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Original Article

Modulation of the antioxidant status of the hearts and brains of genetically modified hypercholesterolemic mice overproducing HDL cholesterol following acute bitter yam supplementation

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Abstract

The bitter yam tuber is consumed as a staple in various regions of the world and is known to possess medicinal properties due to the presence of bioactive compounds such as saponins. These compounds may induce health complications resulting in tissue damage in cases of overconsumption. This study was geared towards determining the effects of Jamaican bitter yam (5%) on tissue damage parameters in mice hearts and brains. Mice were fed diets with or without bitter yam supplementation for a period of four weeks after which they were sacrificed. Lipid peroxidation and antioxidant parameters were determined in the heart and brain. Significant increases in lipid peroxidation were observed in the hearts of mice fed bitter yam supplemented diets and in the brains of hypercholesterolemic mice only. Bitter yam supplementation significantly reduced glutathione peroxidase activities in the hearts of hypercholesterolemic mice and the hearts and brains of normocholesterolemic mice. Significant increase in catalase activity was only seen in the hearts of hypercholesterolemic mice fed a supplemented diet when compared to mice fed an unsupplemented diet. The results indicate that dietary supplementation with the Jamaican bitter yam at a high concentration may induce oxidative stress in mice hearts and brains.

INTRODUCTION

Yams are monocotyledons belonging to the family *Dioscoreaceae* within the order *Dioscoreales* [1]. They are culturally and economically important in tropical and subtropical regions [2] as they are good sources of essential dietary nutrients [3-5] and are also used for medicinal purposes due to the presence of saponins [6]. Research has however revealed that some yam species, particularly wild varieties, contain toxic compounds which may make them bitter and unpalatable and may induce health complications if ingested in large amounts without proper processing [7].

The Jamaican bitter yam (*Dioscorea polygonoides*) is one of the wild yam varieties found in Jamaica. The tuber portion of the plant is traditionally used to make "roots tonic" [8] or sun dried, and then cooked for human consumption [9]. This yam variety is known to possess bioactive factors such as protease inhibitors, tannins, cyanoglucosides, oxalates and phenols [10]. Despite being beneficial to health, some bioactive factors are known to damage organs resulting in significant increases in lipid peroxidation [11-13]. Khajja *et al* [14] stated that plants containing glucosides, acids or alkaloids are used as medicines, however when taken in excess, these plants often have

adverse effects. It was also stated that there is no real limit between a medicine and a poison as a medicine in a toxic dose may be a poison and a poison in a small dose may be medicinal. Paracelsus (1493-1541), the father of toxicology, stated in the sixteenth century that virtually any substance can be harmful at a high concentration [14].

Lipid peroxidation levels and the specific activities of drug metabolizing and antioxidant enzymes can be used as diagnostic indices of peroxidative tissue injury [15]. Lipid peroxidation is a potentially harmful, uncontrolled, self-enhancing, free radical-related process which results in the disruption of membrane lipids and other cellular components. Increased lipid peroxidation is observed in damaged tissues due to the inactivation and leakage of antioxidants from cells as well as the release of metal ions from storage sites and metalloproteins by enzymes from damaged lysosomes [16]. Metal ions such as iron, copper, chromium and vanadium undergo redox cycling while cadmium, mercury, nickel and lead deplete glutathione and protein bound sulfhydryl groups. These reactions result in enhanced lipid peroxidation, alteration of various cellular processes and damage to cells in general [17].

The most important end products of free radical reactions are molondialdehyde (MDA) and conjugated dienes. They have various cytotoxic effects, including enzyme inactivation and inhibition of DNA, RNA and protein synthesis, which may result in various chronic diseases such as atherosclerosis, cancer, cardiovascular diseases, diabetes and rheumatoid arthritis as well as aging and neurodegenerative diseases. [18-20]. It is well documented in literature that the heart and brain are the most susceptible organs to oxidative stress and are ideal targets for free radical attack. The brain has high lipid content, a high metabolic rate and an abundant supply of the necessary transition metals [21]. Increased oxidative stress is observed in hearts that have experienced acute ischemia-reperfusion injury, endothelial damage caused by hyperhomocysteinemia and chronic oxidative damage secondary to lipid peroxidation [22-24]

The biological effects of free radicals are controlled by many antioxidants, including glutathione, beta-carotene, vitamins E and C and antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) [25, 26]. The body's defence mechanism is also comprised of a system of drug metabolizing enzymes such as cytochrome P-450, glutathione transferase (GST) and ethoxyresorufin-O-deethylase (EROD). These enzymes are responsible for the detoxification of a variety of endogenous and exogenous chemicals. Under normal conditions**,** a dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell [17; 27]. Oxidative stress results from an imbalance between formation and neutralization of these ROS.

