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Potato protein hydrolysate attenuates high fat diet-induced cardiac apoptosis through SIRT1/ PGC-1á/Akt signalling

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ABSTRACT

High-fat-diet (HFD)-induced obesity is a major contributor to diabetes and cardiovascular disease. In this research 6 week old male hamsters ($n = 10$) fed with HFD showed significant deterioration in heart function as determined from their cardiac ejection fraction percentage (EF%) and fraction shortening percentage (FS%). The number of apoptosis positive cells and the expression of protein markers of apoptosis drastically increased in the HFD-fed hamsters. However, the effects were significantly reduced following the administration of a lipolysis-stimulating peptide-APPH which was derived from the Alcalase-hydrolysis of potato protein. Fifty days of APPH-treatment was effective in all the concentrations (15, 45 and 75 mg/kg/day) tested. The EF% and FS% measurements in the APPH treatment groups were comparable with that of the control group hamsters. The molecular events associated with the ameliorative effect of APPH were found to be potentially mediated by SIRT1 pathway indicating a restoration from the metabolic disorders induced by HFD.

1. Introduction

Obesity is rapidly becoming a common phenomenon among adults as well as among children prone to high dietary fat intake (Canbakan et al., 2008). While intake of high levels of dietary fat is generally considered as one of the major risk factors of obesity, obesity is reaching epidemic proportions worldwide. Obesity is also associated with other complications such as type 2 diabetes mellitus, dyslipidamia, non-alcoholic fatty liver disease, fatty liver, and various cardiovascular diseases (Artham, Lavie, Milani, & Ventura, 2008; Fried et al., 2008; Marovic, 2008; Pagotto, Vanuzzo, Vicennati, & Pasquali, 2008). Owing to the increasing rates of obesity and associated cardiovascular events, the American Heart Association (AHA) has reclassified obesity as a major, modifiable risk factor for coronary heart disease (CHD) (Eckel, Kahn, Robertson, & Rizza, 2006). Various mechanisms that link obesity with CHD have been reported, strengthening the understanding on obesity as one of the major risk factors for cardiac diseases.

High fat diet (HFD) modulates the conditions in adipose tissue which releases a large number of cytokines and other factors that influence not only body weight homeostasis but also insulin resistance, diabetes, lipid levels, tension, coagulation,

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fibrinolysis, inflammation and atherosclerosis (Van Gaal, Mertens, & De Block, 2006; Zeyda & Stulnig, 2009). This would also cause various morphological adaptations in cardiac structure and haemodynamic function in obese individuals (Poirier et al., 2006; Van Gaal et al., 2006). High-carbohydrate, HFD-induced oxidative stress may also promote chronic inflammation and induce changes in cardiovascular structure and function such as endothelial dysfunction, cardiac hypertrophy, cardiac fibrosis, and ventricular contractile dysfunction (Hilfiker-Klemer, Landmesser, & Drexler, 2006). Reports show that oxidative stress and inflammation induces cellular apoptosis and fibrosis in setting of dilated cardiomyopathy. Further, upward regulation of the molecular markers of apoptosis and fibrosis play a critical role in deteriorating the heart function (Wang et al., 2012). Therefore regulation of the molecular events related to these molecular markers would prove to be a therapeutic strategy in ameliorating the cardiac effects of HFD.

Loss of cardiomyocytes due to apoptosis produces adverse outcomes such as cardiac diseases or heart failure. Apoptosis of cardiomyocytes is well known to be governed by two mechanisms. The extrinsic apoptotic pathway is triggered by formation of death-inducing signal complex initiated by Fas ligand on binding to the Fas receptor. Fas activation further recruits the adaptor protein Fas-associated death domain (FADD), to the complex, which bridges caspase-8 to the cytoplasmic death domain of Fas. (Bishopric, Andreka, Slepak, & Webster, 2001). Further, on activation the active form of caspase 8 cleaves procaspase 3 to form active caspase 3, a principle effector of apoptosis (Bishopric et al., 2001; Green, 1998) . Whereas the intrinsic pathway involves the disruption of mitochondrial membrane potential and thereby releases the cytochrome c that in turn triggers caspase 9. Bcl2, an anti-apoptotic protein, prevents cytochrome c release whereas Bax, proapoptotic proteins, enhance cytochrome c release from the mitochondria and executes the apoptotic program (Bishopric et al., 2001; Brown & Borutaite, 1999; Lee et al., 2013).

Bioactive peptides with various physiological functions have been identified and their applications are extensively studied (Chiang et al., 2014; Lee, Jeon, & Byun, 2014; Ngo, Kang, Jung, Byun, & Kim, 2014; Shi, Kovacs-Nolan, Jiang, Tsao, & Mine, 2014). In animals as well as in human, gastrointestinal digestion of proteins to simpler peptides enhances the actions of parent proteins (Korhonen & Pihlanto, 2003). During the process of enzymatic hydrolysis, proteins are cleaved to smaller peptides and free amino acids thereby improving the nutritional quality and safety of products (Kamnerdpetch, Weiss, Kasper, & Scheper, 2007). A potato protein hydrolysate, APPH, derived from alcalase treatment, with lipolysis-stimulating activity, was found to have potential to act as an efficient anti-obesity diet ingredient. However, the effect of APPH against HFD related cardiac damages are unclear. This study was carried out to find the potential of APPH in ameliorating HFD induced cardiac damages and in providing protection from cardiac dysfunction.

