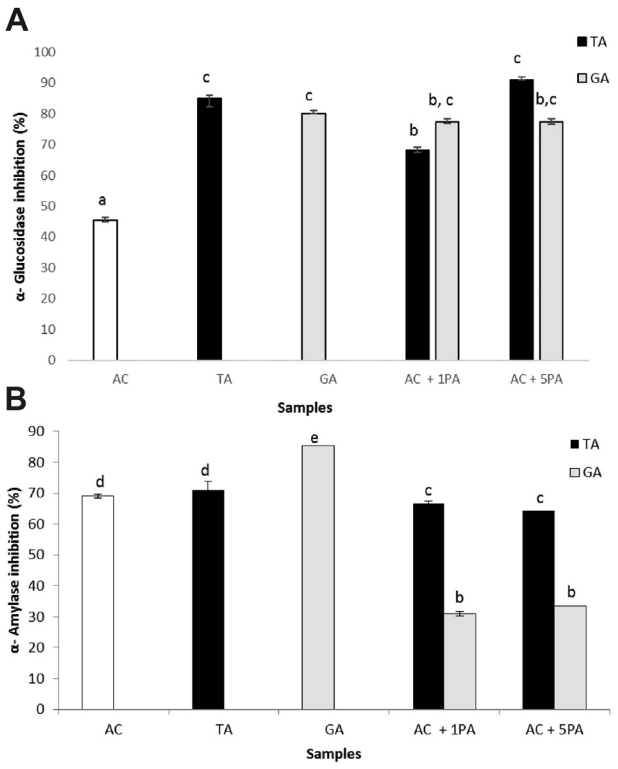

Fig. 1 Effect of GA and TA on: (A) alpha Glucosidase; (B) alpha Amylase Inhibitory Abilities of Acarbose. Bars represent mean ± SD of duplicate readings. Values with different alphabet are significantly different ($p < 0.05$). Abbreviations used: AC: Acarbose (0.5 mg/ml); GA: Gallic acid (1 mg/ml); TA: Tannic acid (1 mg/ml); AC + 1 PA: Acarbose + 1 mg/ml Phenolic acid (GA or TA); AC + 5 PA: Acarbose + 5 mg/ml Phenolic acid (GA or TA).

alpha-glucosidase activity respectively compared to 45.46 ± 0.6% inhibition exhibited by acarbose.

Similarly, acarbose, GA and TA exhibited significant alpha-amylase inhibitory effects. In addition, the combinations of each phenolic acid at different concentration with acarbose produced antagonistic effects by reducing the alpha-amylase inhibitory effect of acarbose. Specifically, at the highest combination ratios, acarbose combined with 5 mg/ml of TA and GA produced 64.29 ± 1.6% and 33.33 ± 3.2% inhibition of alpha-amylase activity respectively compared to 69.05 ± 1.6% inhibition exhibited by acarbose.

The DPPH free radical scavenging abilities of acarbose, GA and TA as well as their various combinations are presented in [Fig. 2A]. This showed that both GA and TA had significantly higher ($p < 0.05$) scavenging abilities than acarbose. Similarly, both phenolic acids synergistically increased the scavenging ability of acarbose; however, TA produced the higher increase in the scavenging ability of acarbose than GA at both concentrators (1 and 5 mg/ml) of phenolic acids tested.

Furthermore, the abilities of acarbose, GA and TA, as well as their various combinations to scavenge hydroxyl (OH) radicals is presented in [Fig. 2B]. This showed that there was no significant difference ($p > 0.05$) in the scavenging abilities of acarbose and GA, but TA had a significantly higher ($p < 0.05$) scavenging ability than acarbose. Regarding the various combinations, both phenolic acids showed significant increase in the scavenging ability of acarbose at the highest concentration used (5 mg/ml).

The result of the Fe^{2+} chelating ability as shown in [Fig. 2C], revealed that both GA and TA exhibited significantly higher ($p < 0.05$) chelating abilities than acarbose. In respect to the various combinations, GA at all concentrations (1–5 mg/ml) had no significant ($p > 0.05$) effect on the chelating ability of acarbose. However, TA significantly ($p < 0.05$) increased the chelating ability of acarbose; nevertheless, this influence reduced as concentration of TA increased (1–5 mg/ml).

Incubation of rat's pancreas homogenates in the presence of Fe^{2+} induced a significant ($p < 0.05$) increase (150.0 ± 1.2%) in the malondialdehyde (MDA) content [Fig. 2D]. However, introduction of all samples inhibited lipid peroxidation in the pancreatic tissue homogenate by causing a significant ($p < 0.05$) reduction in the MDA content; the combinations of the phenolic acids at the highest concentration (5 mg/ml) with acarbose produced the highest significant reduction in MDA production.