High density lipoproteins (HDL) also display antioxidant properties due to the presence of apolipoprotein A-I (apoA-I), and enzymes such as paraoxonase-1 (PON1), glutathione selenoperoxidase, platelet-activating factor acetylhydrolase (PAF-AH) and lecithin-cholesterol acyltransferase (LCAT) [28-31]. These enzymes cleave oxidised lipids while apoA-I removes the oxidised lipids from LDL thereby protecting it from oxidation *in vivo* [32]. Other HDL apolipoproteins that function as antioxidants include apoA-II, apoA-IV, apoC and apoE [33-35]. The C57BL/6-Tg(APOA1)1Rub/J strain used in this study was created by inserting a human apoA-I gene into the genome of the C57BL/6 inbred mice [36]. These mice show a two fold increase in both total apolipoprotein A1 and HDL cholesterol and an overall increase in circulating total cholesterol [36] and should therefore be better protected from oxidative stress than the inbred strain.

On the basis of the above literature, we presume that bitter yam supplementation may elicit adverse effects on mice organs in the form of increased oxidative stress. These effects however, may be less pronounced in the genetically modified mice due to the antioxidant potential of HDL. This present study therefore evaluates the effects, if any, of bitter yam supplementation at a concentration of 5% on selected organs of C57BL/6 (normocholesterolemic) and C57BL/6-Tg(APOA1)1Rub/J (genetically modified hypercholesterolemic) mice strains. The results obtained from this study can be used to determine the effects of overdosing as well as determining the protective effects of increased circulating HDL cholesterol in the blood.

MATERIALS AND METHODS

Materials

Acetic acid, sodium hydroxide, thiobarbituric acid, n-butanol, pyridine, reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), ethylene diamine tetraacetic acid (EDTA), sodium azide (NaN_3), reduced nicotinamide adenine dinucleotide phosphate (NADPH), GR, cumene hydroperoxide, sucrose, tris-HCl, oxidized glutathione, sodium dodecyl sulphate (SDS), tris base and potassium phosphate were purchased from Sigma Chemical (Maryland, USA).

Animals and treatment

Thirty-two male mice (3-5 weeks old) were obtained from Jax Laboratories (600 Main Street Bar Harbor,

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Maine, USA). All mice were housed in a room maintained at 25°C with a 12 h light/dark cycle. Sixteen were genetically modified hypercholesterolemic mice [C57BL/6-Tg(APOA1)1Rub/J], and sixteen were inbred mice (C57BL/6J). Upon arrival at the facility, the mice were tagged, grouped and caged according to the respective diets that they would be fed. They were divided into four groups of eight mice each. The groups were as follows: inbred mice fed a basal rat chow diet (4.5% cholesterol) [normocholesterolemic control]; inbred mice fed basal rat chow (4.5% cholesterol) supplemented with the bitter yam (5%, wt/wt); hypercholesterolemic mice fed a high cholesterol rat chow diet (6.5% cholesterol, as was instructed by the animal breeders from which the mice were purchased) [hypercholesterolemic control]; hypercholesterolemic mice fed a bitter yam supplemented (5%, wt/wt) high cholesterol rat chow diet (6.5% cholesterol). The mice were fed their respective diets for six weeks. Food intake was recorded daily and body weight change weekly. At the end of the feeding period, the mice were sacrificed and the heart and brain were excised, washed with saline solution to remove excess blood, frozen in liquid nitrogen and stored at –80ºC until required for analysis.

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medical Sciences, University of the West Indies, Mona Campus.

Lipid Peroxidation Assessment

Lipid peroxidation was determined using a previously described method [37]. Weighed organ samples were chilled and washed in ice cold 0.9% sodium chloride. Tissue homogenates were then prepared by homogenizing 0.1 g of wet tissue with 0.9 ml of 1.15% KCl using a Teflon Potter-Elvehjen homogenizer.

