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Evaluation of the effects of bitter yam tuber supplementation on serum parameters used to assess hepatotoxicity and nephrotoxicity in transgenic mice

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Article info:

Received: 23 January 2013

Accepted: 6 April 2014

ABSTRACT

The Jamaican bitter yam (*Dioscorea polygonoides*) (ITIS) is known to possess potent antidiabetic and hypocholesterolemic properties and can therefore be exploited for associated nutraceutical/pharmaceutical purposes. It however possesses bioactive compounds known to promote organ damage when ingested in excess. This study investigates the effects of bitter yam consumption at a concentration of 5% on liver and kidney damage/function parameters. Normocholesterolemic mice fed bitter yam supplemented diets experienced significant increases in serum aspartate aminotransferase activity and bilirubin, magnesium and phosphorus concentrations. Significant increases were also observed in serum aspartate aminotransferase activity and blood urea nitrogen concentration of the genetically modified hypercholesterolemic mice fed supplemented diets. These results suggest mild kidney damage in both mice species and a significant increase in the rate of erythrocyte haemolysis in the normocholesterolemic mice.

Key words: Toxicity, organ damage, bitter yam, transgenic mice, experimental hypercholesterolemia, serum biochemical parameters

Introduction

Dioscorea (true yams) is a large genus containing more than 600 species (Mignouna *et al.*, 2009) of which only seven are edible (Jayakody *et al.*, 2007). It is of socioeconomic importance in many developing countries due to its use as a major staple and famine food (Mignouna *et al.*, 2009). The tuber is nutritious due to its protein, lipid, vitamin and mineral contents (Lasztity *et al.*, 1998) and is also used for medicinal purposes due to the presence of phytosterol/phytoestrogen and steroidal saponin precursors (Subiah, 1973; Poornima & Ravishankar, 2009). In addition

to their beneficial components, yam tubers contain various toxic/antinutritional substances that affect both human and animal health (Polycarp *et al.*, 2012). Consumption of the raw or improperly cooked form can result in irritation and inflammation of the buccal cavity and throat, gastrointestinal disturbances, vomiting and diarrhea (Bhandari & Kawabata, 2004). Some antinutritional factors present include polyphenols, oligosaccharides (α -galactosides), lectins, proteases and amylase inhibitors (Medoua *et al.*, 2007).

The Jamaican bitter yam, a wild yam variety, has been shown to possess antidiabetic and hypocholesterolemic properties and is therefore suitable for nutraceutical/

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pharmaceutical purposes (McAnuff *et al.*, 2005a). The tuber however contains some anti-nutritional compounds such as tannins and other phenolic compounds, protease inhibitors, cyanoglucosides and lectins (McAnuff *et al.*, 2005b). These “natural toxins” have been shown to have adverse physiological effects on the body when ingested (Liener, 1980; Cheeke, 1998; Inuwa *et al.*, 2011).

The liver and kidneys play crucial roles in the metabolism and excretion of xenobiotics, thus making these organs highly susceptible to their adverse and toxic effects. It is generally assumed that an increase in the activities of enzymes such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase reflect active inflammation and necrosis of hepatic cells, whereas subsequent reductions indicate a decline of hepatic inflammation and may lead to morphological improvement (Hwang *et al.*, 2000). Elevations in serum alanine aminotransferase are more specific for detecting liver abnormalities since it is primarily found in the liver (Dufour *et al.*, 2000a, 2000b). Aspartate aminotransferase however is less specific for hepatocellular injury due to its ubiquitous nature (Dufour *et al.*, 2000a, 2000b; Ozer *et al.*, 2008). It is found in highest concentrations in the heart compared to other tissues, and may be used to signify abnormalities in the heart, muscle, brain or kidney.

Increases in both total and conjugated bilirubin levels are measures of overall liver function (Reuben, 2004). Significant increases in transaminase levels and in bilirubin to more than double the normal levels are considered an ominous marker for hepatotoxicity (Reuben, 2004). Plasma proteins, such as albumins and globulins, can also be used as a marker of hepatic function and can indicate hepatocellular injury (Thapa & Walia, 2007).

