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Optimal dosage determination of a hypocholesterolemic bitter yam proprietary preparation in diet-induced hypercholesterolemic mice.

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Abstract: Coronary heart disease, a condition associated with dyslipidemias including hyperlipidemia and low HDL-C levels, has been an increasing problem in the developing world. Conventional treatment for hyperlipidemia often present with unfavourable side effects, leading to the need for development of drugs from natural products. The hypoglycemic and hypocholesterolemic properties of the Jamaican bitter yam have previously been demonstrated however consumption at a high dosage presents with various adverse effects. This study is therefore geared towards the determination of an optimal dosage for the consumption of a proprietary preparation made from this yam species. Hypercholesterolemic mice were fed the preparation at various dosages (4, 2, 1, 0.5%) for 3 weeks after which they were phlebotomized then euthanized. Organs were stored at -80°C until required for analysis. The optimal dosage for supplementation, which resulted in significant decreases in serum cholesterol and oxidative stress without eliciting adverse effects, was found to be 2%. The results from this study points to the need for future in-depth studies involving dietary supplementation at the 2% supplementation level.

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INTRODUCTION:

Coronary heart disease (CHD) has had a devastating impact on both developed and developing countries worldwide (Pandya *et* al 2006; Gaziano et al, 2010). It is an atherosclerosis-induced condition associated with dyslipidemias, including hyperlipidemia (hypercholesterolemia) and low levels of high-density-lipoprotein cholesterol (HDL-C) (Bersot, 2011). Hypercholesterolemia is multifactorial in its origin and most forms are characterised by increased concentrations of LDL cholesterol (Grundy, 1991; Vega et al., 1991).

Conventional drugs used to treat hypercholesterolemia include statins, cholesterol absorption blockers, bile acid sequestrants and nicotinic acid. They may be used singly or in combination if the single drug is not effective enough. These drugs, however, display various side effects in patients (Sowmya and Ananthi, 2011), hence there is increased interest in the development of lipid lowering drugs from natural products.

The Jamaican bitter yam, *Dioscorea polygonoides*, a wild yam variety traditionally used to make "roots tonic", has been shown to possess phytochemicals which display bioactivity against hyperglycemia and hypercholesterolemia (McAnuff et al, 2003; McAnuff et al, 2005). The major phytochemicals thought to be responsible for the bioactivity against these conditions are the saponins. These are high molecular weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone. They are constituents of many plant drugs and folk medicines and so great interest has been shown in their characterization and in the investigation of their pharmacological and biological properties.

Recent work conducted in our laboratories however pointed to possible adverse effects of consumption of the Jamaican bitter yam at a relatively high concentration (5%). Oxidative stress was induced in the tissues of mice fed bitter yam supplemented diets (Stennett *et* al, 2013; Stennett *et al*, 2014). The current study therefore investigates the optimal dosage for consumption of a proprietary preparation (BYPP) made from the Jamaican bitter yam (Stennett and Asemota, 2010).

METHODOLOGY:

Reagents

Sodium Dodecyl Sulphate (SDS – 8.1%), Acetic acid (20 %), Sodium hydroxide, Thiobarbituric acid (0.8 %), n-butanol, Pyridine, Chloroform, Methanol, Cyclohexane, Cholic acid, Cholesterol, Standard cholesterol solution (1 mg/mL)

Ferric chloride solution: 10.0 g of ferric chloride was dissolved in 100 mL of glacial acetic acid.

Colour reagent: 2.0 mL of ferric chloride solution was diluted to 200 mL with concentrated sulphuric acid.

Animal treatment

Forty-eight (48) mice were obtained from the animal-house of the University of the West Indies. They were weighed and initially divided into two groups. Group 1 (8 mice) consisted of mice fed a basal rat chow diet only (normocholesterolemic controls). Mice in group 2 (40 mice) were made hypercholesterolemic by adding cholesterol (1%) and cholic acid (0.5%) to the diet for a period of three weeks. At the end of the three week period blood was collected via the ocular cavity for serum total cholesterol determination in order to obtain a hypercholesterolemic baseline. The hypercholesterolemic group was then subdivided into five groups containing eight mice each. The groups were as follows: (2a) hypercholesterolemic mice fed a hypercholesterolemic diet – hypercholesterolemic control, (2b) hypercholesterolemic mice fed a hypercholesterolemic diet supplemented with 4% BYPP, (2c) hypercholesterolemic mice fed a hypercholesterolemic diet supplemented with 2% BYPP, (2d) hypercholesterolemic mice fed a high hypercholesterolemic diet supplemented with 1% BYPP and (2e) hypercholesterolemic mice fed a hypercholesterolemic diet supplemented with 0.5% BYPP. The mice were fed their respective diets for four weeks. Dietary supplementation involved directly adding the BYPP to the diet. At the end of the feeding period, blood was again collected via the ocular cavity and analysed for total-cholesterol levels. The mice were then weighed and sacrificed using an overdose of sodium pentobarbital and the hearts, livers, kidneys, brains and intestines excised, weighed and stored at -80°C until required for analysis.