A reaction mixture containing 0.1 ml of 10% (w/v) tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 using sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA) was made up to 4 ml with distilled water and refluxed at 95ºC for 60 min. After refluxing, 1 ml of distilled water was added followed by 5 ml of a mixture of n-butanol and pyridine (15:1) v/v. The mixture was then vigorously shaken and centrifuged at 4000 rpm for 10 min. The organic layer (top layer) was removed and the absorbance read at 532 nm. A reagent blank was prepared by replacing the 0.1 ml of the homogenate with 0.1 ml of distilled water. The standard used was tetramethoxypropane (TMP) at various concentrations (0 to 8 nmoles).

Enzyme activity assays

Glutathione-S-transferase activity was determined according to a standard method [38]. Weighed frozen tissue samples were partially thawed in 0.9 ml of distilled water, homogenized for 1 min and centrifuged for 90 min at 10,000*g*. The supernatant fluid was then filtered through a plug of glass wool and used to assay for enzyme activity. A reaction mixture containing potassium phosphate (0.1 M), glutathione (1 mM) and 1-chloro-2,4-dinitrobenzene (1 mM) was made up and the pH adjusted to 6.5. The reaction was initiated by the addition of the supernatant and the change in the absorbance followed at 340 nm. A blank was prepared by replacing the homogenate mixture with water.

Glutathione peroxidase activity was determined by a standard method [39]. The organs were washed with 1.14 M NaCl, homogenized in 0.25 M sucrose and a supernatant fraction prepared by centrifugation at 13,000 rpm for 1 h. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 E.U./ml GR, 1 mM GSH, 1.5 mM cumene hydroperoxide in a total volume of 0.8 ml. Supernatant fraction (0.1 ml) was then added to the mixture and allowed to incubate for 5 min at room temperature before initiation of the reaction by the addition of 0.1 ml peroxide solution. Absorbance at 340 nm was then recorded every thirty seconds for 5 min and the activity calculated as µmoles NADPH oxidized per minute. Blank reactions were prepared by replacing the homogenate with distilled water.

Glutathione reductase activity was determined by following the rate of NADPH oxidation at 340 nm [40]. A 20% homogenate (w/v) was prepared in 0.25 M ice-cold sucrose. This was centrifuged for 60 min at 7000*g*. The pH was adjusted to 5.3 by the addition of ice-cold 0.2 M acetic acid. This homogenate was then centrifuged at 15,000*g* for 40 min and the supernatant used to assay for enzyme activity. The pH of the supernatant was then adjusted to 7.4 by the addition of 1 M tris base. A reaction mixture containing 0.1 M potassium phosphate buffer (0.1 M, pH 7.0), EDTA (1 mM), tris-HCl (0.5 mM), NADPH (0.1 mM), oxidized glutathione (20 mM) was then made. The reaction was started by the addition of the supernatant to the reaction mixture. The decrease in absorbance was measured at a temperature of 30°C. The spectrophotometer was blanked using the reaction mixture without the enzyme source.

Catalase was extracted according to a previously described method [41] with slight modifications. Tissue samples were rinsed with phosphate buffered saline (PBS) solution, pH 7.4, to remove any red blood cells or clots, tissue samples (approximately 0.2 g) then homogenized in 1 ml of ice cold 25 mM KH_2PO_4-NaOH buffer, pH 7.0. The homogenates were then centrifuged at 10,000*g* for 15 min at a temperature of 4°C. The resulting supernatants were used to assay for enzyme activity. Catalase activity was determined as

previously outlined [42]. Homogenate (2 ml) was pipetted into a cuvette of light path 1 cm and left to equilibrate at 25°C for 5 min; 1 ml of the hydrogen peroxide solution was then added to the homogenate and the decrease in absorbance (240 nm) at 25°C recorded. A blank solution was prepared by replacing the homogenate with phosphate buffer.

Protein determination

The protein content of the homogenates was determined using a BCA™ protein assay kit from Pierce according to the manufacturer's guideline (Product#: 23227, Lot#:94677).

Statistical Analysis

Data were analysed by means of Analysis of Variance (ANOVA) and Duncan's test was used to test the significance difference of the various groups. Results were expressed as mean ± standard error of the mean (SEM). One-way ANOVA was used to test for significant differences among the groups; $P < 0.05$ was considered significant [43].