The survival curve of flies raised on diets containing TA or GA (0.2 and 2 mg/g) was presented in [Fig. 3A]. This showed that the flies were tolerable to both phenolic acids at both concentrations tested with significantly high survival (0.2 mg/g TA = 87.5 ± 1.4%; 2.0 mg/g TA = 88.8 ± 0.7%;

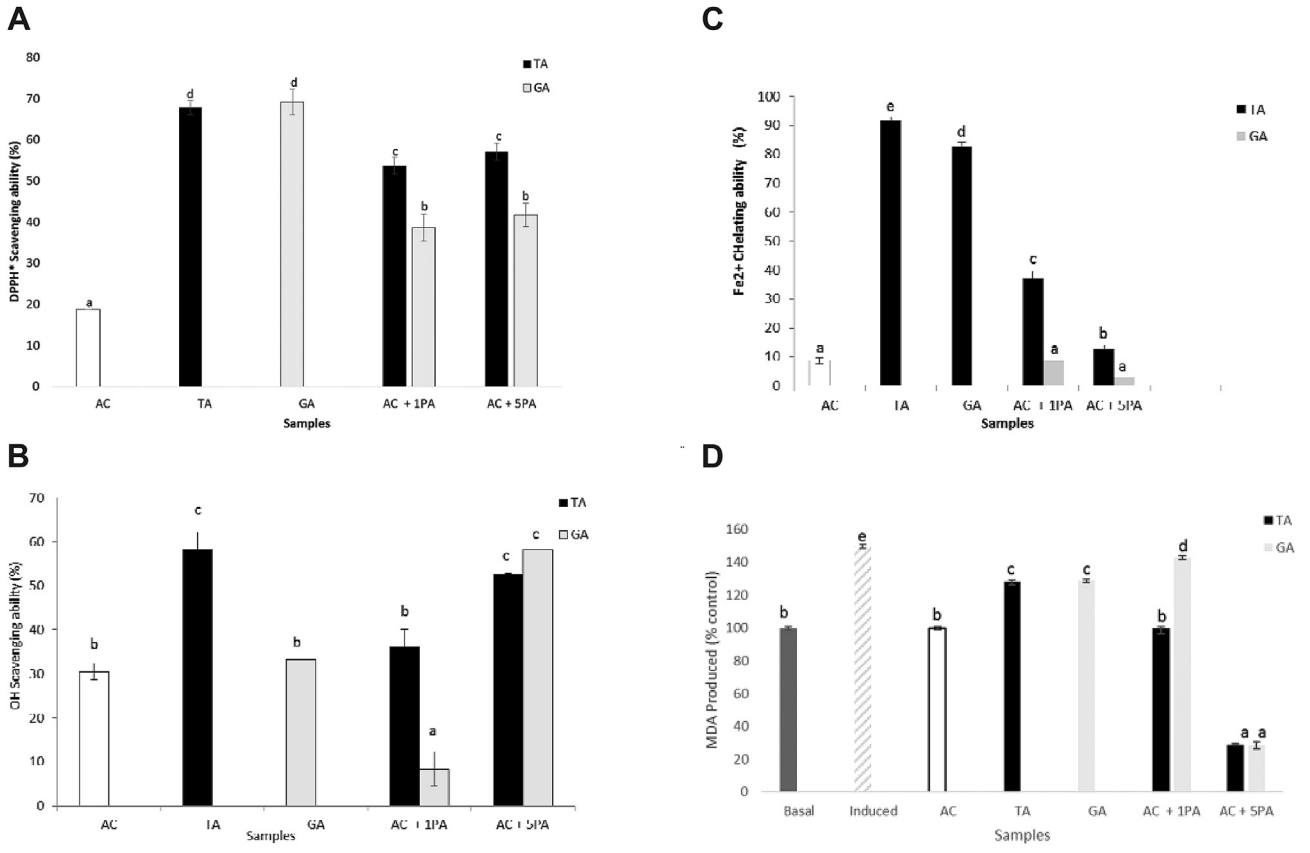

Fig. 2 Effect of GA and TA on: (A) DPPH Radical Scavenging Ability; (B) OH Radical Scavengign Ability; (C) Fe^{2+} Chelating Ability; (D) Inhibition of Fe^{2+} induced Lipid Peroxidation of Acarbose. Bars represent mean ± SD of duplicate readings. Values with different alphabet are significantly different ($p < 0.05$). Abbreviations used: AC: Acarbose (0.5 mg/ml); GA: Gallic acid (1 mg/ml); TA: Tannic acid (1mg/ml); AC + 1 PA: Acarbose + 1mg/ml Phenolic acid (Gallic or Tannic acid); AC + 5 PA: Acarbose + 5 mg/ml Phenolic acid (Gallic or Tannic acid).