Male hamsters that were fed with HFD for 80 days (HFD group) were found to develop symptoms of cardiac dysfunction and progression to apoptosis. The levels of apoptosis related proteins were also found to be significantly lower in APPH treated groups than HFD group. Our results indicate that long term APPH administration attenuates HFD induced cardiac apoptosis in hamsters. Administration of APPH can be therefore considered as a potential therapeutic agent to ameliorate HFD related cardiac damages.

2. Materials and methods

2.1. *APPH preparation*

Alcalase hydrolysate of potato protein was prepared by following methods reported previously on soy protein hydrolysate (Tsou, Kao, Lu, Kao, & Chiang, 2013). Briefly, a reaction mixture containing 2.5% potato (Han-Sient Corporation, Taipei, Taiwan) and 1% alcalase enzyme (Nono Nodisk A/S, Bagsvaerd, Denmark) was used to produce potato protein hydrolysate (~81% protein). Alcalase hydrolysis of potato protein for more than 2 h produced products with degree of hydrolysis greater than 9.5% and with lipolysis stimulating activity when tested on 3T3-L1 adipocytes. Different membrane cut-off filters were used to determine the molecular sizes of the resultant APPH peptides and was found that 5% of the content were >6000 Da, 40% were between 1000 and 6000 Da and 55% were <1000 Da fractions. The amino acid composition was determined by the commercial service provided by the MB Mission Biotech, Taipei, Taiwan (ROC) and is listed in Table 1. For characterization, the APPH was fractionated in reverse phase high performance liquid chromatography (RP-HPLC) and the fraction III was found to have higher lipolysis stimulating activity and was further characterized using LC-ESI-MS/MS for peptide sequence identification. MS/MS ion sequencing of the major peak with corresponding activity performed using DXMS explorer (Sierra Analytics Inc., Modesto, CA, USA) and the sequence was determined as DIKTNKPVIF. The peptide or fragment was associated with Patatin (Accession number Q2MY50) of potato protein (Fig. 1).

2.2. *Animal experiments*

This study was conducted following the IACUC-100-12 protocol and approved by the IACUC ethics committee. The hamsters

Table 1 – Amino acid composition of APPH.

Amino acid	μM/mg
Aspartic acid	3.6442
Threonine	1.3566
Serine	3.2208
Glutamic acid	3.8122
Glycine	1.2890
Valine	1.8370
Cystine	ND
Methionine	0.0462
Isoleucine	0.5820
Leucine	1.8274
Tyrosine	1.0358
Phenylalanine	1.5154
Alanine	2.0007
Tryptophan	0.1900
Lysine	1.2456
Arginine	3.8325
Proline	1.6864
Histidine	0.3661
Asparagine	11.2914

ND, not determined.