RESULTS

Organ weight

Bitter yam supplementation had no significant impact on organ to body weight ratio (Table 1). A significant increase from 0.38 ± 0.006 to 0.41 ± 0.004 g ($P < 0.05$) was however observed in brain weight of normo-cholesterolemic mice fed diet supplemented with the bitter yam when compared to normocholesterolemic mice fed an unsupplemented diet (Fig.1).

Lipid peroxidation

A significant increase ($P < 0.05$) in lipid peroxidation was observed in the hearts of normocholesterolemic mice fed bitter yam supplemented diet when compared with mice fed an unsupplemented diet from 350 ± 10.39 to 567.41 ± 60.26 nmol MDA/g wet wt (Fig.2). Lipid peroxidation was also increased in the hearts and brains of hypercholes-terolemic mice fed bitter yam supplemented diet from 405.52 ± 11.3 to 1839.4 ± 49.94 and 113.57 ± 10.4 to 361.62 ± 9.88 nmol MDA/g wet wt, respectively, when compared with mice fed unsupplemented diets (Fig.2). The level of lipid peroxidation in the brains of hypercholesterolemic mice fed an unsupplemented diet (113.57 ± 10.4 nmol MDA/g wet wt) was significantly lower ($P < 0.05$) than that of normocholesterolemic mice fed an unsupplemented diet (304.94 ± 5.04 nmol MDA/g wet wt).

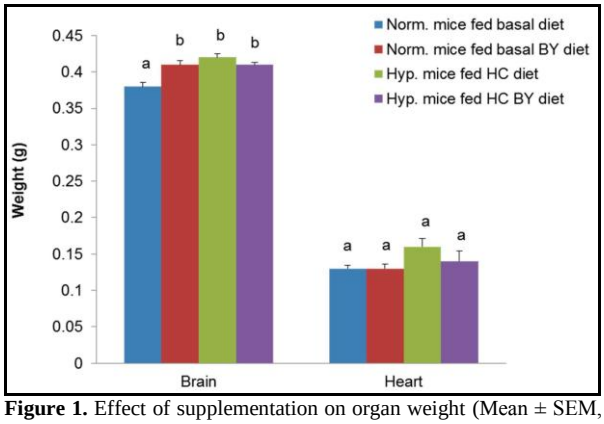

Figure 1. Effect of supplementation on organ weight (Mean ± SEM, n = 8); Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, BY = bitter yam, HC = high cholesterol. Organ weights with different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way ANOVA and the Duncan's multiple range test.

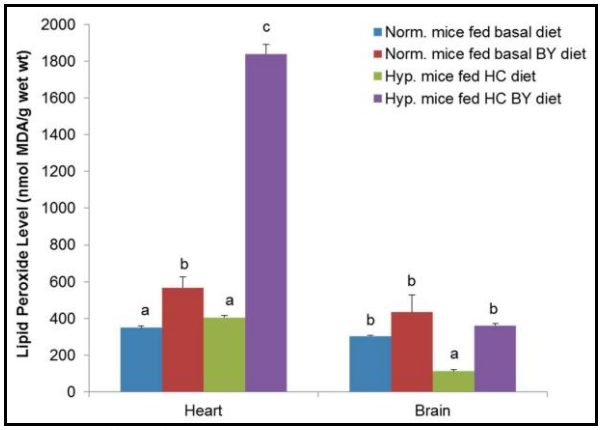

Figure 2. Effect of supplementation on lipid peroxidation in the brain and heart (Mean ± SEM, n = 8); Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, BY = bitter yam, HC = high cholesterol. Organs from different groups with different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way ANOVA and the Duncan's multiple range test. The lipid peroxide level was calculated from the absorbance at 532 nm using tetramethoxypropane (TMP) as an external standard.

Table 1. Organ weight to body weight ratio of brain and heart (Mean ± SEM)

Group	Heart	Brain
Normocholesterolemic mice fed basal diet	0.006 ± 0.0003^{a}	0.018 ± 0.0003^{b}
Normocholesterolemic mice fed basal bitter yam diet	0.006 ± 0.0002^{a}	0.018 ± 0.0004^{b}
Hypercholesterolemic mice fed high cholesterol diet	0.006 ± 0.0007^{a}	$0.016 \pm 0.0009^{a,b}$
Hypercholesterolemic mice fed high cholesterol bitter yam diet	0.005 ± 0.0006^{a}	0.015 ± 0.0004^{a}

Different letter subscripts in columns indicate significant differences ($P < 0.05$). Statistical analyses of table and charts were done using the one way ANOVA and the Duncan's multiple range test.