(Ethical approval for this study was obtained from the Ethics Committee of the University of the West Indies, Mona Campus)

Conjugated dienes determination

Conjugated dienes were determined using the method of Hu et al. (1989). A 1.6 ml aliquot of homogenate was mixed with 6 mL chloroform: methanol (1:2) for 1 minute followed by the addition of 2 mL of chloroform then mixing for a further 30 seconds. Deionized water (2 mL) was then added followed by mixing for 30 seconds and centrifugation for 10 minutes at 1000 × g. Approximately 2 mLs of the chloroform (lower) layer was then removed and dried under nitrogen at 40˚C. The dried extract was dissolved in 1 mL of cyclohexane and conjugated dienes was measured at 233 nm using an extinction coefficient of 27,000 $M^{-1}cm^{-1}$.

Assay of Thiobarbituric Acid Reactive Substances (TBARS)

Homogenates were prepared according to the method of Ibrahim et al. (1997). A 20% homogenate was prepared in ice cold 50 mM potassium phosphate buffer (pH 7.4) containing 1.55 M potassium chloride using a Teflon Potter-Elvehjen homogenizer.

TBARS was determined by the method of Ohkawa et al. (1978). 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 was prepared. The reaction mixture was then made up to 4 mL with distilled water and refluxed at 95ºC for 60 minutes. 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 minutes. 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).

Total cholesterol determination

Total cholesterol was determined according to the method of Zlatkis *et al* (1953). Ferric chloride solution was prepared by dissolving 1.0 g of ferric chloride in 10 mL of glacial acetic acid. A colour reagent was prepared by diluting 2.0 mL of ferric chloride solution to 200 mL with concentrated sulphuric acid.

The sample (0.1 mL) was added to 3.0 mL of glacial acetic acid in a dry test tube. Colour reagent (2.0 mL) was then added and the content of each tube was mixed by vortexing. The tubes were allowed to cool to room temperature and read against a suitable blank at 560 nm using a Cecil 9000 Series spectrophotometer.

A standard cholesterol solution was prepared at a concentration of 1 mg/mL.

Calculations:

Cholesterol concentrations were calculated using the following equation:

$$\frac{Optical\ density\ of\ test}{Optical\ density\ of\ standard} = \frac{Concentration\ of\ test}{Concentration\ of\ standard}$$

RESULTS:

Serum Total Cholesterol

At the end of the testing period, hypercholesterolemic controls had the highest serum total cholesterol 248.63 ± 22.17 mg/dL.

Groups fed bitter yam supplemented (4, 2, 0.5%) hypercholesterolemic diets had significantly lower serum cholesterol ($P<0.05$), 175.18 ± 11.89, 146.84 ± 8.00 and 159.86 ± 13.40 mg/dL respectively, compared to the hypercholesterolemic controls (Fig. 1).

Mice Weights

No significant differences in body weights were observed amongst the various groups (Table 1).

Organ Weights

The absolute kidney weights of mice fed diet supplemented with 4% BYPP (0.25 ± 0.05 g) were significantly lower ($P<0.05$) than normocholesterolemic controls (0.44 ± 0.04 g) (Fig. 2). The livers of hypercholesterolemic controls were significantly larger ($P<0.05$), at 2.64 ± 0.04 g, compared to normocholesterolemic controls, at 1.16 ± 0.13 g.

Table 1 Body Weights of Mice Fed BYPP Supplemented Diets at Varying Concentrations

	Weight (g)		
Group	**Initial**	**Final**	**Total Change (g)**
Hyp BY 2%	16.94 ± 0.46^{a}	19.23 ± 0.80^{a}	$2.29 \pm 0.52^{a,b}$
Hyp BY 4%	15.94 ± 0.28^{a}	18.52 ± 0.82^{a}	$2.58 \pm 0.56^{a,b}$
Hyp BY 1%	16.73 ± 0.72^{a}	19.52 ± 0.79^{a}	$2.79 \pm 0.47^{a,b}$
Hyp BY 0.5%	16.20 ± 0.41^{a}	20.02 ± 0.68^{a}	3.82 ± 0.37^{b}
HYP control	16.74 ± 0.55^{a}	19.30 ± 0.54^{a}	$2.56 \pm 0.17^{a,b}$
Norm control	16.09 ± 0.75^{a}	17.99 ± 0.80^{a}	1.90 ± 0.22^{a}

Values: Mean ± SEM, n = 10, Norm = normocholesterolemic, Hyp = Hypercholesterolemic, BY 4% (2, 1 or 0.5 %) = mice fed a high cholesterol diet supplemented with 4% (2, 1 or 0.5 % respectively) BYPP. Different letter subscripts represent groups that are significantly different ($P<0.05$) in each column. Statistical analysis was done using the one way Analysis of variance (Anova) and the Duncan's multiple range test.