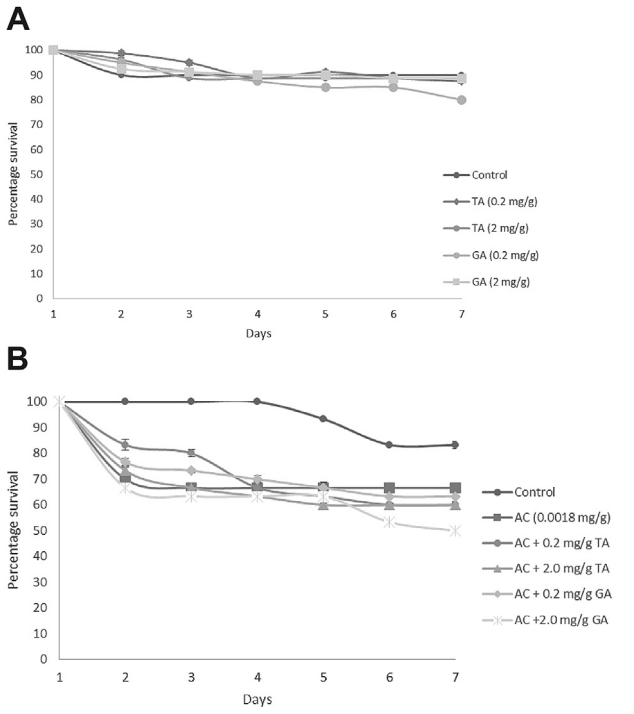

Fig. 3 Survival Curve for: (A) *D. melanogaster* Raised on Diet Containing GA or TA (0.2 and 2.0 mg/g of diet); for seven days; (B) Acarbose Administered D. melanogaster Raised on Diet Supplemented with either of GA or TA (0.2 and 2.0 mg/g of diet) for seven days. Values represent mean ± SD of triplicate experiments.

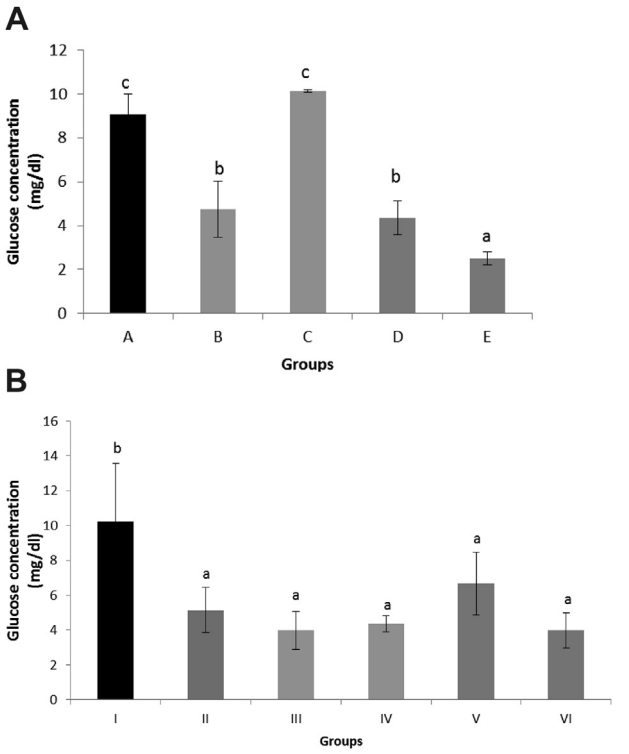

Fig. 4 Whole Fly Body Homogenate Glucose Concentration in: (A) Flies Raised on Diets Supplemented with GA and TA. (B) Acarbose Administered Flies Raised on Diets Supplemented with GA and TA. Bars represent mean ± SD of triplicate experiments. Values with different alphabet are significantly different ($p < 0.05$).
Groups: A Control - Flies raised on basal diet. B - Flies raised on diet supplemented with TA (0.2 mg/g). C - Flies raised on diet supplemented with TA (2.0 mg/g). D - Flies raised on diet supplemented with GA (0.2 mg/g). E - Flies raised on diet supplemented with GA (2.0 mg/g). Groups: I Control - Flies raised on basal diet. II - Flies raised on basal diet and administered 0.0018 mg/g acarbose (AC). III - AC administered flies raised on diet supplemented with 0.2 mg/g TA. IV - AC administered flies raised on diet supplemented with TA (2.0 mg/g). V - AC administered flies raised on diet supplemented with GA (0.2 mg/g). VI - AC administered flies raised on diet supplemented with TA (2.0 mg/g).

