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Hydrolysate of extruded soy proteins concentrate attenuates post myocardial infarction remodeling and improves heart health

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Abstract

Soybean is a good source of dietary proteins associated with numerous nutritional benefits and attenuates metabolic disorders like myocardial infarction (MI). Present study was aimed to explore the role of hydrolysate of extruded soy proteins concentrate (HESPC) intake in mitigation of MI. Isoprenaline induced MI rat groups (3-6) were fed on diet containing 5.07, 10.14, 15.21, 20.30 g proteins from Hydrolysates of extruded soy proteins concentrates (HESPC), respectively. Group 1 (non-induced MI) and group 2 (Isoprenaline induced MI) were fed on casein diet as control. Feed intake, body and heart weight, lactate dehydrogenase (LDH), creatine kinase-MB (CK), troponin, creatinine and urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lipid profile, plasma proteins and homocysteine, cardiac and antioxidant enzymes and histopathology analysis were performed. The diet containing HESPC intake significantly changed body and heart weight. Lowest concentrations of LDH, CK-MB and troponin and highest concentration of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were observed in rats group fed on 20.30 g proteins. Blood cells count was significantly decreased on intake of HESPC in all groups. Plasma lipids, proteins, homocysteine, hepatic enzymes showed decreasing trend in rat groups with increasing percentage of HESPC in diet. Histopathological results showed healing of injured heart tissues was significant on HESPC intake. HESPC diet intake can improve biochemical parameters and cardiac tissues health.

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Introduction

Soybean is glycine rich proteins and utilizes for human consumption in the form of flour, infant formulas, concentrated, protein isolates and textured fibres. The soy enriched foods include tofu, cheese, tempeh, and drinks are used as proteins substitutes and vegetarian food product (Decroos *et al*., 2005; Chauhan and Chauhan, 2007). These proteins are used as milk replacer in many products, but the effect of soy protein consumption reduces as compared to milk proteins due to anti-nutritional, antigenic activities and amount of incorporation (Hagen *et al*., 2009). Based on investigations, epidemiological data and clinical trials, Food and Drug Administration (FDA) has approved health claim related to the soybean for being helpful in reduction of total and LDL cholesterol (Hasler *et al*., 2004). MI is one of the health complications that is not only increasing in western world but also rising rapidly in developing countries (Sabeena *et al*., 2004). MI is categorized as an imbalance of coronary blood demand and supply that leads to myocardial ischemic injury and ultimately damages the cardiomyocytes (Kloner, 2015). Previous studies suggest health boosting potential of soybean proteins against cholesterol, low density lipoprotein and triglycerides, along with increased resistance against lipid oxidation (Pihlanto-Leppälä *et al*., 2000; Zhan and Ho *et al*., 2005).

Potential health claims have led to increase soybean protein based foods as fastest growing category in food industry resulting in demand for manufacturing soy protein ingredients with improved processing characteristics. Heat processing of soybean reduced flatulence and anti-nutritional factors which increases the use of soy proteins in many products. Novel technologies help in reduction of anti-nutritional, anti-genic factors and improve the value addition of these proteins in product development and production of bioactive peptides which are useful for health of cardiac tissues (Pihlanto-Leppälä *et al*., 2000). Bioactive peptides are inactive within the parent protein; however, thermal extrusion processing helps in production of bioactive peptides that can act as physiological modulators of metabolism on their release (Milán-Carrillo *et al*., 2006) In this context, the present study was aimed to assess the nutritional consumption of HESPC incorporated as casein replacer in rat diet and its biological evaluation against post myocardial infarction remodeling and heart health.

Materials and methods

Procurement of material

Soybean seeds of 95-I variety were procured from Ayub Agricultural Research Institute, Faisalabad, Pakistan and transferred to respective laboratory of Government College University, Faisalabad.