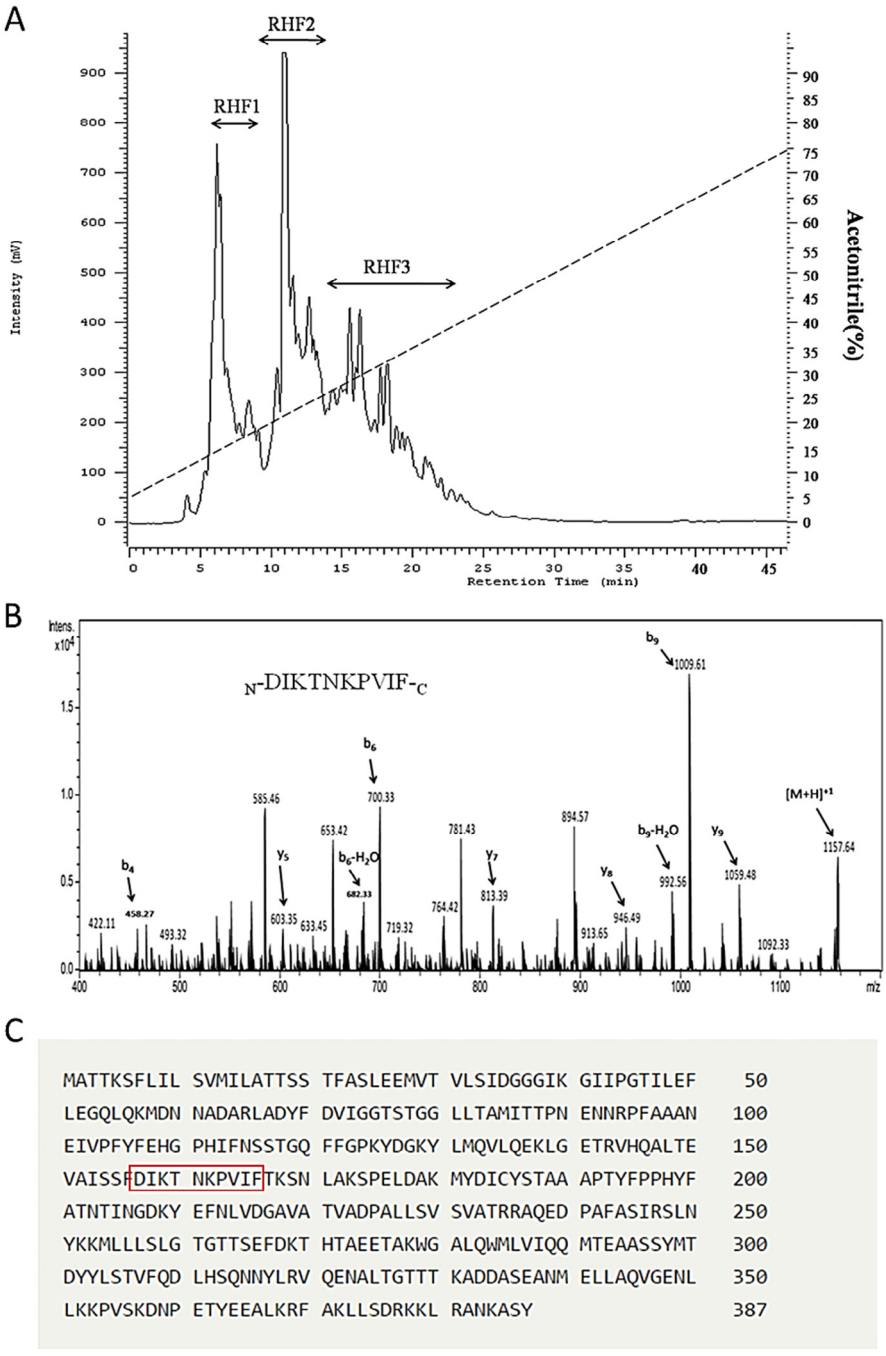

Fig. 1 – Characterization of APPH. (A) The reverse-phase high-performance liquid chromatography of APPH. (B) ESI-MS/MS spectrum of Fraction III obtained from RP-HPLC. (C) The amino acid sequence of Patatin (Accession number Q2MY50).

(6 week old males) were initially allowed to adapt to the environment and the diet for a week. During adaptation, all the animals were individually housed in a room maintained at 24 ± 2 °C and 55 ± 10% humidity with a 12 h light cycle. The hamsters were randomly divided into six groups ($n = 8$): sedentary control fed with standard chow and 75 mg/kg/day BSA (to balance the protein concentration in all the group), HFD fed obese sedentary control, HFD with low dose (15 and 60 mg/kg/day) APPH treatment, HFD fed with moderate (45 and 30 mg/kg/day) APPH treatment and HFD with high (75 mg/kg/day) APPH treatment. The animals were fed with a standard laboratory diet (PMI Nutrition International, Brentwood, MO, USA) and provided with reverse osmosis treated water *ad libitum*. After adaptation obesity was induced during the next 30 days by giving HFD containing 60% of energy as fat. The treatment was administrated orally everyday with specific quantities of APPH on gross weight basis using non flexible needles feeding gavage simultaneously during the next 50 days of HFD feeding course.

2.3. *Haemotoxyline and eosin staining*

For haemotoxyline and eosin (H and E) staining the hearts were excised; soaked in formalin; dehydrated with 100%, 95% and 75% alcohol and were then embedded in paraffin wax. The paraffin embedded tissue blocks were then cut into sections of 0.2 μm thicknesses and de-paraffinized by soaking in xylene. The slices were stained by H and E and rinsed with water. Photomicrographs were obtained using Zeiss Axiophot microscopes (Carl Zeiss Microscopy, Thornwood, NY, USA).

2.4. *TUNEL and DAPI staining*

Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labelling (TUNEL) assay was performed as following to detect cellular apoptosis in the tissue sections. The tissue sections were incubated with proteinase K, washed in PBS, incubated with permeabilization solution and then in blocking buffer, and then washed two times with PBS. The sections were further incubated for 60 min at 37 °C in terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP from an apoptosis detection kit (Roche Applied Science, Indianapolis, IN, USA). Under florescence (excitation wavelength of 460 nm and detection in the range of 515–565 nm) TUNEL-positive nuclei (fragmented DNA) were illuminated in bright green. The tissue sections were also stained with 0.1 μg/ml 4,6-diamidino-2-phenylindole (DAPI) for 5 min, and the nuclei were detected by UV light microscopic observations at 454 nm and photomicrographs were obtained using Zeiss Axiophot microscopes.