0.2 mg/g GA = 80 ± 2.1%; 2.0 mg/g GA = 88.8 ± 0.7%) on day 7. In addition, presented in [Fig. 3B] is the survival curve for flies raised on diets containing acarbose (0.0018 mg/g) and either of TA or GA (0.2 and 2.0 mg/g). This showed that tolerance of the flies to these dietary inclusions ranged from 50.0 ± 1.4% (AC + 2.0 mg/g GA) to 63.3 ± 2.1% (AC + 0.2 mg/g GA) on day 7.

Based on the survival rates, experiments were set up, first to determine the effect of dietary inclusions of GA and TA (0.2 and 2.0 mg/g) on the glucose concentration in the whole fly body homogenate. The result presented in [Fig. 4A] showed that fly groups raised on diet supplemented with GA (0.2 and 2.0 mg/g) or TA (0.2 mg/g) had significantly lower ($p < 0.05$) glucose concentration compared to the control group. In another experiment, flies were raised on diets supplemented with GA and TA (0.2 and 2.0 mg/g) combined with 0.0018 mg/g acarbose, and their whole body homogenate glucose concentration were determined. The result [Fig. 4B] showed that all the fly groups raised on diets supplemented with GA or TA combined with acarbose had significantly lower ($p > 0.05$) glucose concentration compared to the control group.

[Fig. 5A] showed the alpha-glucosidase activity in flies raised on diet supplemented with TA or GA (0.2 and 2.0 mg/g). This showed that alpha-glucosidase activity in flies raised on diets supplemented with TA or GA were significantly lower ($p < 0.05$) to that in flies raised on basal diet. There was however, no significant difference ($p > 0.05$) in the alpha-glucosidase activity between fly groups raised on diet supplement with GA (0.2 and 2.0 mg/g) or TA (0.2 and 2.0 mg/g).

In [Fig. 5B], the alpha-glucosidase activity in acarbose administered flies raised on diets supplemented with GA or TA was presented. This showed that fly groups administered acarbose and raised on basal diet, as well as fly groups administered acarbose and raised on diets supplemented with GA or TA (0.2 and 2.0 mg/g) showed significantly lower ($p < 0.05$) alpha-glucosidase activity compared to the control group. However, apart from acarbose administered flies raised on diet supplemented with 0.2 mg/g of GA, acarbose administered flies raised on basal diet had significantly lower ($p < 0.05$) alpha-glucosidase activity compared to the other groups.

Furthermore, [Fig. 6A] showed the alpha-amylase activity in flies raised on diet supplemented with TA or GA (0.2 and