Preparations of defatted and extruded soybean proteins concentrate

Proteins hydrolysates have bioactive peptides that play important therapeutic role in human health. While thermal extrusion also helps in minimizing anti-nutritional factors and produce anticardiovascular and anti-inflammatory bioactive peptides. The seeds were cleaned, grounded and defatted with n-hexane, desolventized and pulverized to obtain the defatted soy flour. The proteins were concentrated according to described method (Wang *et al*., 2007). Enzymatic hydrolysis of was performed to produce bioactive peptide from protein concentrate using Protamex® (EC 3.4.24.28), Neutrase® 1.5 MG (EC 82 3.4.24.28) and Flavourzyme® 500 MG (EC 3.4.11.1) purchased from Novozymes (Denmark). The ratio of enzymes: substrate was 1:100 using pH and temperature control devices. 1.0 g of enzymes was mixed in a 10 L reactor. The mixture was incubated for 8 hrs at optimal stirring and temperature and then placed into boiling water bath for 10 min to inactivate the enzyme and vacuum dried. Twin screw extruder was used for thermal extrusion (FMHE-36, Hunan Fumake Engineering Technology Co., 90 Ltd., Hunan, China) to produced extruded powder. The optimized conditions of barrel exit temperature (125°C), screw speed (100 rpm), feed flow rate (60 kg/h), feed moisture content (20%) were used in extrusion process.

Composition analysis of soybean seeds and HESPC

The proximate analysis of soybean seeds and HESPC as moisture (AACC Method No. 46-30), crude fat (AACC Method No. 30-25), crude protein (AACC Method 46-10), total ash (AACC Method No. 08-01) and Crud fibre (AACC Method no. 32-10) were conducted according to mentioned procedures of The American Association of Cereal Chemists (AACC, 2000).

Animal handling and MI induction

Rats were purchased from National Institute of Health (NIH), Islamabad and housed in the animal room of Government College University, Faisalabad, in accordance with institutional and National Policies and Guidelines for Care and Use of Laboratory Animals compliance with declaration of Helsinki (Ref.no.gcuf/erc/1886). They were housed in polypropylene cages (47 cm×34 cm×20 cm) lined with husk, changed after every 24 hrs under a 12:12 hrs light dark cycle at 22°C and had free access to tap water and food. The rats were fed on a standard casein diet (Table 1). The rats were divided into 6 groups, each of 12 rats. 5 groups (2-6) were subcutaneously induced by isoprenaline hydrochloride injection (Sigma Aldrich) of 100 mg/kg body weight, once daily for two successive days (Zaafan *et al*., 2013) and MI induction was confirmed by MI enzymes biomarkers of randomly selected from pool of 60 rats.

Feed formulation and study design

Experimental rats were divided into 6 groups, 12 in each group. Four MI induced groups (3-6) were fed on functional diets containing 5.07, 10.14, 15.21, 20.30 g proteins of HESPC while group 1 (non-induced and group 2 (MI induced) were fed on casein diet for 4 weeks as presented in Table 1.

Feed intake and body weight gain

Feed intake of experimental groups was calculated on daily basis by eliminating spilled diet from the total diet consumed during the entire experiment (Wolf and Weisbrode, 2003). Gain in body weight and heart weight of each group were determined accordingly (Seena *et al*., 2006).

Biochemical analysis

The blood was drawn from tail of animals on first day of MI induction and then every week for confirmation of MI induction though troponin level. Animals were anesthetized with chloroform and sacrificed at the end of study for collection of blood plasma and hearts for further analysis. Blood samples were centrifuged at 4000 rpm for 10 min to obtain the serum which was used for determining various biochemical parameters. MI induced was evaluated by measuring markers of heart injury in serum, and antioxidant enzymes in heart tissue, observing pathological changes of tissue. RBCs, WBCs and platelet count were assessed using a Sysmex Cell Counter (Sysmex, Kobe, Japan) (Burr *et al*., 1992). Serum creatinine was measured spectrophotometrically by using commercially available kit (Creatinine Jaffe Ecoline® diagnostic kit Merck). Blood glucose level was determined using diagnosis reagent kit (DiaSys Diagnostic Systems GmbH, Germany) (Husni *et al*., 2016).

