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Antihypertensive Effect of Novel Functional Fermented Milk in Induced High Blood Pressure Rats

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ABSTARCT

The effect of functional fermented milk rich in bioactive peptides on blood pressure, lipid profile, antioxidant status and some organ's in hypertensive-induced rats was studied. Functional fermented milk produced from skimmed milk fortified with 2% SMP:WPC (1:1) and partially proteolyzed by trypsin (2.5 mg/100 g milk for 20 min) before fermintation by using mixed starter culture of *Lb. rhamnosus* B-1445, O-114, YC-X11 and *Lb. helveticus*Lh-B 02 with a 3:2:1:1 ratio, respectively. The main obtained results showed that the daily administration of functional fermented milk (group 4) for 4 weeks lowered the systolic blood pressure (SBP) and diastolic blood pressure (DBP) by 43 and 9 mmHg, respectively compared with a negative control group which administered saline (group 1). Plasma triglycerides, total cholesterol, atherogenic index and cardiac risk factor reduced in group 4 compared to group 1, while low-density lipoprotein, high-density lipoprotein, antithrombotic activity and antioxidant markers did not differ significantly among all groups. Histologically, there were no significant changes in the liver, kidney, heart and aorta tissues of rats in groups 1 and 4. In contrast, there were histopathological changes in these tissues of rats in group 2 which received daily 2 mg captopril /100 g BW.

INTRODUCTION

Hypertension affects more than a billion people in the world (WHO, 2011). About 60% of those people are suffering from related cardiovascular risk factors including hypercholesterolemia, hypertriglyceridemia, atherosclerosis, coronary artery disease, obesity, diabetes and oxidative stress (Wang *et al*. 2009 and Ibrahim 2013). In Egypt the number of hypertensive patients' increases and more than 50% of individuals older than 60 years are suffering from hypertension (Ibrahim, 2013). Medical treatments based on synthetic chemicals with an angiotensin converting enzyme inhibition (ACE-I) molecular mechanism such as captopril are used as an effective pharmacological therapy in treating cardiovascular disorders and to regulate blood pressure in hypertensive patients (Sánchez *et al*., 2011 and Seth *et al*. 2016). The food industries, in association with research and public health institutions screening for natural sources of anti-hypertensive compounds increase and the development of novel functional ingredients from fermented milk products were reprted to be effective in this matter (Jauhiainen *et al*. 2007and Contreras *et al*., 2011).

The consumption of milk and dairy foods is effective in decreasing the risk of hypertention (Engberink *et al*., 2009). Dairy products contain several bioactive compounds (Ebringer *et al*., 2008) that can lower blood pressure (van Mierlo *et al*., 2006) espeatially those peptides generated from milk proteins hydrolyzation (Flambard and Johansen, 2007) during gastrointestinal digestion or milk fermentation with lactic acid bacteria (Boutrou *et al*., 2015 and Korhonen, 2009). Recently, our *in vitro* privous works found that milk fermentation by *Lb. helveticus* Lh-B 02 or *Lb. rhamnosus* B-1445 (Abd El-Fattah *et al*. 2016a and El-Kashef, 2017) as well as limited milk proteolysis by trypsin (Abd El-Fattah *et al*. 2016b) are very effective to obtain peptides which possess different ACE-I and antioxidant activities. *In vitro* bioactivity does not generally translate into *in vivo* effects due to gastrointestinal tract considerations comprising the action of digestive enzymes, absorption and bioavailability. Therfore, the aim of this work was to study the effect of functional fermented milk rich in bioactive peptides on high blood pressure in hypertensive-induced rats. Also, study its effect on some related health parameters including antioxidant activity, lipid profile and the histological examination of liver, kedny, heart and aorta.