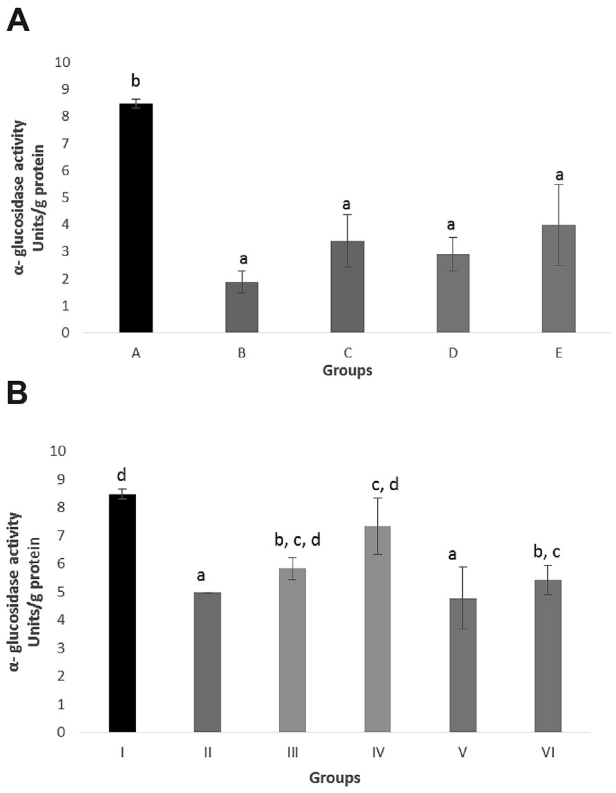

Fig. 5 Alpha Glucosidase Activity in: (A) Flies Raised on Diets Supplemented with GA and TA; (B) Acarbose Administered Flies Raised on Diets Supplemented with GA and TA. Bars represent mean ± SD of triplicate experiments. Values with different alphabet are significantly different ($p < 0.05$).
Groups: A Control - Flies raised on basal diet. B - Flies raised on diet supplemented with tannic acid (0.2 mg/g). C - Flies raised on diet supplemented with tannic acid (2.0 mg/g). D - Flies raised on diet supplemented with gallic acid (0.2 mg/g). E - Flies raised on diet supplemented with gallic acid (2.0 mg/g).
Groups: I Control - Flies raised on basal diet. II - Flies raised on basal diet and administered 0.0018 mg/g acarbose (AC). III - AC administered flies raised on diet supplemented with 0.2 mg/g tannic acid. IV - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g). V - AC administered flies raised on diet supplemented with gallic acid (0.2 mg/g). VI - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g).

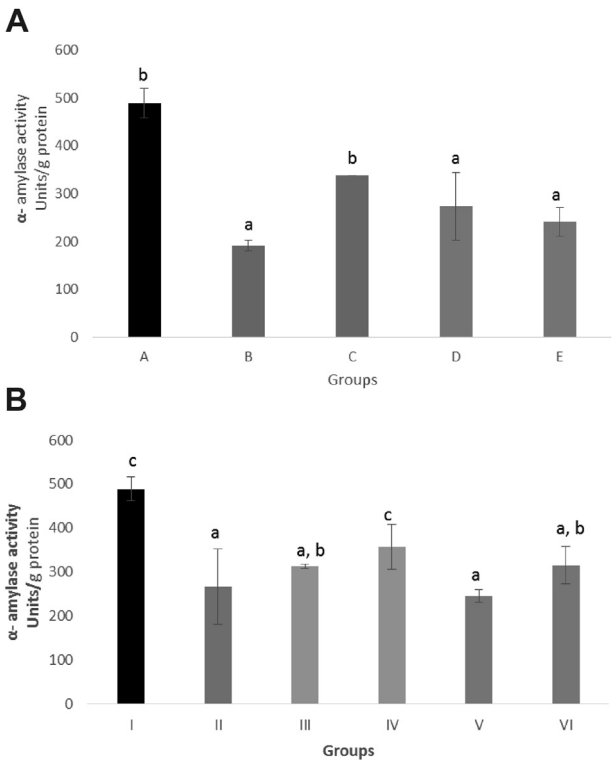

Fig. 6 Alpha Amylase Activity in: (A) Flies Raised on Diets Supplemented with GA and TA; (B) Acarbose Administered Flies Raised on Diets Supplemented with GA and TA. Bars represent mean ± SD of triplicate experiments. Values with different alphabet are significantly different ($p < 0.05$).
Groups: A Control - Flies raised on basal diet. B - Flies raised on diet supplemented with tannic acid (0.2 mg/g). C - Flies raised on diet supplemented with tannic acid (2.0 mg/g). D - Flies raised on diet supplemented with gallic acid (0.2 mg/g). E - Flies raised on diet supplemented with gallic acid (2.0 mg/g).
Groups: I Control - Flies raised on basal diet. II - Flies raised on basal diet and administered 0.0018 mg/g acarbose (AC). III - AC administered flies raised on diet supplemented with 0.2 mg/g tannic acid. IV - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g). V - AC administered flies raised on diet supplemented with gallic acid (0.2 mg/g). VI - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g).

2.0 mg/g). This showed that apart from flies raised on diet supplemented with 2.0 mg/g TA, the alpha-amylase activity in flies raised on diets supplemented with TA or GA were significantly lower ($p < 0.05$) to that in flies raised on basal diet. There was however, no significant difference ($p > 0.05$) in the alpha-amylase activity between fly groups raised on diet supplement with GA (0.2 and 2.0 mg/g) and TA (0.2 mg/g).

In [Fig. 6B] showed the alpha-amylase activity in acarbose administered flies raised on diets supplemented with GA or TA was presented. This showed that fly groups administered acarbose and raised on basal diet, as well as fly groups administered acarbose and raised on diets supplemented with GA or TA (0.2 and 2.0 mg/g) showed significantly lower ($p < 0.05$) alpha-amylase activity compared to the control group. However, apart from acarbose administered flies raised on diet supplemented with 0.2 mg/g of GA, acarbose administered flies raised on basal diet had significantly lower ($p < 0.05$) alpha-amylase activity compared to the other groups.