Serum creatinine concentration is widely interpreted as a measure of the glomerular filtration rate and is used as an index of renal function in clinical practice. In renal disease however, serum creatinine values do not increase significantly until renal function has been considerably impaired (Johnson *et al.*, 2004). Other parameters used to assess renal function/damage include blood urea nitrogen and serum electrolytes (Surks & Sievert, 1995; Hsu & Chertow, 2002; Pamela, 2004; Gowda *et al.*, 2010; Cunningham *et al.*, 2012).

The current study looks at the effects of consumption of the Jamaican bitter yam at a concentration of 5% of the diet, on serum parameters used to assess liver and kidney function. The parameters assessed were aspartate aminotransferase,

alanine aminotransferase, alkaline phosphatase, total protein, albumin, total and direct bilirubin, creatinine, blood urea nitrogen and serum electrolytes. Parameters used to indicate efficiency of glucose metabolism were also investigated as damage to the liver may impair glucose metabolism towards diabetes.

Materials and Methods***Sample preparation***

Yam tubers were obtained from farmers in Trelawny, Jamaica (Voucher numbers, 35620, 35621, and 35622; UWI Mona Herbarium). After washing thoroughly with distilled water to get rid of excess dirt, the tuber was then peeled, diced into small pieces and placed in a drying oven set to relatively low temperature (between 35 and 45°C). The yam pieces were left in the oven until dried as confirmed by constant weight measurements (approximately 48 hours). The dried yam pieces were subsequently ground to fine powder using a laboratory mill (Cole-Palmer IKA continuous feed grinding mill, EW-04301-30, Chicago Illinois USA) mesh size 0.2 mm.

Animals

Thirty two male mice (3 – 5 weeks of age) were obtained from Jackson's Laboratories, U.S.A. and housed at the Animal Core Facility, University of Maryland. The mice strains used were the C57BL/6 inbred strain and C57BL/6-Tg(APOA1)1Rub/J genetically modified strain. The C57BL/6-Tg(APOA1)1Rub/J strain was created by inserting the entire human apolipoprotein A-I gene including the promoter, APOA1, into the genome of the C57BL/6 strain (Rubin *et al.*, 1991). These mice showed a two fold increase in both total apolipoprotein A1 and HDL cholesterol (Rubin *et al.*, 1991). All mice were housed in a room maintained at 25 degrees Celsius with a 12 hour light/dark cycle. They were grouped as follows: (a) normocholesterolemic mice fed the Laboratory Rodent Diet 5001, (b) normocholesterolemic mice fed the Laboratory Rodent Diet 5001 supplemented with bitter yam (5%), (c) hypercholesterolemic mice fed the Laboratory Rodent Diet 5008, and (d) hypercholesterolemic mice fed the Laboratory Rodent Diet 5008 supplemented with the bitter yam (5%). The mice were fed their respective diets for six week. At the end of the feeding period, blood was obtained by orbital sinus venipuncture and sent to an independent laboratory for analysis.

Ethical approval for this study was obtained from the

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Ethics Committee of the Faculty of Medical Sciences, University of the West Indies, Mona Campus.

Biochemical assays

The concentration of total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin, albumin, glucose, creatinine, Blood Urea Nitrogen (BUN) and electrolytes in the serum of mice were determined using the VetTest® Chemistry Analyser at the Indexx Laboratories Inc., U.S.A. according to manufacturer's instructions.

Liver glucose-6-phosphatase activity was determined according to the method of Baginski *et al.* (1974). Briefly, a liver homogenate, prepared in sucrose solution (0.25 M), was added to a reaction mixture containing sucrose (0.25 M), glucose-6-phosphate (0.1 M) and cacodylate buffer (0.1 M; pH 6.5). This was incubated at 37°C for 5 minutes after which the reaction was stopped by the addition of TCA solution (10%) followed by immersion in an ice bath for approximately 10 minutes to allow for precipitate formation. The precipitate was removed by centrifugation and the resulting supernatant used to determine the liberated phosphate according to the method of Goldenberg & Fernandez (1966).