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Table 2. Glutathione peroxidase specific activity in the heart and brain(Mean ± SEM)

Group	Specific activity (U/mg protein)	
	Heart	Brain
Normocholesterolemic mice fed basal diet	4.28 ± 0.07^{b}	1.06 ± 0.12^{b}
Normocholesterolemic mice fed basal bitter yam diet	0.47 ± 0.15^{a}	0.44 ± 0.09^{a}
Hypercholesterolemic mice fed high cholesterol diet	6.81 ± 0.23^{c}	$0.66 \pm 0.08^{a,b}$
Hypercholesterolemic mice fed high cholesterol bitter yam diet	3.71 ± 0.48^{b}	0.40 ± 0.02^{a}

Organs from different groups that share different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way ANOVA and the Duncan's multiple range test. Glutathione peroxidase specific activity is expressed as Units/mg of protein; one unit is defined as the amount of enzyme that will cause the oxidation of 1 nmol of NADPH to $NADP^{+}$ per minute at room temperature.

Glutathione peroxidase

Specific activity of GPx was determined in the hearts and brains of test mice. Bitter yam supplementation at 5% resulted in significant reductions ($P < 0.05$) in the specific activity of GPx in the hearts of normocholesterolemic and hypercholesterolemic mice from 4.28 ± 0.07 to 0.47 ± 0.15 Units/mg of protein and 6.81 ± 0.23 to 3.71 ± 0.48 Units/mg of protein, respectively, when compared with mice fed unsupplemented diets. GPx specific activity was significantly higher ($P < 0.05$) in the hearts of hypercholesterolemic control mice than in normocholesterolemic control mice (Table 2). The brains of normocholesterolemic mice fed a supplemented diet had significantly lower ($P < 0.05$) GPx specific activity (0.44 ± 0.09 Units/mg of protein) than normocholesterolemic mice fed an unsupplemented diet (1.06 ± 0.12 Units/mg of protein).

Glutathione reductase and glutathione S-transferase

The specific activities of GR (Fig.3) and GST (Fig.4) in the brain and heart were not affected by bitter yam supplementation.

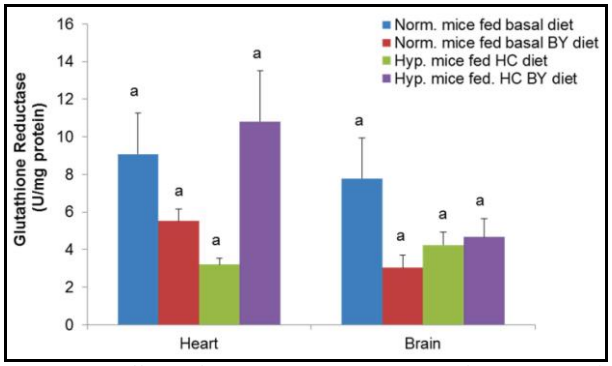

Figure 3. Effect of supplementation on specific activity of glutathione reductase in the heart and brain (Mean ± SEM, n = 8); Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, BY = bitter yam, HC = high cholesterol. Different letter subscripts from organs in different groups represent specific activities that are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way ANOVA and the Duncan's multiple range test. GPx specific activity is expressed as units/mg of protein; one unit is defined as the amount of enzyme that will cause the oxidation of 1 nmol of NADPH to $NADP^{+}$ per minute at 30°C.

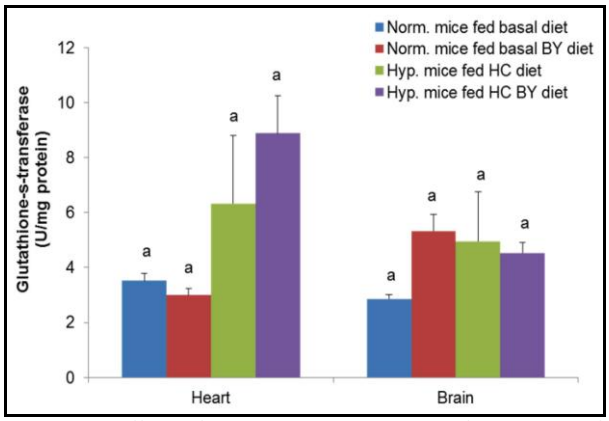

Figure 4. Effect of supplementation on specific activity of glutathione S-transferase in the heart and brain (Mean ± SEM, n = 8); Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, BY = bitter yam, HC = high cholesterol. Different letter subscripts for organs from different groups represent specific activities that are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way ANOVA and the Duncan's multiple range test.