The serum total cholesterol (TC) and total triacylglycerol (TG) were determined by using DiaSys Diagnostic Systems USA reagent kit method. High density lipoprotein cholesterol (HDL-C) was determined by reagent kit method (Randox, Randox Laboratories LTD, UK). Calculation was done according to given equation for LDL-cholesterol [LDL-cholesterol = (Total cholesterol) – Triglycerides – (HDL- cholesterol)]. Very low density lipoprotein (VLDL) was calculated using following equation: VLDL-C = TG/5. Total plasma protein (Bradford, 1976) and homocysteine in blood plasma were determined though high performance liquid chromatography (Durand *et al*., 1996).

Cardiac biomarker enzymes LDH, CK-MB and troponin were assayed using commercial kits according to previously described methods (Okinaka *et al*., 1961; King, 1965). Serum ALT and AST were measured using commercially available kits (Reference # BT294QY; Randox Laboratories, United Kingdom). Heart homogenate was prepared according to method of (Noori *et al*., 2009) using

Hematoxylin and eosin staining. Heart tissue CAT, SOD and GPx were measured through commercially available kits (Reference # KA0882, Abnova Corporation, Taiwan using method of Bancroft and Gamble. Similarly, histopathology analysis of heart tissues were also performed according to cited method (Bancroft and Gamble, 2002). The section mounted, and histological images were captured using 40x and100x magnification of light microscope.

Statistical analysis

All experiments were performed in triplicate and results are presented as means ± SD. The statistical analyses were performed using SPSS (version 20.0, IBM Corporation, NY, USA) and MS excel. Biochemical data were analysed using one-way ANOVA followed by t-test. Least Significant Difference test was used to determined significance difference at p value of < 0.05 (Steel *et al.*, 1997).

Results and discussion

Feed intake, body weight gain and heart weight

The means values of feed intake, body weight gain and heart weight are presented in Table 2. The results show insignificant increase in feed intake in all groups except group 2.

The highest feed intake (16.9±1.06 g/day) was observed in group 6 and lowest feed intake (16.03±1.26 g/day) was observed in group 2. HESPC intake resulted in significant increase ($p<0.05$) in body weight. Highest body weight gain was observed in group 6 feeding on 20.3 g HESPC proteins diet.

Heart weight change was significant in all groups feeding on HESPC diets as compared to control group. Highest heart weight (3.2±0.3 g) and lowest (2.60±0.2 g) was observed in groups 2 and 6, respectively.

Table 1. Composition (%) of soybean seed, HESPC and rat diet.

Soybean seed and HESPC					
Composition (%)	Moisture	Protein (Nx6.25)	Fat	Crude fibre	Ash
Soybean seed	4.6±0.14	34.52±0.34	21.03±0.37	7.41±0.16	6.15±0.13
HESPC	3.91±0.36	73.08±0.25	1.88±0.25	3.27±0.17	6.93±0.30
Rat groups and diet					
Rat groups	Groups 1	Groups 2	Groups 3	Groups 4	Groups 5	Groups 6
Diet type	Standard diet		HESPC diet		
composition (g/100g)	D_0	D_1	D_2	D_3	D_4
Corn starch	39.74	39.74	39.74	39.74	39.74
Casein proteins	20.30	15.22	10.16	5.09	0.0
Proteins (HESPC)	0	5.07	10.14	15.21	20.30
Carbohydrate	13.2	13.2	13.2	13.2	13.2
Fat	7.0	6.87	6.74	6.61	6.48
Fat from HESPC	0	0.13	0.26	0.39	0.52
Sucrose	10	10	10	10	10
Fibre	5.0	4.73	4.55	4.32	4.09
Fibre (HESPC)	0	0.27	0.45	0.68	0.91
Vitamin mixture	1.0	1.0	1.0	1.0	1.0
Mineral mixture	3.5	3.5	3.5	3.5	3.5

Casein proteins were replaced with hydrolysate of extruded soy proteins concentrate (HESPC) D_0: Standard diet (casein diet), D_{1-4}: diets containing 5.07, 10.14, 15.21, 20.30 g proteins of HESPC, respectively, replaced with casein proteins. Each group had 12 rats; group 1 was not induced for myocardial infarction (MI) while groups 2-6 were induced for MI.