Glucokinase activity of the liver was determined according to the spectrophotometric method described by Davidson & Arion (1987). Briefly, liver homogenates were prepared in buffer containing tris-HCl (50 mM; pH 7.4), potassium chloride (100 mM), mercaptoethanol (10 mM), and EDTA (1 mM). The homogenate was centrifuged and the supernatant used to determine enzyme activity. The reaction was carried out at 37°C and initiated by the addition of ATP (5 mM) to a reaction mixture containing tris-HCl (100 mM), magnesium chloride (7.5 mM), NAD (1 mM), glucose-6-phosphate dehydrogenase (5.5 units), glucose (10 mM) and supernatant.

Protein was determined using a BCA™ protein assay kit according to manufacturer's instructions (product # 23227, lot # GC 94677, Pierce, USA).

Statistical analysis

The results were expressed as mean value ± the standard error of the mean (SEM). Statistical analysis was done using the one way analysis of variance (Anova) ($p < 0.05$). All statistical analyses were done using the statistical program, SPSS version 16 (2007).

Results

No significant changes were observed in the levels of serum total proteins in mice fed diets supplemented with bitter yam when compared to mice fed diets without supplementation (Figure 1).

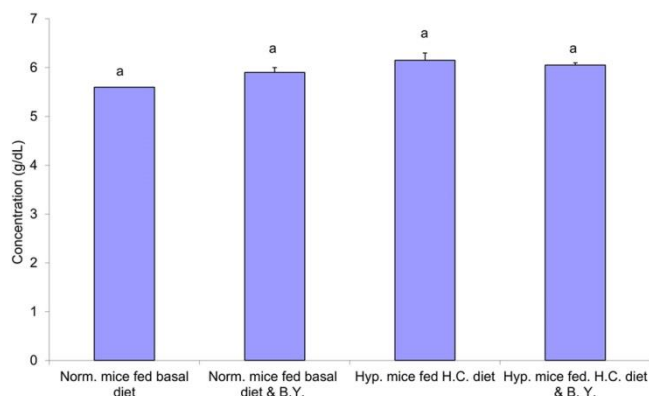


Figure 1. Total protein concentration in mice serum. Values: Mean ± SEM, $n = 8$, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

Bitter yam supplementation resulted in significant increases ($P < 0.05$) in serum aspartate aminotransferase of normocholesterolemic mice (from 116.50 ± 32.50 to 235.00 ± 16.00 U/L) and hypercholesterolemic mice (from 156.00 ± 17.00 to 231.50 ± 1.50 U/L) when compared to controls (Figure 2). Bitter yam supplementation had no effect on alanine aminotransferase or alkaline phosphatase activities in the mice serum (Figure 2).

Serum total bilirubin was significantly increased in normocholesterolemic mice fed diets supplemented with bitter yam ($P < 0.05$), from 0.35 ± 0.05 to 0.85 ± 0.05 mg/dL, when compared to normocholesterolemic mice fed a basal diet (Figure 3). No significant changes were seen in total bilirubin concentration in the serum of hypercholesterolemic mice fed a supplemented diet (Figure 3). Serum albumin was not affected by bitter yam supplementation (Figure 4).

Bitter yam supplementation had no effect on serum creatinine levels of mice however caused a significant increase in serum BUN levels of hypercholesterolemic mice only, from 26.00 ± 1.00 to 32.50 ± 0.50 mg/dL (Table 1).

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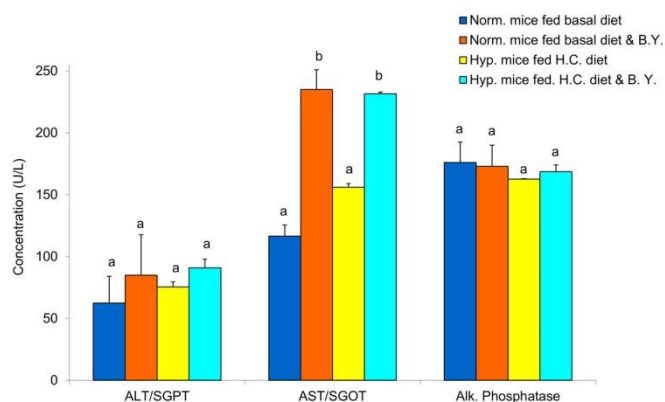