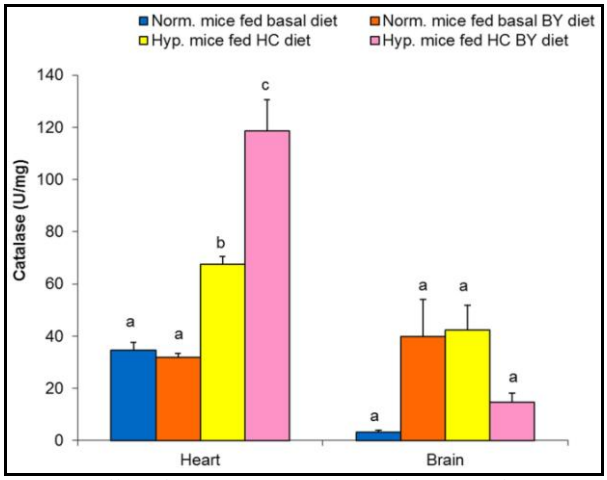

Figure 5. Effect of supplementation on specific activity of catalase in the heart and brain (Mean ± SEM, n = 8); Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, BY = bitter yam, HC = high cholesterol. Organs in different groups with different letter subscripts represent specific activities that are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way ANOVA and the Duncan's multiple range test.

Catalase

Bitter yam supplementation significantly increased ($P < 0.05$) the specific activity of CAT activity in the hearts of hypercholesterolemic mice from 67.54 ± 2.88 to 118.53 ± 11.92 U/mg (Fig.5). The hearts of hypercholesterolemic mice had significantly higher levels ($P < 0.05$) of CAT activity than normocholesterolemic mice.

DISCUSSION

Previous studies have indicated that the Jamaican bitter yam possesses potent antidiabetic and hypocholesterolemic properties, with lipoprotein redistribution, due to the presence of saponins [44]. Lipoprotein redistribution involved an increase in HDL (good) cholesterol and a decrease in low density lipoprotein LDL (bad) cholesterol levels [44, 45], thus making this wild yam variety suitable for exploitation within the nutraceutical industry. The Jamaican bitter yam, however, also contains various bioactive factors [10] known to elicit adverse effects on organs of the body [12, 46]. The current study therefore investigates the effects of consumption of the yam at a concentration of 5% on the brains and hearts of two mice strains (C57BL/6 and the C57BL/6-Tg(APOA1)1Rub/J strains).

Both organs investigated are susceptible to oxidative stress which can possibly lead to the development of neurodegenerative disorders and cardiovascular diseases. Organ to body-weight ratio indicated that mice fed bitter yam supplemented diets experienced no significant changes in heart or brain weight. However, despite having no effect on organ weight, bitter yam supplementation resulted in significant increases in lipid peroxidation in the hearts and brains of hypercholesterolemic mice and the hearts of normocholesterolemic mice. This increase in lipid peroxidation may have been as a result of increased oxidative stress brought about by the presence of bioactive factors such as cyanogenic glucosides, alkaloids and oxalates, which can be toxic to tissues [46-48]. Cyanogenic glucosides have displayed acute toxicity and have been linked to neuropathy and even death [49, 50]. Alkaloids may cause gastrointestinal and neurological disorders [51] and oxalates may induce toxicity if an unprocessed food is consumed [52].

Lipid peroxidation occurs by a radical chain reaction which once initiated rapidly propagates [53]. Proteins may also be damaged by ROS leading to structural changes and loss of enzyme activity [53, 54]. This could possibly be a contributing factor to the significant reductions seen in enzyme activities in the tissues of mice fed bitter yam supplemented diets. Significant reductions were observed in the specific activity of GPx in the hearts and brains of mice fed bitter yam supplemented diets when compared with mice fed unsupplemented diets. The main biological role of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and hydrogen peroxide to water [55]. A reduction in the activity of this enzyme would therefore possibly result in further increases in lipid peroxides.