The previous studies have shown that increased protein content in soy protein diet results in intake of specific amino acids that were useful in mediation of myocardial infarction. The heart weight increased in isoprenaline treated rat groups was significantly decreased ($p < 0.05$) because of amino acid present in diet given to infarcted rat groups (Moradi-Arzeloo *et al.*, 2016). Ahmad and Ahmed also observed weight gain in soybean fed groups of rats from initial to final day of study (Ahmad & Ahmed, 2014).

Table 2. Effect of HESPC intake on feed intake, physiological and biological parameters of rats.

Physical parameters		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Feed Intake (g/day)		16.68±0.58^{a}	16.03±1.26^{b}	16.26±0.94^{ab}	16.27±1.3^{ab}	16.29 ±0.86^{ab}	16.9 ±1.06^{a}
Body weight (g)		264.7±8.1^{a}	225.3±6.3^{e}	239.6±7.1^{d}	253.31±5.6^{c}	255.73±6.01^{bc}	257.94±1.2^{b}
Heart weight (g)		2.3±0.3^{d}	3.21±0.3^{a}	3.09±0.19^{a}	2.93±0.27^{b}	2.68±0.32^{c}	2.60±0.2^{c}
Haematological parameters of rat groups							
Blood Cell Count	RBCs (10^{6}/µL)	5.67±0.24^{e}	11.25±0.68^{a}	8.61±0.44^{b}	7.95±0.71^{c}	7.12±0.54^{d}	5.73±0.38^{e}
	WBCs (10^{3}/L)	3.57±0.25^{e}	9.2 ± 0.4^{a}	7.56±0.17^{b}	6.31±0.48^{c}	4.01±0.31^{d}	3.95 ±0.06^{d}
	Platelets (10^{5}/mm^{3})	2.96±0.25^{e}	4.72±0.49^{a}	4.16±0.53^{b}	3.98±0.82^{b}	3.36±0.46^{c}	3.11±0.62^{d}
CMP	Urea (mmol/L)	9.8±1.35^{f}	45.40±2.55^{a}	35.82±1.46^{b}	28.56±1.7^{c}	21.62±2.56^{d}	11.6±1.92^{e}
	Creatinine (µmol/L)	46±0.04^{a}	10.95±2.89^{f}	15.31±3.05^{e}	23.88±2.06^{d}	36.02±3.01^{c}	41.63±2.46^{b}
	ALT (U/L)	20.58±3.16^{e}	48.5±2.3^{a}	41.47±2.39^{b}	37.36±3.26^{b}	28.26±2.18^{c}	22.10±1.07^{d}
	AST (U/L)	48.74±4.00^{e}	96±5.24^{a}	85.47±3.02^{b}	69.42±3.24^{c}	48.47±4.65^{d}	37.01±3.8^{e}
Blood lipids, proteins profile and glucose level							
Serum Glucose (mg/dL)	Glucose	92.91±1.81^{f}	170±5.83^{a}	157.5±5.49^{b}	142.00±5.03^{c}	136.33±4.51^{d}	121.73±4.01^{e}
Lipid Profile (mg/dL)	TC	82.19±4.13^{d}	141.14±4.1^{a}	130.81±6.14^{b}	124.47±5.95^{b}	115.32±3.90^{c}	110.15±5.8^{c}
	HDL-C	56.58±2.14^{a}	18.5±2.1^{e}	44.71±1.82^{d}	48.48±2.97^{c}	50.56±3.18^{bc}	53.79±3.25^{b}
	LDL-C	43.28±1.95^{e}	85.6±3.5^{a}	71.12±2.1^{b}	68.79±2.9^{b}	63.57±1.73^{c}	52.47±3.1^{d}
	VLDL	20.84±3.08^{b}	26.4±0.6^{a}	24.97±1.29^{a}	24.01±0.91^{a}	23.02±1.1^{ab}	22.2±1.8^{b}
	TG	106±8.03^{c}	132.69±8.1^{a}	126.73±6.3^{ab}	121.31± 7.82^{b}	116.71±6.04^{bc}	110.5±5.2^{c}
Plasma proteins	Protein (g/L)	6.35±0.29^{c}	4.19±0.13^{d}	6.09±0.21^{c}	7.04± 0.23^{b}	7.98±0.19^{ab}	8.8±0.26^{a}
	Homocysteine (µmol/L)	8.9±0.4^{e}	22.72±0.4^{a}	14.9 ±0.2^{b}	12.7±0.71^{c}	11.53±1.53^{c}	9.8±0.82^{d}