The total thiol contents in flies raised on acarbose, the phenolic acids and combination of the two are presented in [Fig. 7]. This showed that while administration of acarbose significantly ($p < 0.05$) reduced the flies' total thiol contents compared to control group, this was significantly ameliorated by the inclusions of the phenolic acids (GA and TA). However,

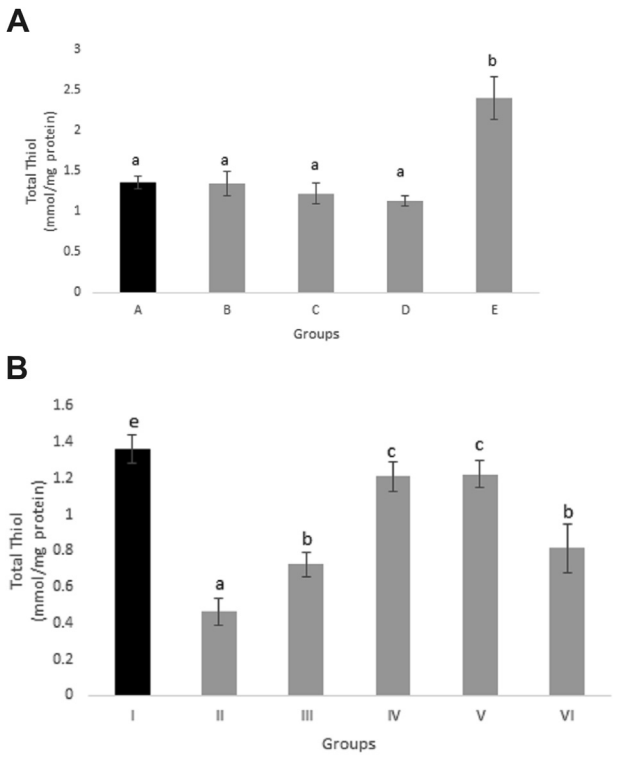

Fig. 7 Total Thiol Content in: (A) Flies Raised on Diets Supplemented with GA and TA; (B) Acarbose Administered Flies Raised on Diets Supplemented with GA and TA. Bars represent mean ± SD of triplicate experiments. Values with different alphabet are significantly different ($p < 0.05$). Groups: A Control – Flies raised on basal diet. B - Flies raised on diet supplemented with tannic acid (0.2 mg/g). C - Flies raised on diet supplemented with tannic acid (2.0 mg/g). D - Flies raised on diet supplemented with gallic acid (0.2 mg/g). E - Flies raised on diet supplemented with gallic acid (2.0 mg/g). Groups: I Control – Flies raised on basal diet. II - Flies raised on basal diet and administered 0.0018 mg/g acarbose (AC). III - AC administered flies raised on diet supplemented with 0.2 mg/g tannic acid. IV - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g). V - AC administered flies raised on diet supplemented with gallic acid (0.2 mg/g). VI - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g).