2.5. *Echocardiography*

M-mode echocardiographic examination was performed using a 6–15 MHz linear transducer (15–6 L) via a parasternal long axis approach. Left ventricular M-mode measurements at the level of the papillary muscles included left ventricular internal end-diastolic dimensions (LVIDd), left ventricular internal end-systolic dimensions (LVIDs), interventricular septum (IVS), posterior wall thicknesses (LVPW), ejection fraction (EF) and fractional shortening (FS). EF% was calculated by (EDV-ESV)/EDV × 100 and FS% was calculated by [(LVIDd − LVIDs)/LVIDd] × 100.

2.6. *Tissue protein extraction*

Tissue extracts were obtained by homogenizing the heart tissue in lysis buffer (100 mg/ml). The homogenates were placed on ice and then centrifuged at 12,000 *g* for 40 min. The supernatants were collected and stored at −80 °C for further experiments.

2.7. *Western blot*

Protein concentration of the extract was determined by the Lowry's protein assay method. Protein samples were separated in a 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) using 75 V of constant power supply. Proteins were then transferred to PVDF (GE Healthcare Life Sciences, Pittsburgh, PA, USA) membranes using 50 V current for 3 hours. The membranes were incubated in 3% bovine serum albumin (BSA) in TBS buffer and the primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were added onto the membranes for conjugation with specific proteins. Horseradish peroxidase-labelled secondary antibodies were used for detection and pictures were finally taken with Fujifilm LAS-3000 (GE Healthcare Life Sciences).

2.8. *Statistical analysis*

The results shown are the means ± SD of three independent experiments. Statistical analysis was performed by one-way analysis of variants. For paired samples, the Student's *t* test was applied.

3. Results

3.1. *Cardiac features*

The HFD intake induced several notable changes in the function of hamster hearts. The prominent cardiac characteristics of the HFD fed hamsters deteriorated significantly when compared with that of the control group, but cardiac characteristics of hamsters treated with APPH showed that they remain relatively healthier. The ratio of the whole heart weight (WHW, g) to that of the tibia length (mm), however, reveal that APPH or the probucol treatment in HFD fed hamsters do not have any significant influence on the weight of HFD fed hamster hearts (Fig. 2).

3.2. *Cardiac echocardiography*

Cardiac echocardiography was used on hamsters to record the heart ejection fraction % (EF%, Teich) and fraction shortening % (FS %) measurements. After 30 days of HFD feeding EF% and FS% of the control hamster hearts were 85.3 ± 8 and 49.9 ± 9

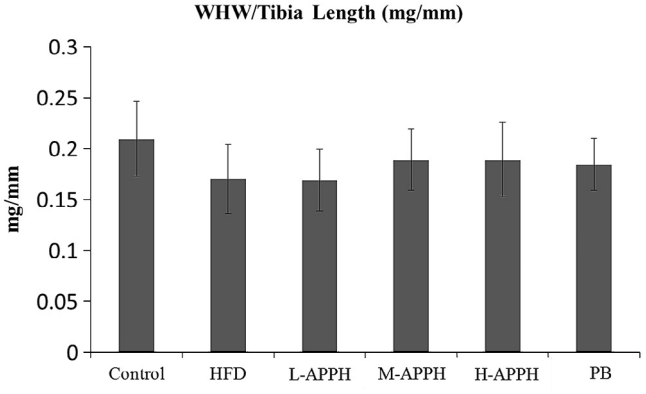

Fig. 2 – Weight measurements of the hamster hearts with respect to the tibia length. Heart weights of Control, HFD fed hamsters (HFD), HFD fed hamsters treated with low dose of APPH (L-APPH), HFD fed hamsters treated with moderate dose of APPH (M-APPH), HFD fed hamsters treated with high dose of APPH (H-APPH) and HFD fed hamsters treated with probucol (PB).

Table 2 – Echocardiographic assessment on the effects APPH.

	Ejection fraction (Teich)	Fraction shortening (%)
Normal chow fed hamster	85.5 ± 1.6	49.1 ± 1
HFD fed hamsters	74 ± 5***	37.7 ± 4.2***
HFD with low dose APPH treatment	83.4 ± 2.6###	46.6 ± 3###
HFD with moderate dose APPH treatment	81.2 ± 5.1###	44.6 ± 5###
HFD with high dose APPH treatment	82.9 ± 4.7###	46.4 ± 5.1###
HFD with probucol	85.3 ± 6.8###	49.8 ± 8.7###

*** $P < 0.001$ compared with control group; ###$P < 0.001$ compared with High fat diet fed group.

whereas those of the HFD fed hamster hearts were 83.4 ± 2 and 46.6 ± 3 revealing the signs of cardiac dysfunction after HFD feeding. Further feeding on HFD for the next 50 days reduced the cardiac function significantly; however, the hamsters treated with different concentrations of APPH were shown to withstand the effects of HFD on cardiac function (Table 2). Surprisingly the effect of low dose APPH treatment was very close to the effect of the positive control probucol. Average EF (Teich) of the control group hamsters was 85.5 ± 1.6% and their average FS was 49.1 ± 1%. Average EF (Teich) of HFD group hamsters was 74 ± 5% and average FS was 37.7 ± 4.2%. Average EF (Teich) and FS of low, moderate and high dose APPH treatment group hamsters were 83.4 ± 2.6 and 46.6 ± 3%; 81.2 ± 5.1 and 44.6 ± 5%; and 82.9 ± 4.7% and 46.4 ± 5.1% respectively. Further in the probucol treatment group the EF (Teich) was 85.3 ± 6.8% and average FS was 49.8 ± 8.7%).