Values are expressed as mean ±S.D. Different superscripts show the significant difference ($p < 0.05$) within row. HESPC: hydrolysate extruded soy proteins concentrate. Group 1, 2: Fed on casein diet, Group 3-6: fed on diets containing 5.07, 10.14, 15.21, 20.30 g proteins of HESPC, respectively, replaced with casein proteins. Values are expressed as mean ±SD. Different superscripts show the significant difference ($p < 0.05$) within row. RBCs: Red blood cells, WBCs: White blood cells, ALT: Alanine transaminase, AST: Aspartate transaminase, CMP: Comprehensive metabolic panel, TC: total cholesterol, HDL-C: high density lipoproteins cholesterol, LDL-C: low density lipoproteins cholesterol, VLDL: very low density lipoproteins cholesterol, TG: Triacyl glycerides.

Haematological parameters

The haematological results of WBC, RBC and platelets are presented in Table 2. A significant decrease of WBC, RBC and platelets was observed in MI induced group. Highest level of WBC, RBC and platelets count were 7.56±1.17 x10^{3}/□L, 8.61±0.44 x106/□L and 4.16±0.53 x 105/mm^{3}, respectively in group 3 and lowest (5.73±0.38 x 10^{3}/□L, 3.95±0.06 x 106/□L and 3.11±0.62 x 105/mm^{3}) respectively, were observed in group 6. The studies show that increased RBCs, WBCs and platelets on MI induction can be reversed on consumption of soy proteins. In present research, we observed an increase of RBCs, WBCs and platelets after isoprenaline administration were gradually decreased in rats group consuming HESPC diet. Mean values of blood proteins, urea and creatinine are also presented in Table 2. A significant difference ($p<0.05$) was observed in all MI groups. The highest and lowest urea level decrease was observed in group 3 and 6, respectively. The mean comparison analysis of MI induced groups fed on HESPC diet show decreasing trend in urea level with increased HESPC proteins in diets. Creatinine level was significantly increased ($p<0.05$) with increasing HESPC in diet. The highest (41.63±2.46 □mol/L) and lowest (15.31±3.05 □mol/L) values of creatinine were observed in group 6 and 3, respectively.

The creatinine level in MI infarcted rats was significantly improved on intake of HESPC in all rats groups. Previous studies show that serum creatinine and urea concentrations were significantly lowered in rats fed on soy protein based diet (Aukema and Housini, 2001). They proposed that soybean protein diet decreased urea concentrations in comparison to casein diet (Okafor and Ebuehi, 2016).

Table 3. Effect of HESPC intake on biomarkers in rat groups.