MATERIALS AND METHODS

Materials

Fresh skimmed cow milk was prepared after collecting from the Technology Center of Agricultural Production, Faculty of Agriculture, Cairo University. Spray dried Skimmed milk powder (SMP, 1. 5% fat and 5% moisture) was brought from Dina For Agriculture Industries Co. (Dina farms, Egypt). Whey protein concentrate (WPC, 53%) was supplied by CP Kelco, a Huber Company (Georgia, USA). Bovine trypsin (EC: 3.4.21.4) with activity ≥ 250 Units/mg protein was purchased from Sigma-Aldrich (Egyptian International Centre for Import, Cairo, Egypt). The DVI commercial starters of high viscosity yogurt culture YC-X11, O-114 and Lb. helveticus Lh-B 02 were obtained from Chr Hansen laboratories, Hoersholm, Denmark and Lb. rhamnosus B-1445 were procured from NRRL, USA. Triglycerides (TGs), total cholesterol (TC), high-density lipoproteins-cholesterol (HDL-C) and low-density lipoproteins-cholesterol (LDL-C) Spectrum kits were purchased from Egyptian Company for Biotechnology (Spectrum, Cairo, Egypt). Plasma total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GPx) Biodiagnostic Kits were obtained from Biodiagnostic Company, Giza, Egypt. Leptin enzyme-linked immunosorbent assay (ELISA) kit sandwich was purchased from DRG Instruments GmbH Company, Germany.

Mixed starter preparation

The activated starters of *Lb. rhamnosus* B-1445, O-114, YC-X11 and *Lb. helveticus*Lh-B 02 was mixed at ratio of 3:2:1:1, respectively to avoid the predominant of strains each others.

Fermented milk preparation

Based on our findings in previous study (El-Kashef, 2017), two treatments of non-fat stirred fermented milk were manufactured at laboratory scale. Skim milk was fortified with SMP: WPC (1:1) at level

of 2% (w/v) and was heat-treated at 90ºC/10 min in water bath, then was cooled to 37ºC and then divided into two parts. The first part was inoculated with 2% (v/v) yogurt starter YC-X11 (traditional yogurt). The second part was hydrolyzed using trypsin at 2.5 mg/100 g for 20 min at 37ºC, then trypsin was inactivated by heating at 100ºC/5 min in boiling water bath, followed by cooling to 37ºC and then inoculated with 2% (v/v) mixed starter of *Lb. rhamnosus* B-1445, O-114, *Lb. helveticus* Lh-B02 and YC-X11 at ratio of 3:2:1:1, respectively (functional fermented milk rich in bioactive peptides). After about 5 h of fermentation period at 37ºC the final fermented milk from each treatment was kept under cooling for oral administration of rat groups.

Animal experiment

This experiment was approved by the Committee of Scientific Ethics at animal house, Biological experiments Center, Faculty of Medicine, KasrAlAiny, Egypt and the rats were treated according to its guidelines. Thirty-two male Wistar rats weighing 150 – 160g (7 weeks-old) were procured from Biological experiments Center, Faculty of Medicine, Kasr AlAiny, Egypt and nursed in the animal house in the same place. They were maintained under standard conditions at 22±1ºC and 55% relative humidity, with a 12 h light: dark cycle and fed on a standard diet (60% corn starch, 20% casein, 10% corn oil, 5% cellulose, 4% salt mixture and 1% vitamins mixture) to adapt for 7 days. After the adaptation period all rats subcutaneously injected with dexamethasone (30µg/kg/day) for 14 days to induce hypertension as recommended by Zhang *et al*. (2009). Then, the hypertensive-induced rats were divided into 4 groups of 8 rats each. The first group (group1) served as a negative control group (received daily 1.5 ml saline/100 g of body weight, BW). The second group (group 2) served as a positive control group (received daily 2 mg captopril /100 g BW). The third (group3) and fourth (group 4) groups received daily traditional yogurt or functional fermented milk, respectively at 1.5 ml/100 g BW. All groups were orally administered by oral gavage and fed on normal rat diet and water *ad libitum* during the experimental period (4 weeks). The rats were observed continuously and their BW and blood pressure were recorded weekly.