3.3. *Heart biopsy*

Haematoxylin and eosin stain of heart tissue slides of HFD-induced hamsters showed that the arrangement of cardiomyocytes was highly disordered (Fig. 3). The cardiomyocytes in APPH treatment groups were comparatively well arranged and was not much different from control group hamsters.

3.4. *Nucleic acid stain*

To investigate the effect of APPH on HFD-induced cardiac apoptosis, the number of apoptosis-positive cardiac cells in the excised hearts of hamsters was determined by TUNEL assay. We found that left ventricle stained with TUNEL assay showed increased TUNEL-positive cardiac cells in the HFD fed hamsters compared with the control group. However, the APPH treatment reduced the cardiac apoptosis effect of HFD in hamsters (Fig. 4). The low and moderate dose treatments were effective in reducing HFD-induced cardiac apoptosis.

3.5. *Protein analysis*

The expression levels of crucial heart proteins were analyzed by Western blot analysis of heart tissue extract. As the results show HFD down-regulated the survival proteins such as PI3K

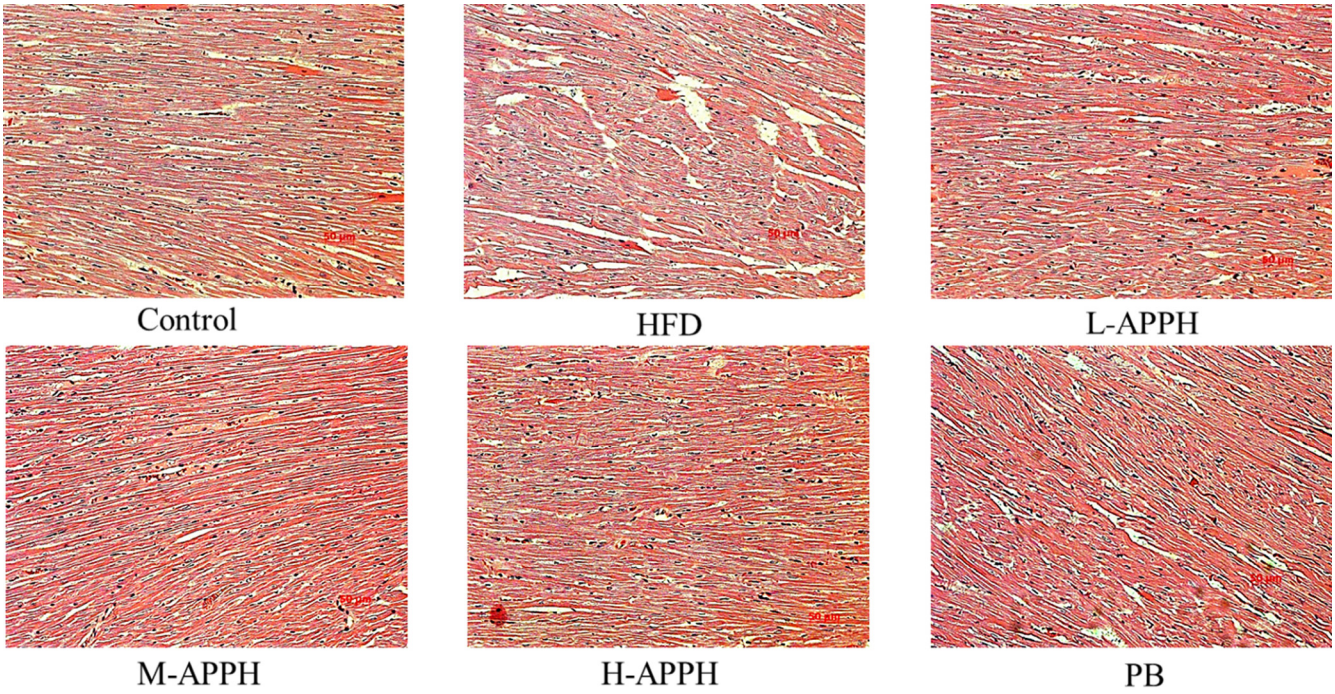

Fig. 3 – Haematoxylin and eosin (H&E) stain of heart slides. Histopathological analysis of cardiac sections from left ventricles of Control, HFD fed hamsters (HFD), HFD fed hamsters treated with low dose of APPH (L-APPH), HFD fed hamsters treated with moderate dose of APPH (M-APPH), HFD fed hamsters treated with high dose of APPH (H-APPH) and HFD fed hamsters treated with probucol (PB). Haematoxylin stains basophilic structures such as nucleus in blue and the eosin stains eosinophilic structures in bright pink.