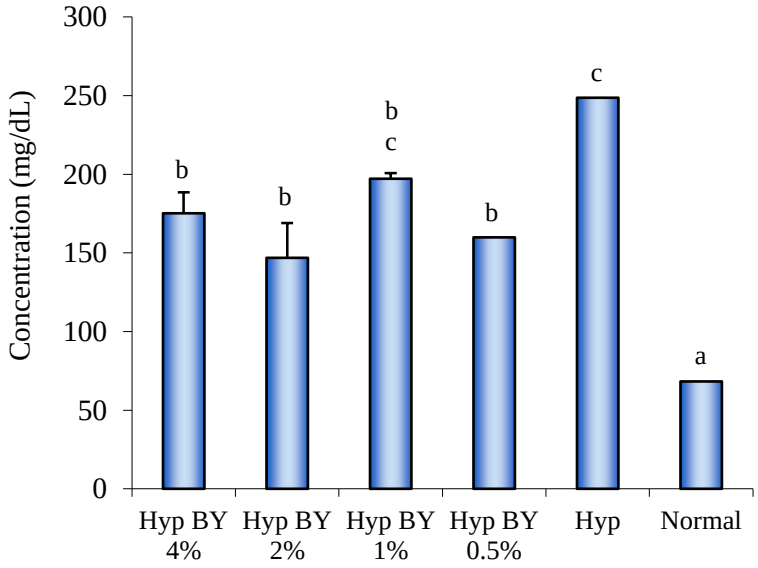

Fig. 1 Mice Serum Total Cholesterol in Mice Fed BYPP Supplemented Diets at Varying Concentrations

n = 10, Hyp = hypercholesterolemic mice fed a hypercholesterolemic diet, Hyp BY 4% (2, 1 or 0.5 %) = Hypecholesterolemic mice fed a high cholesterol diet supplemented with 4% (2, 1 or 0.5 % respectively) BYPP. Different letter subscripts for each group represents cholesterol levels that are significantly different ($P<0.05$). Statistical analysis was done using the one way Analysis of variance (Anova) and the Duncan's multiple range test.

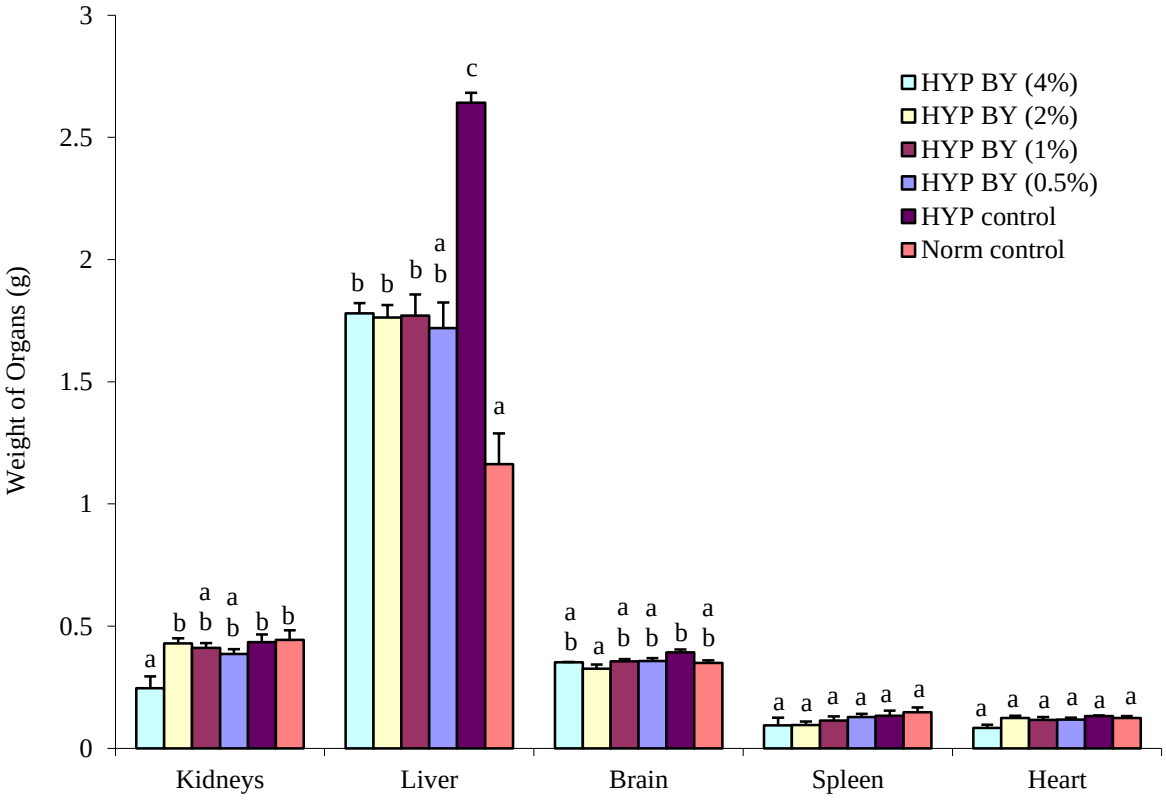

Fig. 2 Organ Weights of Mice Fed BYPP Supplemented Diets at Varying Concentrations

n = 10, Norm = normocholesterolemic, Hyp = Hypercholesterolemic, BY 4% (2, 1 or 0.5 %) = mice fed a high cholesterol diet supplemented with 4% (2, 1 or 0.5 % respectively) BYPP. Different letter subscripts for each organ represent significant differences (P<0.05). Statistical analysis was done using the one way Analysis of variance (Anova) and the Duncan″s multiple range test.