Preparation of blood plasma and tissues

At the end of experimental period, the blood samples were collected from each group in heparinized tubes and the rats were euthanized using diethyl ether and the liver, kidney, heart and aorta were removed after sacrifice. Collected blood samples were centrifuged at 3500 x*g* for 10 minutes at 4ºC and plasma aliquots were collected and stored at -40ºC until analysis of blood chemistry parameters. The tissues were fixed in 10% formalin and embedded in paraffin for histological examination.

Blood pressure measurement

Both SBP and DBP of the rats were measured once per week using the tail-cuff method (LE 5001 pressure Meter, Letica SA, Barcelona, Spain). To ensure that the pulsations of the tail arteries detectable, all rats were maintained at 37ºC for 10 minutes prior to the measurement process.

Body weight and body mass index

Body weight was recorded weekly and weight gain was calculated. Body mass index (BMI) was calculated as follows: BMI = BW (g) / length^2 (cm^2).

Blood chemistry parameters

Triglycerides (TG), total cholesterol (TC), HDL-C, LDL-C and leptin levels as well as total antioxidant capacity (TAC), superoxide dismutase (SOD) activity and glutathione peroxidase (GPx) activity were measured according to kit manufacturer's instructions. The antithrombotic activity was determined in plasma with chromogenic substrates as described by Abildgaard *et al*. (1977). The atherogenic index (AI) and the cardiac risk factor were calculated using the following formulas: AI = TC-HDL-C/HDL-C (Hostmark *et al*. 1991) and cardiac risk factor = TC/HDL-C (Kim and Sho, 2007).

Histological examination

Changes in the histological structure of different rat's tissues were investigated at the end of the experimental period. Liver, kidney, heart and aorta tissues of rats from different groups were embedded in paraffin blocks and small sections (3-5 µm thick) from each were taken and fixed on poly-lysine-coated glass slides, deparaffinised and stained with hematoxylin and eosin (H&E) as described by Bancroft and Stevens (1996) and Photographs were taken by digital camera for rat's organs from different group under this study.

Statistical analysis

Three replicates from each parameter were statistically analyzed and the data were expressed as the mean ± standard deviation (SD). The Mstat-C software was used to carry out both randomize complete block design and the analysis of variance of factorial methods. The calculation of least significant differences (L.S.D.) was used in comparing between the significant differences and the mean of different treatments (Snedecor and Cochran, 1982). Results were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Body weight and plasma leptin of rats

The mean body weight, mean weight gain, body mass index (BMI) and plasma leptin of hypertensive-induced rats are tabulated in Table (1). From the obtained results, the body weight of all groups increased at the end of the experiment with no significant differences in BMI among the 4 groups. The gain in body weight was significantly lower in the groups 3 and 4 which received traditional yogurt and functional fermented milk, respectively followed by group 2 which received captopril (positive control) and group 1 which received saline (negative control).

Adipose tissue produces leptin and releases it into the blood stream and as fat deposits grow, blood leptin levels tend to increase (Considine *et al*., 1996). The results in Table (1) demonstrated that there were no significant differences in plasma leptin concentration among all groups. Unexpected, these results suggested that reduction in body weight gain is not associated with a reduction in leptin and this may be attributed to the decrease in adipose tissue and fat deposits grow which related to produce and release of leptin into bloodstream. In

contrast, a decrease in the body weight and plasma leptin of rats was noted by the administration of fermented skim milk with *Lb. gasseri* (Sato *et al.*, 2008) and fermented whey beverage with *Lb. plantarum* (Hong *et al*., 2015).

Table 1. Effect of functional fermented milk on body weight, rate of weight gain, BMI and plasma leptin of hypertensive-induced rats[a].