Biomarkers	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
LDH (IU/L)	362±10.43^{e}	794±11.53^{a}	702±17^{b}	563±21^{c}	474±20^{d}	359±23^{e}
CK-MB (IU/L)	768.1±23.2^{e}	1104.4±64.3^{a}	943±11.8^{b}	886±12.46^{c}	799.53±26.8^{d}	715±20.2^{f}
Troponin (ng/dL)	7.3±0.31^{e}	15±0.23^{a}	11±0.12^{b}	9.3±0.26^{c}	8.9±0.02^{d}	6.4±0.15^{f}
SOD (U/mg prot)	42.6±2.6^{a}	24.61±1.91^{d}	26.9±2.4^{d}	28.6±2.3^{cd}	31.16±1.16^{c}	34.20±0.95^{b}
CAT (pmol/mg prot)	83.74±1.48^{a}	22.64±2.40^{d}	75.1±4.7^{c}	77.41±3.61^{bc}	78.07±3.55^{b}	79.13±3.64^{ab}
GPx (nmol/min/mg prot)	14.6±2.3^{a}	9.54±2.05^{c}	12.4±1.2^{bc}	13.11±1.50^{b}	14.08±1.92^{ab}	15.42±1.99^{a}

Values are expressed as mean ± S.D. Different superscripts show the significant difference ($p < 0.05$) within row. HESPC: hydrolysate of extruded soy flour proteins, Group 1, 2: Fed on casein diet, group 3-6: fed on diets containing 5.07, 10.14, 15.21, 20.30 g proteins of HESPC, respectively, replaced with casein proteins. LDH: Lactate dehydrogenase, CK-MB: Creatine kinase-MB, SOD: superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidases.

The significant ($p<0.05$) decrease in all MI induced groups was observed. Highest values of AST and ALT (85.47±3.02 IU/L) and (41.47±2.39 IU/L), respectively, were observed in group 3 and lowest (37.01 ±3.8 IU/L) and (22.10±1.07IU/L), respectively, were observed in group 6. The serum levels of liver biomarkers in experimental rats after 4 weeks of administration of hard wheat bread and wheat soy breads were significantly decreased in experimental rats fed on wheat-soy bread compared to control group. In present finding, glucose level was increased after isoprenaline treatment that was remarkably decreased on consumption of HESPC diet. Anosike *et al.* described that glucose level in soybean supplemented rats was significantly lower than those without soybean supplementation. This may imply that soybean could reduce the incidence of hyperglycemia through production of antioxidant peptides (Anosike *et al.*, 2008).

Blood lipids, proteins and glucose level

The mean values of blood lipids, plasma proteins in Table 2 show that TC, HDL-C, LDL-C, VLDL and TG levels in all MI groups changed significantly. Highest decrease in TC, LDL-C, VLDL and TG and increase in HDL-C was observed in group 6. Maximum level of TC, LDL-C, VLDL and TG and minimum level of HDL-C was observed in group 3. These results are in accordance with the findings that after feeding on soy proteins result in TC, LDL-C, VLDL, and TG decrease. Our current findings show that increased soy protein in diet increased plasma protein levels, as like the results of Hagen *et al.* proposed that soybean diets produced significant increases in the total plasma protein especially at 25%, 50% and 75% milk protein replacement (Hagen *et al.*, 2009).The plasma proteins show significant difference ($p<0.05$) in total proteins and homocysteine levels in all MI groups. Maximum increase in proteins level and maximum decrease in homocysteine were observed in group 6. Minimum level of total proteins and maximum level of homocysteine were observed in group 3.

Cardiac biomarkers

Mean values of cardiac biomarkers LDH, CK-MB and troponin and antioxidant enzymes SOD, CAT and GPx are presented in Table 3.The results show a significant decrease in level of these enzymes. Intake of 20.3 g HESPC diet reduced maximum level of LDH, CK-MB and troponin while maximum level of these enzymes was observed in MI control group. Increase in heart weight might be associated with the increased water content, oedematous intramuscular space, increased protein content and infiltration of inflammatory cells to damaged areas that was decreased in group 3-6. Isoprenaline treated groups have increased the LDH, CK-MB and troponin levels and intake of HESPC diet decreased the level of these enzymes. Previous results showed decreasing trend in heart biomarkers on consumption of soybean flour within one week (Bertinchanta *et al.*, 2000). Hamed *et al.* (2010) also worked on effects of a soybean proteins diet in MI female albino rats. They concluded that soy protein diet intake increases CK-MB resulting in protection of heart against acute MI attack.