Reduced trace mineral bioavailability may also provide a possible explanation for the increase seen in lipid peroxidation and decrease in GPx activity. Bioactive factors may affect the bioavailability of trace elements. Two major antinutrients responsible for inhibiting the absorption and/or retention of some trace elements are phytic acid and dietary fibre [56]. It has been found that dietary fibre may reduce selenium absorption from the gut [57]. This could possibly negatively impact the production of the selenium-containing enzyme, GPx, and may have been one of the factors responsible for the significant decrease seen in its activity. Dietary fibre may also inhibit the bioavailability of both manganese and zinc. In the presence of oxalic acid, minerals such as copper and zinc may become trapped in fibre-copper-oxalate or fibre-zinc-oxalate complexes [56]. These complexes are even less readily degraded in the digestive tract [58]. Further research would however be required to determine whether there was decreased zinc absorption by mice fed bitter yam. Zinc is important for immune and antioxidant functions, gene expression, DNA synthesis, hormone synthesis, storage and release of neurotransmitters and apoptosis [59, 60]. Zinc deficiency has been reported to induce cellular oxidative stress [61, 62]. It is not only the core constituent of free radical scavenging enzymes such as SOD, but is also thought to block lipid peroxidation by displacing transition metals such as iron and copper from catalytic sites [63]. Phytic acid may also reduce the bioavailability of zinc as it complexes with divalent cations resulting in them becoming unavailable for absorption through the intestinal walls [64].

Catalase activity was generally higher in the hearts of hypercholesterolemic mice. This may have been an adaptive mechanism aimed at protecting HDL from oxidation, thereby not becoming atherogenic. Zheng *et al* [65] demonstrated that apolipoprotein A1 and HDL are selective targets for myeloperoxidase (MPO) catalysed oxidation resulting in the inhibition of cholesterol efflux from macrophages. The transgenic mice however exhibit enhanced cellular cholesterol efflux in macrophages *in vivo* [66]. Macrophage activation generates reactive oxidant species which are rapidly converted to hydrogen peroxide by SOD [67]. This increase in peroxide generation may result in the upregulation of catalase gene expression, effectively converting the excess peroxide generated to water. Since MPO utilizes hydrogen peroxide as a substrate to

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form reactive oxidant species [68, 69], increased attack upon apoA-1 and HDL would therefore have been prevented. Overall, the significant increase in catalase activity may also explain the reduced susceptibility of the transgenic mice to atherosclerosis. The accumulation of oxidized lipids in the walls of arteries has been implicated in the development of the condition. There is evidence that hydrogen peroxide released by vascular cells is involved in the formation of oxidized LDL [70, 71]. The overexpression of catalase may have reduced LDL oxidation thereby reducing atherosclerosis.

The human apoA-I is known to be highly expressed in the genetically modified mice [36]. While it is a major apolipoprotein of the cerebrospinal fluid, it is not synthesized in the central nervous system [72, 73]. Balazs *et al* [74] hypothesizes that ApoA-I rich cerebrospinal fluid is most probably derived from plasma HDL that enters the central nervous system by crossing the blood-brain barrier. Since the genetically modified mice used in this study had significantly elevated HDL cholesterol levels it is possible that there may have been increased HDL cholesterol transport across the blood-brain barrier resulting in elevated cerebrospinal fluid HDL cholesterol levels. HDL cholesterol has demonstrated neuroprotective properties by binding excess amyloid-β and by directly decreasing oxidative stress [75] which could possibly explain the significantly lower lipid peroxidation seen in the brains of genetically modified mice when compared with the normocholesterolemic mice.

Significant increases in serum HDL levels may also boost the antioxidant defense system. Catalase and GPx activities were significantly greater in the hearts of transgenic mice than their inbred counterparts. Oxidative stress plays a significant role in the development of atherosclerotic legions and cardio-vascular diseases [70, 76]. The increased expression of these antioxidant enzymes as well as the antioxidant properties of HDL cholesterol could possibly explain the decreased susceptibility of the genetically modified mice to the development of atherosclerosis.

It can be concluded that supplementation of the diet with the Jamaican bitter yam at a high concentration (5%) may have adverse effects on the heart and brain of C57BL/6 and C57BL/6-Tg(APOA1)1Rub/J mice strains and may alter the expression of some enzymes involved in protection from oxidative stress. We also conclude from the results that HDL cholesterol may have played some protective role against oxidative stress in the brains of the genetically modified mice. These results point to the necessity of conducting further investigations to determine an effective dosage and also to unravel the mechanisms of oxidative damage/mechanisms of enzyme down-regulation.

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CONFLICTS OF INTEREST

None to declare

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