Parameters	Group 1	Group 2	Group 3	Group 4	LSD at 0.05
Mean weight at 1 day (g)	157.30±7.02[a]	154.60±11.54[a]	150.60±2.31[a]	153.30±1.15[a]	14.51
Mean weight at last day (g)	217.30±1.15[a]	201.30±21.38[ab]	182.60±6.11[b]	177.30±20.43[b]	34.55
Rate of weight gain (%)	38.14±3.71[a]	30.21±2.33[b]	21.25±1.62[c]	15.66±1.77[d]	1.902
BMI (g/cm^2)	0.62±0.02[a]	0.62±0.03[a]	0.66±0.05[a]	0.63±0.07[a]	0.1094
Leptin (ng/ml)	13.10±2.19[a]	11.93±1.5[a]	11.47±0.94[a]	11.09±1.89[a]	3.642

[a]values are means ± standard deviation BMI: body mass index.

Blood pressure of rats

The antihypertensive effect of administration of functional fermented milk, traditional yogurt and captopril (ACE inhibitor) was assessed comparing with the negative control (0.9% NaCl) in hypertensive-induced rats (Figs. 1 and 2).

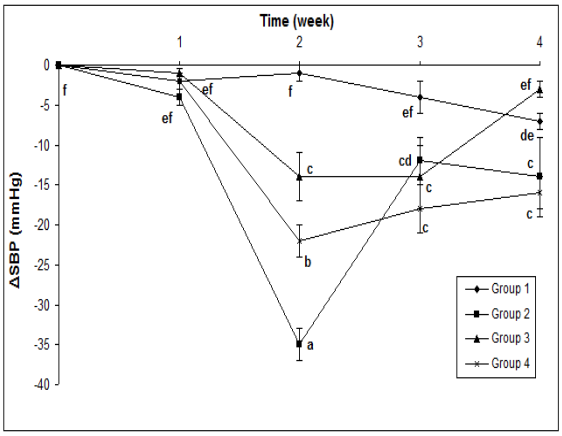

Fig. 1. The effect of functional fermented milk on the systolic blood pressure (SBP) of hypertensive-induced rats. LSD at 0.05 = 5.615.

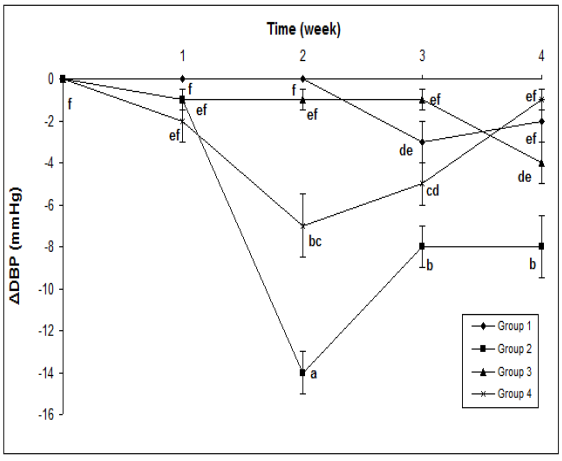

Fig. 2. The effect of functional fermented milk on the diastolic blood pressure (DBP) of hypertensive-induced rats. LSD at 0.05 = 2.821.

At the beginning of the experiment, the hypertensive-induced rats (n=32) had SBP and DBP values of 203±2.7 and 123±1.3 mmHg, respectively. Throughout the 4 weeks of the experiment, both of SBP and DBP of the positive control (group 2) were significantly lower than the other groups. This antihypertensive effect of captopril was expected where it prevents diversion of angiotensin I to the vigorous vasoconstrictor angiotensin II and inhibits continuously the bradykinin as vasodilator (Odaka and Mizuochi, 2000). A significant antihypertensive effect on both of SBP and DBP was observed after 2 weeks of the continuous administration of functional fermented milk (group 4) as compared with the negative control (group 1) and traditional yogurt (group3) groups (Figs. 1 and 2). Relative to the negative control group, the reduction rates of SBP and DBP in rats of group 4 were 20 and 8 mmHg, respectively, after oral administration for 2 weeks. This trend was continuous until the experiment ended (4 weeks) with high reduction rates. In this regard, Chen *et al*. (2014) reported that a significant antihypertensive effect for both SBP and DBP of spontaneous hypertensive rats was observed after the administration of *Lb. helveticus* H9-fermented milk, as compared with the control and commercial yogurt groups. Also, Inoue *et al*. (2003) found that fermented milk by *Lb. casei* Shirota and *Lactococcuslactis* YT2027 and containing - aminobutyric acid led to decrease SBP by 17 mmHg in 35 mild hypertensives.