Figure 2. Serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase concentrations. Values: Mean \pm SEM, $n = 8$, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

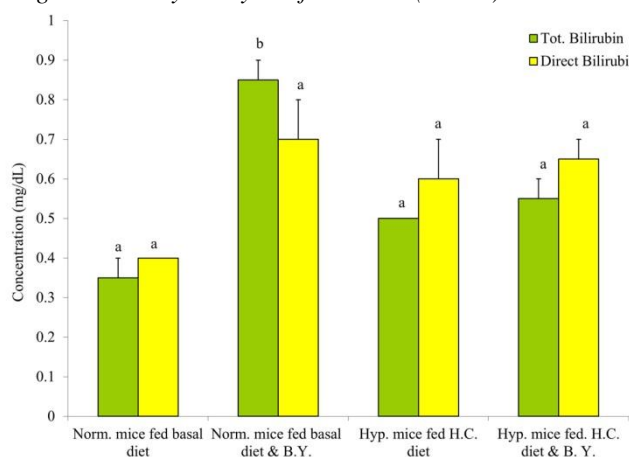


Figure 3. Total and direct bilirubin levels in mice serum. Values: Mean \pm SEM, $n = 8$, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

Magnesium concentration was significantly increased ($P < 0.05$) in the serum of normocholesterolemic mice fed a diet supplemented with the bitter yam, from 2.20 ± 0.00 to 4.65 ± 0.35 mmol/L, when compared to normocholesterolemic mice fed a basal diet (Table 2). No significant differences were observed in the concentration of magnesium in the serum of genetically modified

hypercholesterolemic mice fed diet supplemented with bitter yam when compared to hypercholesterolemic controls (Table 2). Dietary supplementation with the bitter yam did not result in any significant changes in serum potassium concentrations in either normocholesterolemic or hypercholesterolemic mice (Table 2).

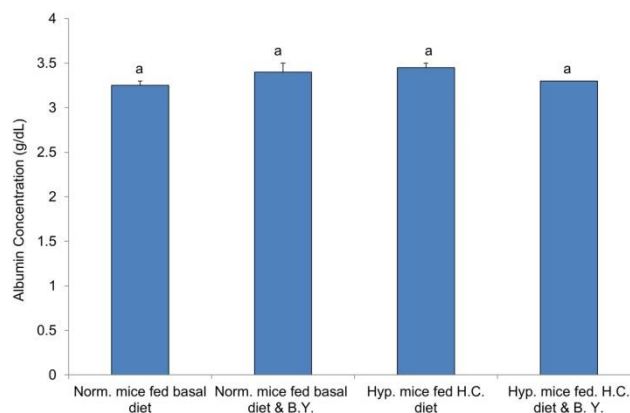


Figure 4. Albumin levels in mice serum. Values: Mean \pm SEM, $n = 8$, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

Table 1. Creatinine and blood urea nitrogen levels in mice serum

Group	Creatinine (mg/dL)	BUN (mg/dL)
Norm. mice fed basal diet	0.40 ± 0.00^a	25.00 ± 1.00^a
Norm. mice fed B.Y. diet	0.45 ± 0.05^a	$28.00 \pm 1.00^{a,b}$
Hyp. mice fed basal diet	0.50 ± 0.00^a	26.00 ± 1.00^a
Hyp. mice fed B.Y. diet	0.45 ± 0.05^a	32.50 ± 0.50^b

Values: Mean \pm SEM, $n = 8$, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different ($P < 0.05$). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

No significant changes were seen in the concentrations of iron (Table 3), sodium or chloride ions (Figure 5) in the serum of mice fed bitter yam supplemented diets compared to control mice fed diets without supplementation. A significant increase ($P < 0.05$) was observed in the concentration of phosphorus in the serum of normocholesterolemic mice fed basal diet supplemented with bitter yam compared to normocholesterolemic controls (Table 3).