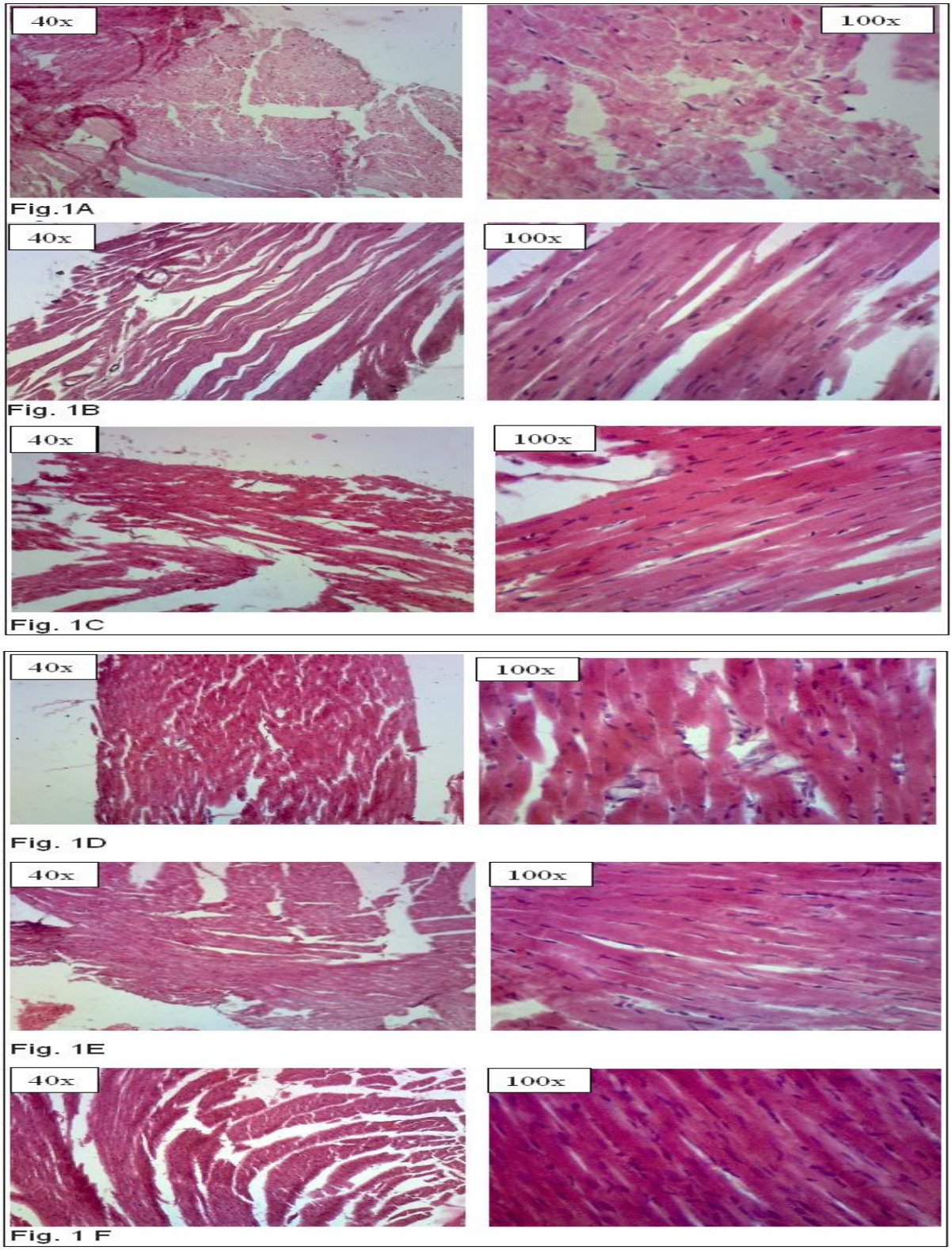

Fig. 1. Histopathological higher magnification (40x, 100X) images of cardiac muscles showing MI in control (A) and isoprenaline myocardial infarcted induced rats groups (B-F). **A**= regular arranged cardiac myocytes with branching of rat group fed on standard casein diet, with a pale acidophilic cytoplasm and single vesicular nucleus of the cardiac muscle fibers. B-F= Rats were isoprenaline induced MI and fed on hydrolysate of extruded soy protein concentrate 5.07, 10.14, 15.21, 20.30 g proteins. Myocardial infarction heart tissues damage was observed showing cardiac myocytes with areas of irregular arrangement and necrosis in B-F that was improved in strength and tissue structure gradually after intake.

Similarly, troponin levels were increased in disease induced group that showed decreased trend when HESPC diets were given to groups 3-6 with different concentrations. Significant difference ($p<0.05$) was observed in all MI groups on intake of HESPC. Minimum activity of SOD, CAT and GPx (26.9±2.4 U/mg prot), (75.1±4.7 pmol/mg prot) and (12.4±1.2 nmol/min/mg prot), respectively, was observed in group 3. Maximum activity of SOD (34.20±0.95 U/mg prot), CAT (79.13±3.64 pmol/mg prot) and GPx (15.42±1.99 nmol/min/mg prot) was observed in group 6. Antioxidant enzymes of heart tissues such as SOD, CAT, GPx were decreased after MI induction and significantly increased on HESPC intake. Hagen *et al.*, worked on comparative effect of soy protein isolate and casein diet in MI rats. They showed that SOD, CAT and GPx were increased in isolated soy protein fed group of rats as compared to casein.

Histopathological study