Lipid profile, antithrombotic activity and atherogenic indices of hypertensive-induced rats

The lipid profile and atherogenic indices of rats are shown in Table (2). The obtained results showed that there were no significant differences between groups 3 and 4 in TG, TC, LDL-C and HDL-C, while TG and TC values were decreased significantly in group 4 compared to group 1. The level of LDL-C and HDL-C did not differ significantly in all groups. Several studies showed that fermented milk with LAB including *Bifidobacterium longum* (Xiao *et al*., 2003), *Lb. acidophilus* (Park *et al*., 2007) and *Lc. lactis* (Rodrígues-Figueroa *et al*., 2013) had hypocholesterolemic effects in both rat and human. This effect may be attributed to lactic acid bacteria which inhibit cholesterol synthesis enzymes, eliminate of cholesterol in feces, bind of cholesterol to bacterial cells and interfere with the recycling of bile salt (a metabolic product of cholesterol) (An *et al*., 2011).

Atherogenic index (AI) and cardiac risk factor are considered to be important parameters of atherosclerosis. These parameters indicate the risk of deposition of foam cells, plaque and fatty infiltration or lipids in the heart, coronaries, aorta, liver and kidney (Basu *et al*., 2007). These parameters were calculated

and tabulated in Table (2). The results showed that the highest values of AI (0.65) and cardiac risk factor (1.65) were recorded for rats of group 2 compared to other groups. The values of AI and cardiac risk factor decreased significantly in group 4 compared to group 1. Kawase *et al*. (2000) observed the similar trend in healthy adult men, showing that the intake of fermented milk with *Lb. casei* and *St. Thermophilus* and supplemented with WPC led to decrease the AI compared to placebo group. Also, Mohania *et al.* (2013) found a remarkable decrease in AI of rats fed with probiotic dahi preparing by *Lc. lactis* ssp. *cremoris*, *Lc. lactis* ssp. *lactis*biovar*diacetylactis* and *Lb. plantarum*.

The results in the same Table (2) demonstrated that the antithrombotic activity did not change significantly in the rats among all groups. On the other hand, antithrombotic activity was found with the κ-casein-derived undecapeptide and the lactoferrin derived tetrapeptide (Drouet *et al*.,1990) or oral intake of *Lb. pentosus* JCM 8333, *Leuconostoc oeni* Elios 1 and *Lb. fermentum* NBRC 3961 (Ooe *et al*., 2014).

Table 2. Effect of functional fermented milk on plasma lipid profile, atherogenic indices and antithrombotic activity of hypertensive-induced rats[a].

Groups	TG (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	AI	Cardiac risk factor	Antithrombotic activity (%)
Group 1	70.38±11.73^{a}	66.60±8.76^{b}	82.75±6.1^{a}	57.93±7.66^{a}	0.15±0.01^{b}	1.15±0.1^{b}	35.0±2.0^{a}
Group 2	55.72±4.69^{ab}	90.88±1.09^{a}	74.39±8.05^{a}	55.05±2.11^{a}	0.65±0.05^{a}	1.65±0.05^{a}	39.0±6.0^{a}
Group 3	63.93±1.76^{ab}	56.07±4.34^{c}	80.18±8.93^{a}	52.63±2.22^{a}	0.07±0.001^{c}	1.07±0.01^{c}	36.0±4.0^{a}
Group 4	51.61±8.21^{b}	58.82±0.75^{c}	71.29±7.99^{a}	55.77±3.03^{a}	0.05±0.002^{d}	1.05±0.01^{c}	37.5±6.5^{a}
LSD at 0.05	17.1	5.254	18.97	9.034	0.00632	0.0894	10.45

[a]Values are means ± standard deviation.