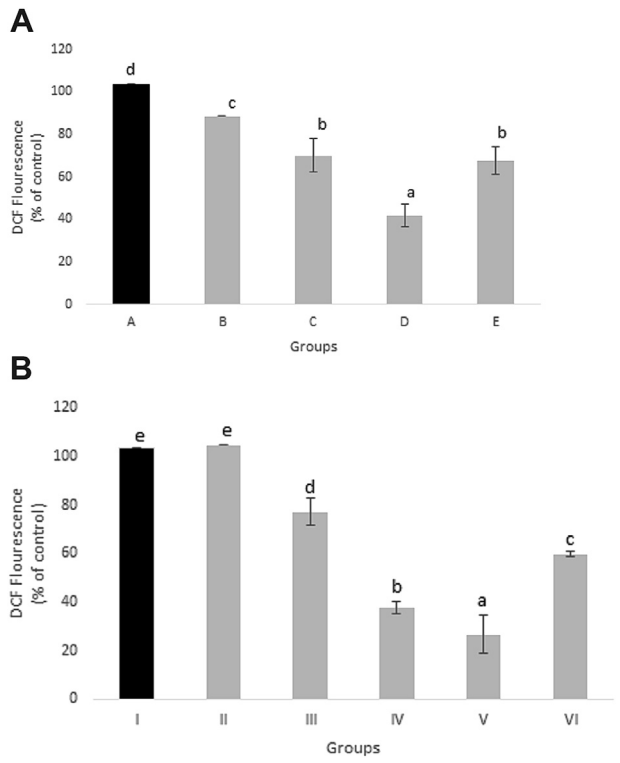

Fig. 8 ROS level in: (A) Flies Raised on Diets Supplemented with GA and TA; (B) Acarbose Administered Flies Raised on Diets Supplemented with GA and TA. Bars represent mean ± SD of triplicate experiments. Values with different alphabet are significantly different ($p < 0.05$). Groups: A Control – Flies raised on basal diet. B - Flies raised on diet supplemented with tannic acid (0.2 mg/g). C - Flies raised on diet supplemented with tannic acid (2.0 mg/g). D - Flies raised on diet supplemented with gallic acid (0.2 mg/g). E - Flies raised on diet supplemented with gallic acid (2.0 mg/g). Groups: I Control – Flies raised on basal diet. II - Flies raised on basal diet and administered 0.0018 mg/g acarbose (AC). III - AC administered flies raised on diet supplemented with 0.2 mg/g tannic acid. IV - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g). V - AC administered flies raised on diet supplemented with gallic acid (0.2 mg/g). VI - AC administered flies raised on diet supplemented with tannic acid (2.0 mg/g).

flies fed on diet supplemented with the phenolic acids alone produced no significant difference ($p > 0.05$) in their total thiol content, except for dietary inclusion of 2.0 mg/g of TA in which the flies exhibited significantly higher total thiol content compared to control group.

Lastly, the ROS level in flies raised on acarbose, the phenolic acids and combination of acarbose and the phenolic acids are presented in [Fig. 8]. This showed that acarbose administered flies fed diet supplement with the phenolic acids (GA and TA) significantly reduced the ROS level ($p < 0.05$) compared to both acarbose treated and control groups (which are not significantly different in their ROS levels), In addition, flies fed on diet supplemented with the phenolic acids alone also exhibited ↘significantly lower ($p < 0.05$) ROS level compared to control group.

Discussion

In the clinical management of T2DM, acarbose is a common antidiabetic drug which functions to reduce hyperglycemia by retarding glucose release from foods via inhibition of alpha-amylase and alpha-glucosidase which are major carbohydrate metabolizing enzymes [4,5]. Although acarbose is relatively effective, its use is often limited due to its attendant side effects, mainly abdominal pain, diarrhea and flatulence which are often dose-dependent [31]. Hence, combinatory therapy

are often advocated to help mitigate the side effects of acarbose while maintaining or possibly potentiating its antihyperglycemic effects [13,31]. Consequently, this study revealed that GA and TA significantly increased the alpha-glucosidase inhibitory effect of acarbose *in vitro*. It should also be noted that TA, a polymeric form of GA, with more distributed phenolic groups increased the alpha-glucosidase inhibitory effect of acarbose than GA at the highest concentration tested (5 mg/ml). However, as for *in vitro* alpha-amylase inhibitory effect of acarbose, there seems to be an antagonistic effect between acarbose and the phenolic acids as combinations of acarbose with either of the phenolic acids resulted in a significantly lower alpha-amylase inhibition. While the reason(s) for this trend might not be entirely clear, it holds significant clinical potentials as higher alpha-glucosidase and mild alpha-amylase inhibitors are desirable for management of T2DM-induced hyperglycemia [32]. This is more so because side effects of acarbose are often associated with excessive alpha-amylase inhibition [33]. These findings are in agreement with earlier studies; Oboh et al. [9] reported that GA significantly increased the alpha-glucosidase and alpha-amylase inhibitory properties of acarbose, while Adisakwattana [31] reported that condensed tannin-rich extracts from the bark of Cinnamon spp. produced additive effect in combination with acarbose to inhibit the activities of intestinal alpha-glucosidase and pancreatic alpha-amylase.

In vivo in *D. melanogaster*, it was observed that flies raised on diets containing acarbose, GA and TA, as well as their various combinations resulted in reduction in alpha-glucosidase and alpha-amylase activities in whole fly tissue homogenates. Flies raised on diets supplemented with either GA or TA had significantly lower activities of alpha-glucosidase and alpha-amylase compared to control flies. However, there was no significant difference in the enzymes' activities between flies raised on diet supplemented with GA and those supplemented with TA. This may be attributed to possible lack of metabolic machinery needed to hydrolyze TA to free gallate monomers in *D. melanogaster*; metabolism of TA has been reported in several mammalian models, showing that GA, 4-o-methyl-gallic acid, pyrogallol, resorcinol and ellagic acid are the metabolites of TA [34]. Furthermore, low absorption of TA in fly gut could also have limited its observed physiological effects. This is more so because previous studies have shown that high molecular weight phenolic acids such as TA are poorly absorbed in the gastrointestinal tract of rats, compared to low molecular weight phenolic acids such as GA [35] This could also account for the inverse proportionality between the concentration of TA in flies' diets and the reduction in the activities of both enzymes in the flies. Hence, further studies into absorption, distribution and metabolism of polyphenols in *D. melanogaster* is highly essential.