Mice fed diets supplemented with 0.5, 1, 2, and 4% BYPP had significantly reduced liver weights (P<0.05), 1.72 ± 0.11, 1.77 ± 0.09, 1.76 ± 0.05 and 1.78 ± 0.04 g respectively compared to hypercholesterolemic controls, 2.64 ± 0.04 g (Fig. 2).

The brains of mice fed diet supplemented with 2% BYPP were significantly smaller (P<0.05), 0.33 ± 0.02 g, than brains of hypercholesterolemic controls, 0.39 ± 0.33 g.

Organ to Body Weight Ratio

Mice fed diet supplemented with 4% BYPP had a significantly lower (P<0.05) kidney to body weight ratio, 0.0131 ± 0.0020, than hypercholesterolemic controls, 0.0200 ± 0.0005 (Fig. 3). Hypercholesterolemic controls had a higher liver to body weight ratio, 0.1364 ± 0.0005, than the normocholesterolemic controls, 0.0645 ± 0.0011 (Fig. 3). Mice fed diets supplemented with 4, 2, 1 and 0.5 % BYPP had significantly lower (P<0.05) liver to body weight ratios from 0.0969 ± 0.0018, 0.0834 ± 0.0018, 0.0910 ± 0.0027 and 0.0853 ± 0.0042 respectively, than hypercholesterolemic controls, 0.1364 ± 0.0005 (Fig. 3).

The heart and kidney to body weight ratios, 0.0044 ± 0.0005 and 0.0131 ± 0.0020 respectively, were lower in groups fed diets supplemented with 4% BYPP than in normocholesterolemic controls, 0.0069 ± 0.0001 and 0.0244 ± 0.0005 respectively (Fig. 3).

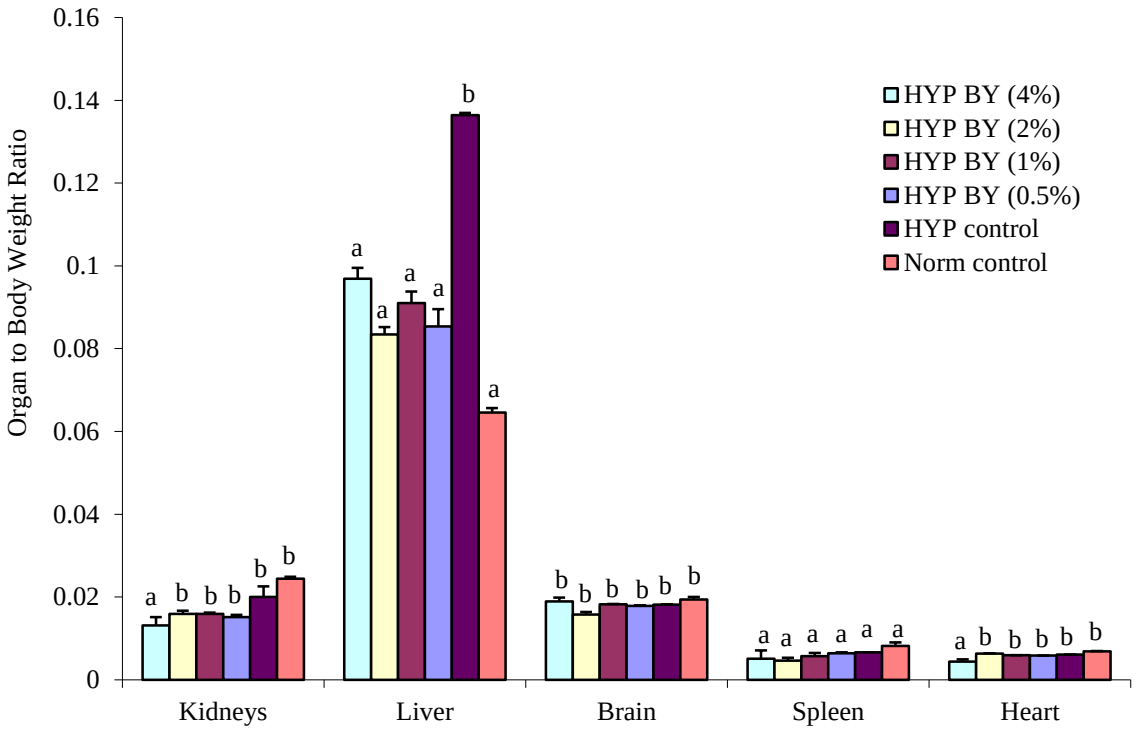

Fig. 3 Organ to Body Weight Ratio at Varying Concentrations

n = 10, Norm = normocholesterolemic, Hyp = Hypercholesterolemic, BY 4% (2, 1 or 0.5 %) = mice fed a high cholesterol diet supplemented with 4% (2, 1 or 0.5 % respectively) BYPP. Different letter subscripts for each organ represent significant differences (P<0.05). Statistical analysis was done using the one way Analysis of variance (Anova) and the Duncan's multiple range test.