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Table 2. Potassium and magnesium levels in mice serum

Group	Potassium (mmol/L)	Magnesium (mEq/L)
Norm. mice fed basal diet	8.35 ± 0.25 ^a	2.20 ± 0.00 ^a
Norm. mice fed B.Y. diet	8.55 ± 0.05 ^a	4.65 ± 0.35 ^b
Hyp. mice fed basal diet	8.95 ± 0.55 ^a	2.45 ± 0.05 ^a
Hyp. mice fed B.Y. diet	8.65 ± 0.05 ^a	2.60 ± 0.20 ^a

Values: Mean ± SEM, n = 8, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different (P<0.05). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

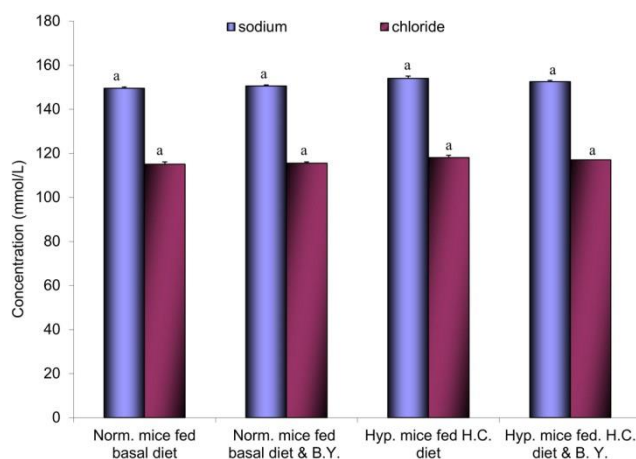


Figure 5. Sodium and chloride ion concentrations in mice serum. values: Mean ± SEM, n = 8, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different (P<0.05). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova)

Bitter yam supplementation had no effect on serum fasting glucose concentration (Table 4) or the activities of glucokinase and glucose-6-phosphatase (Table 5).

Discussion

Based on an overall assessment of serum parameters used to indicate liver damage/dysfunction, it can be postulated that bitter yam supplementation did not induce liver damage in the mice despite significant increases in serum aspartate aminotransferase activity. Due to the widespread nature of this enzyme, significant increases in its concentration in serum represent non-specific tissue damage (Dufour *et al.*,

2000a, 2000b; Ozer *et al.*, 2008).

Table 3. Total iron and phosphorus levels in mice serum

Group	Total Iron (µg/dL)	Phosphorus (mg/dL)
Norm. mice fed basal diet	185.50 ± 12.50 ^a	7.30 ± 0.10 ^a
Norm. mice fed B.Y. diet	191.50 ± 8.50 ^a	15.70 ± 1.20 ^b
Hyp. mice fed basal diet	153.00 ± 17.00 ^a	9.20 ± 0.10 ^a
Hyp. mice fed B.Y. diet	162.00 ± 36.00 ^a	8.40 ± 0.00 ^a

Values: Mean ± SEM, n = 8, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different (P<0.05). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

Table 4. Serum fasting glucose concentrations

Group	Serum fasting glucose concentrations (g/L)
Norm. mice fed basal diet	172.5 ± 12.5 ^a
Norm. mice fed B.Y. diet	193.0 ± 7.0 ^a
Hyp. mice fed basal diet	197.5 ± 1.5 ^a
Hyp. mice fed B.Y. diet	187.0 ± 7.0 ^a

Values: Mean ± SEM, n = 8, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different (P<0.05). Statistical analyses of table and charts were done using the one way Analysis of variance (Anova).