Furthermore, the reduction in the activities of both alpha-glucosidase and alpha-amylase in flies raised on diets supplemented with either GA or TA could justify the observed reduction in the glucose concentration in these fly groups compared to the control flies. Previous studies have characterized the activities of these two enzymes in *D. melanogaster* and associated them with glucose homeostasis in the flies [15,16]; reduction in activities of these carbohydrate catabolizing enzymes could lead to retardation in glucose release from diet and thus reduce flies' glucose concentration especially in the haemolymph. However, the possible low absorption of TA in fly gut and/or absence of TA hydrolysing enzyme could also be responsible for the fact that flies raised on diets supplemented with GA (2.0 mg/g) had the lowest significant glucose concentration, while there was no significant difference between control flies and flies raised on 2.0 mg/g of TA.

Although flies raised on diets containing the combination of acarbose and either of GA or TA did not produce an additive or synergistic effect at reducing flies' glucose concentration, the interaction was neither antagonistic. Noteworthy is the fact that the glucose concentration in flies raised on 0.2 mg/g TA or GA (0.2 and 2.0 mg/g) alone was not significantly different from those raised on diets containing acarbose alone. Therefore, there is possibility of achieving significant antihyperglycemic effects in combining either of these phenolic acids with reduced dose of acarbose. Reduction in the dosage of acarbose could help reduce its attendant side effects which are often dose-dependent [31]. Furthermore, the food–drug interactions between acarbose and GA or TA could confer additional antioxidant benefits in the therapeutic use of acarbose. As observed in this study, combination of acarbose with either of GA or TA significantly improved the *in vitro* antioxidant properties of acarbose by augmenting its free radicals scavenging abilities and increasing its ability to prevent Fe^{2+} induced lipid peroxidation. Antioxidant therapy is another important strategy for the management of T2DM and previous studies have reported the antioxidant properties of numerous phenolic acids including GA [9] and TA [8]. This study also agrees with earlier studies [9] which reported that GA improved the *in vitro* antioxidant properties of acarbose. TA especially have been reported to be an excellent chelator of Fe^{2+} due to its ten galloyl groups [11]. Thus, TA makes Fe^{2+} unavailable to participate in fenton reaction and mediate in hydroxyl radical formation which is important in initiation and propagation of lipid peroxidation chain reaction [11].

As earlier discussed, the management of T2DM often involves multi-therapeutic approaches, involving one or more of the following; use of antidiabetic drugs/insulin therapy, advocating for lifestyle adjustment, and dietary regulations. Dietary regulations not only involve cutting down on high caloric diets but encourage functional foods with antioxidant and antihyperglycemic properties; many functional foods with these properties are rich in dietary polyphenols such as GA and TA [8,9,36]. Antioxidant therapy is essential in the management of T2DM because they are essential for attenuating oxidative stress which have been implicated in the pathogenesis and progression of T2DM and its complications [36,37]. Therefore, as observed in this study, the combination of acarbose with either of GA or TA exhibited higher antioxidant properties and could confer additional therapeutic benefit on the use of acarbose for the management of T2DM. This is further justified by the fact that acarbose administered flies fed diets supplemented with both GA and TA exhibited reduced level of ROS level, when compared to both acarbose administered and control groups. Both TA and GA also

augmented the flies total thiol content which was significantly depleted by acarbose supposedly as a result of commitment of endogenous thiol-containing molecules to the metabolism of this drug.

Conclusively, this study has revealed that both gallic acid and tannic acid could help improve the therapeutic properties of acarbose by enhancing its antioxidant properties. Specifically, the combination of acarbose with either of the tested phenolic acids significantly reduced the production of ROS and ameliorated depletion in total thiol contents induced by acarbose in *D. melanogaster*. Therefore, diets rich in these phenolic acids could exhibit antioxidant properties and hypoglycemic effects on their own, as well as confer additional antioxidant benefits on the use of acarbose as antidiabetic drug, but may not significantly increase its enzyme inhibitory properties. Nevertheless, further studies including clinical trials are essential.

Conflicts of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2019.01.005.

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