TBARS

Hypercholesterolemic mice had significantly higher liver lipid peroxidation levels (P<0.05) (308.58 ± 14.02 nmol MDA/g wet wt.) than normocholesterolemic controls (211.34 ± 10.37 nmol MDA/g wet wt.) (Fig. 4). Mice fed diets supplemented with 4, 2, 1 and 0.5 % BYPP had significantly lower liver lipid peroxidation levels (140.39 ± 10.23, 177.72 ± 18.47, 166.23 ± 18.41 and 173.23 ± 25.26 nmol MDA/g wet wt. respectively) than hypercholesterolemic controls (308.58 ± 14.02 nmol MDA/g wet wt.) (Fig. 4).

Lipid peroxidation was significantly higher (P<0.05) in the hearts of hypercholesterolemic controls (742.12 ± 81.50 nmol MDA/g wet wt.) compared to the normocholesterolemic controls (529.28 ± 44.28 nmol MDA/g wet wt.). The hearts of hypercholesterolemic mice fed diets supplemented with BYPP (4%) had significantly higher lipid peroxidation levels (923.16 ± 46.10 nmol MDA/g wet wt.) than both control groups (Fig. 4). The hearts of mice fed diets supplemented with 2, 1, 0.5% BYPP had significantly lower levels of lipid peroxidation (P<0.05), of 489.33 ± 31.89, 387.43 ± 44.97 and 470.08 ± 31.16 nmol MDA/g wet wt. respectively, compared to the hypercholesterolemic control group (742.12 ± 81.50 nmol MDA/g wet wt.).

Lipid peroxidation was significantly higher (P<0.05) in the brains of hypercholesterolemic controls than in the brains of normocholesterolemic control mice. Groups fed diets supplemented with 4, 2, 1 and 0.5 % had significantly lower lipid peroxidation (P<0.05) in the brains (325.03 ± 43.57, 306 ± 15.91, 318.68 ± 32.24 and 214.32 ± 13.38 nmol MDA/g wet wt. respectively) compared to the hypercholesterolemic control group with an average lipid peroxide level of 472.03 ± 56.29 nmol MDA/g wet wt. (Fig. 4).

The spleen of hypercholesterolemic mice fed diets supplemented with 0.5% BYPP had significantly lower lipid peroxidation (155.21 ± 10.03 nmol MDA/g wet wt.) compared to both control groups (Fig. 4).