The histopathological images (Figure 1) show that the intake of HESPC improves the tissue structure of injured heart. The results indicate that heart tissues of group 1 arranged regularly the cardiac myocytes branching with a pale acidophilic cytoplasm and single vesicular nucleus of the cardiac muscle fibres. The broken tissues due to MI can easily be observed in figure 1B, the heart tissues of group 2 were highly damaged showing irregularly arranged cardiac myocytes with areas of irregular arrangement and necrosis. The figure 1C shows heart tissues of group 3 started to improve the strength that gradually increased in group 4, 5and 6 with increase intake of HESPC as shown in figure 1(D,E,F), respectively. Soybean protein can be valuable in protecting the heart against the attack of acute MI. A significant improvement in the ventricular function was examined after MI and consequently preventing the progression towards a severe heart failure by using isolated soy proteins in diet. We observed that the preventive effect of HESPC products in the terms of post MI dysfunction concluding that favourable effect is possibly secondary to the antioxidant activity of soy protein and bioactive peptides produced during hydrolysis and extrusion (Hagen *et al.*, 2009).

Conclusion

Soy proteins can be used for preparation of functional food and its efficacy can further be enhanced by food processing technology. The extrusion processing of soy flour and enzymatic hydrolysis result in production of bioactive peptides that can be effectively used for MI ailment. The intake of defatted HESPC significantly improves the biological parameter against MI. HESPC diet containing 5.07, 10.14, 15.21, 20.30 g proteins can effectively decrease glucose, LDL-C, VLDL, TG and TC, increase HDL-C and improved haematological and CMP levels in rats. The MI induced was reversed with the help of HESPC diet intake though improved tissues structures of heart and cardiac health and antioxidant enzymes. It is suggested that HESPC could be used food tool for treating MI induced hyperlipidemia and hyperglycemia.

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