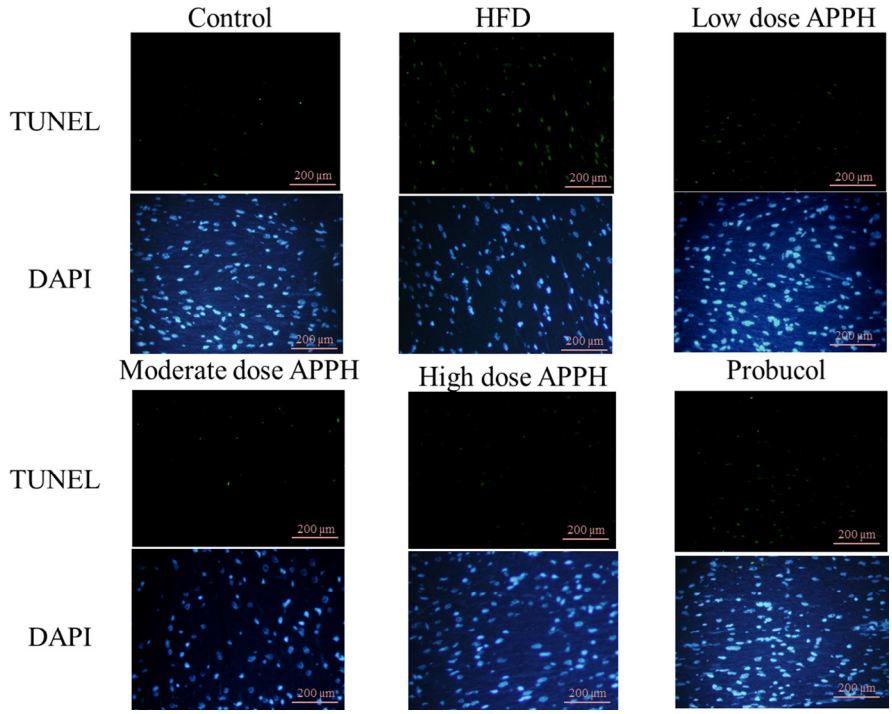

Fig. 4 – DAPI and TUNEL staining to detect apoptosis. DAPI and TUNEL stained in the heart sections of Control, HFD fed hamsters (HFD), HFD fed hamsters treated with low dose of APPH (L-APPH), HFD fed hamsters treated with moderate dose of APPH (M-APPH), HFD fed hamsters treated with high dose of APPH (H-APPH) and HFD fed hamsters treated with probucol (PB). The nuclei were stained in blue colour after DAPI staining, and DNA fragments caused by apoptosis process were stained in green colour after TUNEL assay.

and Akt whereas APPH feeding enhanced the activation of PI3K and Akt (Fig. 5a). While the expression of pro-survival proteins declined in the hearts of the HFD fed hamsters, the levels of apoptosis related proteins such as Bax, BAD and the caspase 3 significantly increased (Fig. 5b). However in the APPH treatment group the levels of apoptosis related proteins were found to be regulated and were comparable with that of the control. Meanwhile the level of HSP27, which was suppressed in HFD group hamster hearts, was enhanced in the hearts of APPH treated hamsters. Further, the key protein of the mitochondrial biogenesis PGC-1α and its regulator SIRT1 was found to be down-regulated in HFD fed hamsters and treatment with APPH reduced the effect of HFD by enhancing the expression of PGC-1α and SIRT1 (Fig. 5c).

4. Discussion

Excess dietary fat and hypercholesterolaemia are significant risk factors of cardiac damage and dysfunction. The use of dietary supplements or herbal medicine for the treatment of various disorders including heart diseases has a long and extensive history. Lipolysis, or the process involving degradation of fat cells to release their content of triacylglycerols, is a potential therapeutic target for obesity (Langin, 2006; Wang et al., 2008). Various lipolysis stimulating peptides of plant origin and their modified products have been assessed for their efficiency in providing protection against obesity or diet related disorders (Chiang, Shih, & Chu, 1999; Tsou et al., 2013; Tsou, Kao, Tseng, & Chiang, 2010).

Potato protein has a high nutritional value and a balanced hydrophilic/hydrophobic amino acid profile (Boody & Desborough, 1981; Gorinstein et al., 1998). The process of protein recovery from potato involves heat treatment that results in loss of solubility and depletion of functional properties of the proteins (Knorr, Kohler, & Betschart, 1977). Therefore, converting the insoluble protein into functionally active, value-added ingredients would enrich it as a functional food. Enzymatic hydrolysis of potato proteins is one of the possible means to produce functionally active peptides from potato protein (Wang & Xiong, 2005). Limited peptide cleavage under controlled conditions has been employed to produce bioactive APPH from potato proteins and their potential activity may be attributed to exposures of reactive amino acid side chains or hydrophobic patches and the diverse fragments of peptides (Wang & Xiong, 2005).