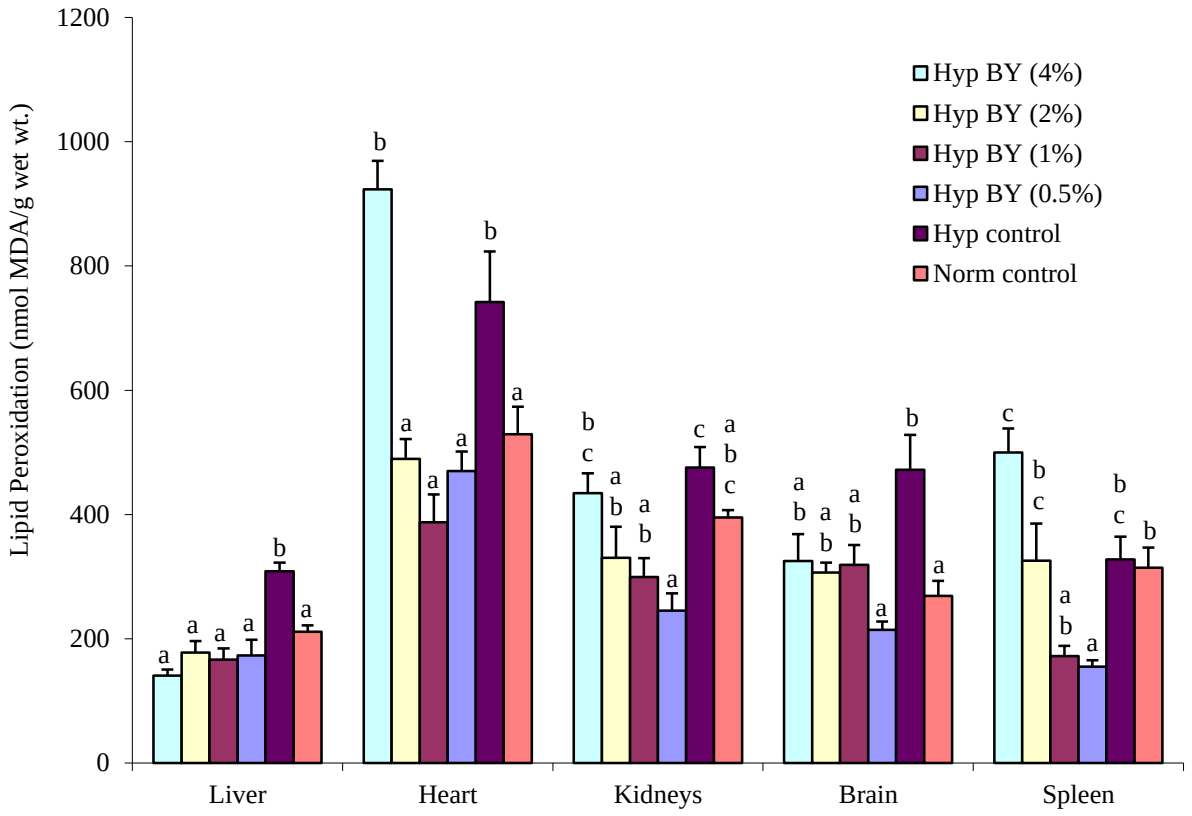

Fig. 4 Lipid Peroxidation (TBARS) in Organs of Mice Fed BYPP Supplemented Diets at Varying Concentrations

n = 10, Norm = normocholesterolemic, Hyp = Hypercholesterolemic, BY 4% (2, 1 or 0.5 %) = mice fed a high cholesterol diet supplemented with 4% (2, 1 or 0.5 % respectively) BYPP. Different letter subscripts for each organ represent significant differences (P<0.05). Statistical analysis was done using the one way Analysis of variance (Anova) and the Duncan's multiple range test.

Conjugated Dienes

Conjugated dienes was significantly lower (P<0.05) in the livers of hypercholesterolemic mice fed diets supplemented with 4, 2, 1 and 0.5 % BYPP, 1.21 ± 0.01, 1.77 ± 0.03, 1.68 ± 0.02 and 1.48 ± 0.01 µmole/g tissue respectively, compared to hypercholesterolemic controls, with a level of 2.20 ± 0.12 µmole/g tissue (Fig. 5).

The hearts of hypercholesterolemic controls and hypercholesterolemic mice fed diet supplemented with 4% BYPP had significantly elevated (P<0.05) conjugated dienes levels, 0.60 ± 0.02 and 0.66 ± 0.05 µmole/g tissue respectively, compared to normocholesterolemic controls (0.42 ± 0.06 µmole/g tissue) (Fig. 5). Groups fed diets supplemented with 2, 1 and 0.5 % BYPP had significantly lower conjugated dienes (P<0.05) in the hearts, 0.60 ± 0.02 to 0.40 ± 0.02, 0.44 ± 0.02 and 0.34 ± 0.02 µmole/g tissue respectively, compared to hypercholesterolemic controls (Fig. 5).

Conjugated dienes was significantly higher in the kidneys of hypercholesterolemic controls (0.75 ± 0.04 µmole/g tissue) compared to normocholesterolemic controls (0.50 ± 0.01 µmole/g tissue) (Fig. 5). The kidneys of hypercholesterolemic mice fed diets supplemented with 2, 1, 0.5 % BYPP had significantly lower levels of conjugated dienes (P<0.05), 0.54 ± 0.01, 0.37 ± 0.06 and 0.29 ± 0.01 µmole/g tissue respectively, when compared to hypercholesterolemic controls with a level of 0.75 ± 0.04 µmole/g tissue (Fig. 5).

The brains of the hypercholesterolemic controls had significantly higher (P<0.05) conjugated dienes (0.86 ± 0.03 µmole/g tissue) than normocholesterolemic controls (0.49 ± 0.00 µmole/g tissue) (Fig. 5). Groups fed diets supplemented with 4, 2, 1 and 0.5 % BYPP had significantly lower (P<0.05) levels of conjugated dienes in the brains, 0.51 ± 0.01, 0.48 ± 0.00, 0.46 ± 0.01 and 0.39 ± 0.01 µmole/g tissue respectively, when compared to hypercholesterolemic control mice with an average concentration of 0.86 ± 0.03 µmole/g tissue (Fig. 5).

Conjugated dienes was significantly higher (P<0.05) in the spleen of mice fed a diet supplemented with 4 % BYPP, 0.89 ± 0.01 µmole/g tissue, compared to normocholesterolemic controls,

0.57 ± 0.02 µmole/g tissue (Fig. 5). Groups fed diets supplemented with 1 and 0.5 % BYPP had significantly lower ($P<0.05$) conjugated dienes in the spleen, 0.41 ± 0.01 and 0.36 ± 0.01 µmole/g tissue, compared to hypercholesterolemic controls, with an average concentration of 0.59 ± 0.00 µmole/g tissue (Fig. 5).