Table 5. Specific activity of glucokinase and glucose-6-phosphatase in mice livers

Group	Glucokinase specific activity (U/mg of protein)	Glucose-6-phosphatase specific activity (U/mg of protein)
Norm. mice fed basal diet	2231.84 ± 773.13 ^a	80 ± 0.02 ^a
Norm. mice fed B.Y. diet	1644.34 ± 639.47 ^a	80 ± 0.0 ^a
Hyp. mice fed basal diet	2415.83 ± 840.67 ^a	90 ± 0.0 ^a
Hyp. mice fed B.Y. diet	2315.77 ± 463.15 ^a	120 ± 0.02 ^a

Values: Mean ± SEM, n = 8, Norm. = normocholesterolemic, Hyp. = hypercholesterolemic, B.Y. = bitter yam supplemented, H. C. = high cholesterol. Figures in columns that share different letter subscripts are significantly different (P<0.05). Statistical analyses of table and charts were done using the one way analysis of variance (Anova) and the Duncan's multiple range test. A unit of glucose-6-phosphate activity is defined as the amount of enzyme liberating 1nmole of orthophosphate per minute. A unit of glucokinase is defined as the amount of enzyme that oxidizes 1nmole of NADH to NAD⁺ per minute.

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Other tests must be performed in order to ascertain which organ may have been damaged. The other enzymes assessed were alkaline phosphatase and alanine aminotransferase. These enzymes are mainly found in the liver, but also in smaller amounts in other tissues (Pratt & Kaplan, 2000; Kaplan, 2002). They are released into the bloodstream during liver damage or in diseased conditions (Dufour *et al.*, 2000a; Pratt & Kaplan, 2000; Huang *et al.*, 2006). Their activities however, remained unchanged after bitter yam supplementation suggesting maintenance of liver integrity in all groups. Glucose and protein metabolism remained unaffected in both species after bitter yam supplementation which is also suggestive of an intact and functioning liver.

Other parameters used to assess hepatic damage/dysfunction include serum albumin, total bilirubin, direct bilirubin and total proteins. Hyperbilirubinemia was observed in normocholesterolemic mice after bitter yam supplementation. Saponin, a phytochemical found in Jamaican bitter yam, is known to cause erythrocyte haemolysis by causing irreversible damage to the lipid bilayer (Baumann *et al.*, 2000). Bilirubin is a major product of erythrocyte haemolysis, and could possibly explain the significant increase observed in the serum of normocholesterolemic mice. No significant increase in this parameter was observed in the serum of hypercholesterolemic mice fed bitter yam supplemented diets. These mice were genetically engineered to overproduce both total apolipoprotein A1 and HDL cholesterol (Rubin *et al.*, 1991). Studies have shown that apolipoprotein A1 and HDL help to protect and stabilize cell membranes thereby preventing haemolysis (Epanand *et al.*, 1994). This would enhance erythrocyte protection thereby negating, to some extent, the destructive forces of the saponins thus leaving the erythrocytes intact.

Serum parameters used to assess kidney function revealed that the kidneys of both mice species may have been damaged to different extents by bitter yam consumption (5%). These results corroborate with results from lipid peroxidation analyses in a previous study which indicated that the kidneys of the hypercholesterolemic mice may have been damaged to a greater extent than in the normocholesterolemic mice (Stennett *et al.*, 2014). Damage, however, may have been mild as there were no significant changes in serum creatinine. It is well documented that serum creatinine levels do not increase until at least half of the kidney's nephrons are destroyed or damaged (Bhattacharya *et al.*, 2005).

Hyperphosphatemia and hypermagnesemia were experienced by normocholesterolemic mice fed bitter yam. While kidney damage could have possibly caused the increases seen, the more likely explanation would be erythrocyte haemolysis since a similar increase in bilirubin concentration was seen in these mice after bitter yam supplementation (Gibson, 2005; Tucker & Hornley-Brown, 2013). The concentrations of magnesium and phosphorus are greater in erythrocytes and their destruction could therefore possibly affect serum concentrations.

Based on evidence from previous work done (Stennett *et al.*, 2013, 2014) and currently obtained results, it can be concluded that bitter yam supplemented at a concentration of 5% may have elicited mild damage to mice kidneys. Some haemolysis may also have occurred as a result of supplementation, however, HDL cholesterol may have protected erythrocytes from significant haemolysis in the genetically modified hypercholesterolemic mice. Further research is required to determine the most effective dosage for consumption of the bitter yam.

Acknowledgement

The authors of this paper would like to thank the Office of Graduate Studies and Research, University of the West Indies and the Biology Department, Morgan State University for supporting this research. Special thanks to Dr. Curtis Green for his invaluable contributions to this work.

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