APPH, a lipolysis stimulating product of potato protein hydrolyzed by Alcalase enzyme, when administered to hamsters fed with HFD displayed significant protection to HFD induced cardiac apoptosis and cardiac dysfunction. The HFD fed hamster showed reduced whole heart weight and distorted cardiomyocyte arrangement with a large number of TUNEL positive apoptotic cells. The hamsters that had 50 day APPH administration remained healthy and did not show the deteriorating effects observed in the hamsters fed with HFD alone.

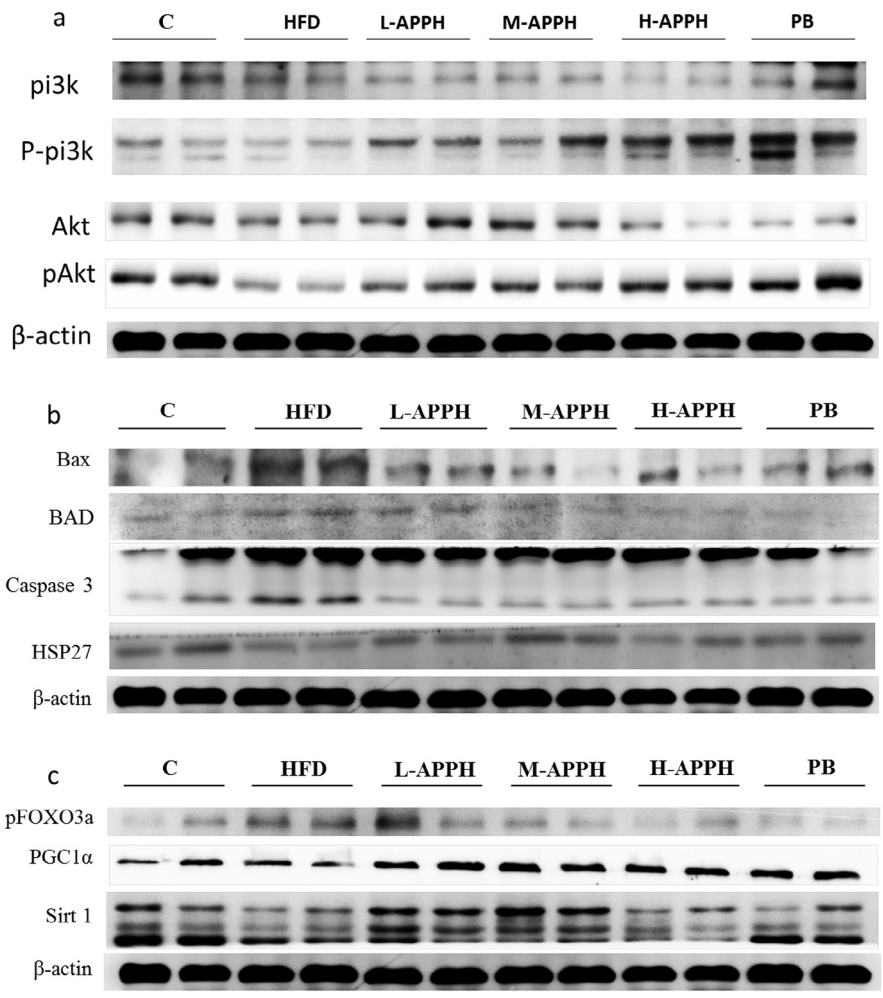

Fig. 5 – Protein expression analysis by western blotting. Levels of apoptosis and survival related proteins in the heart sections of Control, HFD fed hamsters (HFD), HFD fed hamsters treated with low dose of APPH (L-APPH), HFD fed hamsters treated with moderate dose of APPH (M-APPH), HFD fed hamsters treated with high dose of APPH (H-APPH) and HFD fed hamsters treated with probucol (PB). (a) Western blots of survival pathway related proteins, (b) Western blots of apoptosis related proteins, (c) Western blots of the proteins of mitochondrial biogenesis. Data represent results of six animal models and the samples from two animals were pooled together and therefore $n = 3$.

Apoptosis in cardiomyocytes is a critical phenomenon that reduces the cardiomyocyte population per functional unit and causes serious implications like cardiac dysfunction (Haunstetter & Izumo, 1998). The loss in the left ventricle weight in the HFD fed hamster group could be correlated with the progression of heart failure (Porter & Janicke, 1999). On the basis of the quantified TUNEL positivity, apoptosis may be suggested as one of the major form of cell death and a major complication induced by HFD.

To determine the molecular events underlying the effect of HFD the expression of specific protein markers of apoptosis and survival signalling were analysed. Apoptotic death usually involves two different mechanism, one that follows the activation of cell surface death receptors by extracellular ligands and the other as a consequence of the activation of mitochondrial-related pro-apoptotic mechanisms in response to unfavourable changes in the intracellular environment (Gonzalez et al., 2003). Increased expression levels of caspase-3 in the left ventricular tissues, as determined by Western blotting analysis, reveals the possible involvement of caspase-3 in the cardiomyocyte apoptosis induced by HFD. Caspase-3 modulates both mitochondria-dependent and Fas-death-receptor-dependent apoptotic pathways, and it is therefore an important molecular marker of apoptosis (Huang et al., 2012).