DISCUSSION:

The hypocholesterolemic action of saponins has been extensively studied and well documented (McKoy *et al*, 2004; McAnuff *et al*, 2003; Oakenfull and Sidhu, 1990). Previous studies carried out in our laboratories have also strongly indicated the hypocholesterolemic nature of the Jamaican bitter yam (*Dioscorea polygonoides*), a wild yam species with a high saponin content (McAnuff *et al*, 2003; McKoy *et al*, 2004; McAnuff *et al*, 2005). The consumption of this yam species, however, at a concentration of 5% displayed adverse effects on various organs (Stennett *et al*, 2013; Stennett *et al*, 2014a; Stennett *et al*, 2014b). This study was therefore undertaken to determine an effective dosage for the consumption of a proprietary preparation (Stennett and Asemota, 2010) made from this wild yam species, beyond which there are no additional benefits and below which there are significantly less or no clinical benefits.

Hypercholesterolemia was induced in test mice by dietary means, and confirmed by significant increases in total serum cholesterol. Supplementation of the diet with BYPP, however, resulted in significant decreases in serum cholesterol levels when compared to hypercholesterolemic controls. The greatest decrease was observed in mice fed diet supplemented with 2% proprietary preparation. This hypocholesterolemic action may, in part, be due to the action of saponins. They interfere with cholesterol excretion, absorption and metabolism by the liver, thereby effectively reducing cholesterol concentration in the blood (Harwood *et al*, 1993; Khanna *et al*, 2002; Balasubramanian *et al*, 2008).

Lipid peroxidation biomarkers were used to evaluate oxidative stress in the organs tested. The effects of lipid peroxidation can be assessed at different stages of lipid degradation. The products measured include conjugated dienes, lipid hydroperoxides and substances reactive with thiobarbituric acid (TBARS) considered as MDA-like peroxides (Dalle-Donne *et al*, 2006; Guteridge, 1995). Conjugated dienes and TBARS were used as oxidative stress biomarkers in this study. These parameters were chosen due to the fact that an exposure to stress situations, such as pathological conditions, may result in increased free radical generation, ultimately leading to increased lipid peroxidation, protein oxidation, DNA damage and possible cell death (Grotto, 2009). Hypercholesterolemia induction led to significant increases in lipid peroxidation in the livers, hearts and brains of mice, suggesting possible injury to these organs. Supplementation of the diet with BYPP at all concentrations tested resulted in significant reductions in the oxidative stress in the liver. Lipid peroxidation analysis suggested dietary supplementation with 4% BYPP negatively impacted mice heart, kidneys and spleen when compared to controls. Similar results were obtained in previously conducted work in our laboratories where mice were fed diets supplemented with 5% BYPP (Stennett *et al*, 2013; Stennett *et al*, 2014a). Lipid peroxidation analyses also indicated that dietary supplementation with 0.5%, 1% and 2% BYPP had positive effects on organs tested, specifically the liver, heart and brain, with values trending towards those of the normocholesterolemic controls. The beneficial effects of supplementation was particularly observed in the spleen of mice fed diets supplemented with 0.5% BYPP where lipid peroxidation was significantly lower than normocholesterolemic controls. These results corroborate well with results obtained from conjugated dienes analysis.

Hypercholesterolemic mice fed diet supplemented with 0.5% BYPP experienced significant increases in body weight; this dosage is therefore not optimal for supplementation. Hypercholesterolemic control mice experienced hepatomegaly at the end of the study. These results corroborate with work carried out by Matos *et al* (2005) which showed significant increases in liver weights of hypercholesterolemic mice fed various hypercholesterolemic diets. This increase was reported to have occurred as a result of an increase in the fat content of the liver as well as the proliferation of hepatocytes stimulated by overexpression of squalene synthase, the first committed enzyme in cholesterol biosynthesis (Okazaki *et al*, 2006). Supplementation of the diets of hypercholesterolemic mice with the BYPP at all concentrations reversed the condition in these mice. This may have been as a result of the saponins present in the preparation, which is known for its hepatoprotective properties (Abe *et al*, 1980; Kiso *et al*, 1984; Safayhi and Sailer, 1997; Matsuda *et al*, 1997; Yoshikawa *et al*, 1997).

Renal and cardiac atrophy was evident in hypercholesterolemic mice fed a hypercholesterolemic diet supplemented with 4% BYPP. Atrophy is an adaptive response of tissues to physiologic stress or pathogenicity, resulting in decreased size and/or number of cells. Function is also often diminished. Other responses include hypertrophy, hyperplasia and metaplasia. Cell death may occur if injury is severe and irreversible (Sirica, 1989; Muscari *et al*, 1996).