Heat shock protein 27 (HSP27) is a regulatory protein of the small molecular weight heat shock protein (HSP) family (12–43 kDa). During chemical stress conditions HSP27 acts as an anti-apoptotic agent by affecting both the intrinsic as well as extrinsic pathway of apoptosis (Vidyasagar, Wilson, & Djamali, 2012). HSP27 is particularly involved in protection from programmed cell death by inhibition of caspase-dependent apoptosis (Vidyasagar et al., 2012). HSP27 also interacts with Bax and cytochrome c, thereby it prevents mitochondria dependent apoptosis (Bruey et al., 2000; Havasi et al., 2008). Our results reveal that HFD feeding suppresses the expression of HSP27 and APPH administration in hamsters reverses the effect of HFD on HSP27. Moreover, in septic mice HSP27 has been shown to activate the PI3K/Akt pathway to attenuate cardiac

dysfunction (Ghayour-Mobarhan, Saber, & Ferns, 2012; Havasi et al., 2008; You et al., 2009). The deduced HFD induced pro-apoptotic mechanism was also accompanied by decrease in the levels of the active cell survival proteins such as pAkt and pPI3K; however in APPH treated hamsters the levels of pAkt and pI3K remained normal. The results therefore, indicate the development of a protection mechanism, induced by APPH, which ameliorates the effect of the HFD on cell viability and survival.

HFD feeding also affected the expression of PGC-1α and SIRT1, indicating an adverse effect on the mitochondria of the cardiomyocyte that eventually leads to apoptosis. PGC-1α is a transcriptional co-activator that facilitates the activation of transcription and enhances gene expressions that are related to fatty acid oxidation and mitochondrial functions (Sugden, Caton, & Holness, 2010). Enhanced activity of PGC-1α protects cells against metabolic disorders and enhances mitochondrial functional capacities; it also protects cells against apoptotic injury (Valle, Alvarez-Barrientos, Arza, Lamas, & Monsalve, 2005). APPH treatment enhances PGC-1α expression indicating a protective effect established in the cardiomyocytes against HFD. In our results the HFD induced down-regulation of PGC-1α may be rationally interpreted to reduced mitochondrial biogenesis and mitochondrial density. During obesity and other inflammatory conditions the level of palmitic acid released from the adipose tissue generally tends to be higher. The elevated palmitic acid level reduces cardiomyocyte viability and increases cardiovascular complications (Wilson-Fritch et al., 2004). Plamitic acid in cardiomyocytes down-regulates the expression of PGC-1α that is essential for the normal functioning of the heart (Arany et al., 2006; Handschin & Spiegelman, 2006). SIRT1 regulates the activity of PGC-1α via deacetylation thus increases the PGC-1α transcriptional activity (Chong, Wang, Shang, & Maiese, 2012; Levine & Levine, 2013). Thus SIRT1 activates mitochondrial biogenesis by inducing PGC-1α. High fat diet feeding in hamsters reduced the expression of the SIRT1 and APPH administration effectively restored the essential SIRT1 expression. Sirtuin and the IGF/Akt pathways are often related to the amelioration of aging effects. Reports show that health benefits of calorie restriction are mediated through the activation of sirtuins. The role of sirtuin is much relevant as HFD is known to accelerate aging and cause metabolic syndromes (Honma et al., 2012). Moreover, sirtuins are known to be effective regulators of Akt signalling and SIRT1 directly deacetylates Akt and promotes its activation (Pillai, Sundaresan, & Gupta, 2014). The down-regulation of SIRT1 in response to HFD and its corresponding up-regulation in the hamsters administered with APPH show that SIRT1 plays an important role in HFD induced apoptosis and the restoration of SIRT1 by APPH is responsible for providing the required cardioprotection.

In humans, like other Foxo factors FoxO3a is one of the major substrates of Akt and is suppressed by activation of the PI3K/Akt pathway (Levine & Levine, 2013). The down-regulation of SIRT1 in response to HFD and its corresponding up-regulation in the hamsters administered with APPH show that SIRT1 plays an important role in HFD induced apoptosis and the restoration of SIRT1 by APPH is responsible in providing the required cardio protection. HFD feeding is known to elevate the levels of phosphorylated (p)-FoxO3a. APPH treatment significantly suppressed the HFD induced p-FoxO3a expression revealing that APPH provides effective cardio protection from HFD damages (Dong, Li, Sreejayan, Nunn, & Ren, 2007).

In this study, the potential of APPH to attenuate HFD-induced apoptotic effects in the cardiac tissue was determined using HFD fed hamster animal model. HFD induced effects, such as changes in the heart weight, muscle architecture, lipid levels and modulations in apoptosis related events were ameliorated by APPH administration. Thereby, APPH administration could potentially rescue the heart from obesity related loss of cardiomyocyte.

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