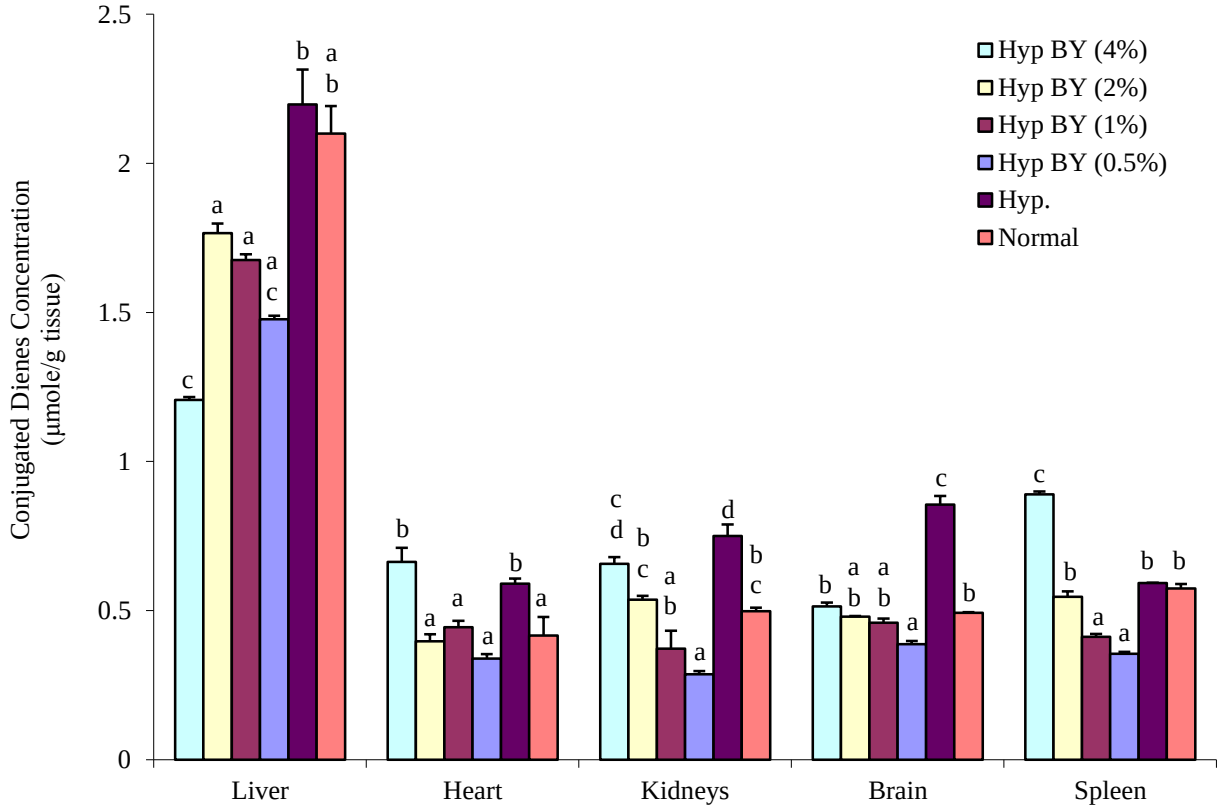

Fig. 5 Conjugated Dienes Levels in Organs of Mice Fed BYPP Supplemented Diets at Varying Concentrations

n = 10, Norm = normocholesterolemic control, Hyp = Hypercholesterolemic control, BY 4% (2, 1 or 0.5 %) = mice fed a high cholesterol diet supplemented with 4% (2, 1 or 0.5 % respectively) BYPP. Different letter subscripts for each organ represent significant differences (P<0.05). Statistical analysis was done using the one way Analysis of variance (Anova) and the Duncan's multiple range test.

Possible causative factors include increased catabolism of cell organelles, apoptosis, decreased protein synthesis or increased protein degradation (Goljan, 2013). In excess, the bioactive factors present in the bitter yam preparation may have introduced insults which eventually led to tissue damage followed by subsequent atrophy once enough cells were affected. Plant secondary metabolites, despite their therapeutic properties, have been shown to display toxicity and can affect virtually any physiological process of a living organism (Wink, 2009).

A possible link can be made between the reduction in organ size and the significant increase seen in lipid peroxidation. Brown atrophy, a condition marked by atrophy of a tissue or organ and an intracellular accumulation of lipofuscin pigment (aging pigment), may be displayed by organs, such as the heart and liver (Goljan, 2014). Lipofuscin is an indigestible product of unsaturated fatty acid oxidation which accumulates within lysosomes as granules (Sulzer *et al*, 2008). This intracellular deposition of the lipofuscin pigment results in the tissues acquiring a brown colouration. Other constituents of the lipofuscin network include oxidized proteins and bound sugar residues (Höhn and Grune, 2013).

CONCLUSION:

Overall, dietary supplementation at a dosage of 2% proved to be optimal after full assessment of all pertinent parameters. At this dosage, supplementation resulted in significant reductions in serum cholesterol and oxidative stress associated with the hypercholesterolemic state. Supplementation at 1% did not effectively reduce serum cholesterol levels while 4% elicited atrophic changes in the heart and kidneys and mice fed diets supplemented at 0.5% gained significantly more weight than mice in other groups.

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