AI: atherogenic index; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein- cholesterol; HDL-C: high-density lipoprotein- cholesterol.

Antioxidant status of hypertensive-induced rats

The toxicity effect as a result of harm caused by oxidative stress, especially in obese patients was clearly reported by Baskol et al. (2007). Also, association of some enzymes with the blood circulatory system diseases or oxidative stress related biomarkers have been developed. Total antioxidant capacity (TAC) could be a reliable biomarker of diagnostics and prognostics (Kusano and Ferrari, 2008). As a scavenger substance, Superoxide dismutase (SOD) was known to play a vital role with its antioxidant effect that prevents body cells from oxidative damages (Li et al., 1995). Moreover, glutathione peroxidase (GPx) the glutathione-utilizing peroxidase is playing an important role as an antioxidant enzyme which protects organelles from oxidative damage (Esposito et al., 2000). Antioxidant markers in plasma of hypertensive-induced rats are presented in Table (3).

Table 3. Effect of functional fermented milk on plasma antioxidant markers of hypertensive - induced rats[a].

Groups	TAC (mM/L)	SOD (U/ml)	GPx (mU/ml)
Group 1	2.93±0.41^{a}	241.67±28.87^{a}	1.69±0.4^{a}
Group 2	2.84±0.5^{a}	233.30±14.43^{a}	1.69±0.24^{a}
Group 3	3.11±0.44^{a}	241.67±28.87^{a}	1.78±0.17^{a}
Group 4	3.58±0.72^{a}	250.00±25.0^{a}	1.98±0.65^{a}
LSD at 0.05	1.189	57.07	0.8382

[a]Values are means ± standard deviation.

TAC: total antioxidant capacity; SOD: superoxide dismutase; GPx: glutathione peroxidase.

After 4 weeks of the experiment, there were not significant differences in TAC, SOD and GPx activity among all groups. In this respect, Wang *et al*. (2009) found that administration of pigs with *Lb. fermentum* for 8 weeks led to increase TAC, SOD and GPx activities in serum. Moreover, Kumar *et al*. (2017) observed that feeding diabetic rats with probiotic fermented milk with *Lb. fermentum* for 60 days led to increase the activity of catalase, SOD and GPx comparing with normal control and diabetic groups.

Histological evaluation

Histological evaluation is a necessary tool for determining the healthy status of different tissues and for affirmation the results of blood analysis. The histological changes of liver, kidney, heart and aorta of hypertensive-induced rats orally administered with traditional yogurt or functional fermented milk comparing with negative or positive control are shown in Figs. (3, 4, 5 and 6 respectively).

Liver tissues

Liver of negative control rats showed the normal histological structure of hepatic lobule (Fig. 3, A). The hepatic lobules are normal, each lobule consists of the normal hepatocytes arranged in hepatic strands, and each lobule contains blood vessels and bile ducts. However, the liver of rats administered captopril (positive control) revealed focal hepatic necrosis associated with inflammatory cells infiltration and cystic dilatation of the bile duct and fibroplasia in the portal triad (Fig. 3, B and C). Our results supported by Moinuddin *et al.* (2011) who observed that the liver of captopril treated rat showed that congestion, dilation of the central vein, mild hemorrhage and a mild degree of vacuolations in the sub-borders of the hepatic parenchyma. Also, Seth *et al*. (2016) found that the liver of treated rat with captopril showed high level of mononuclear cell infiltration confirming that captopril causes damage to liver. The liver of rats administered traditional yogurt exhibited apparent normal hepatic parenchyma (Fig. 3, A). However, liver of rats administered functional fermented milk showed normal histological structure and kupffer cells activation (Fig. 3, D).

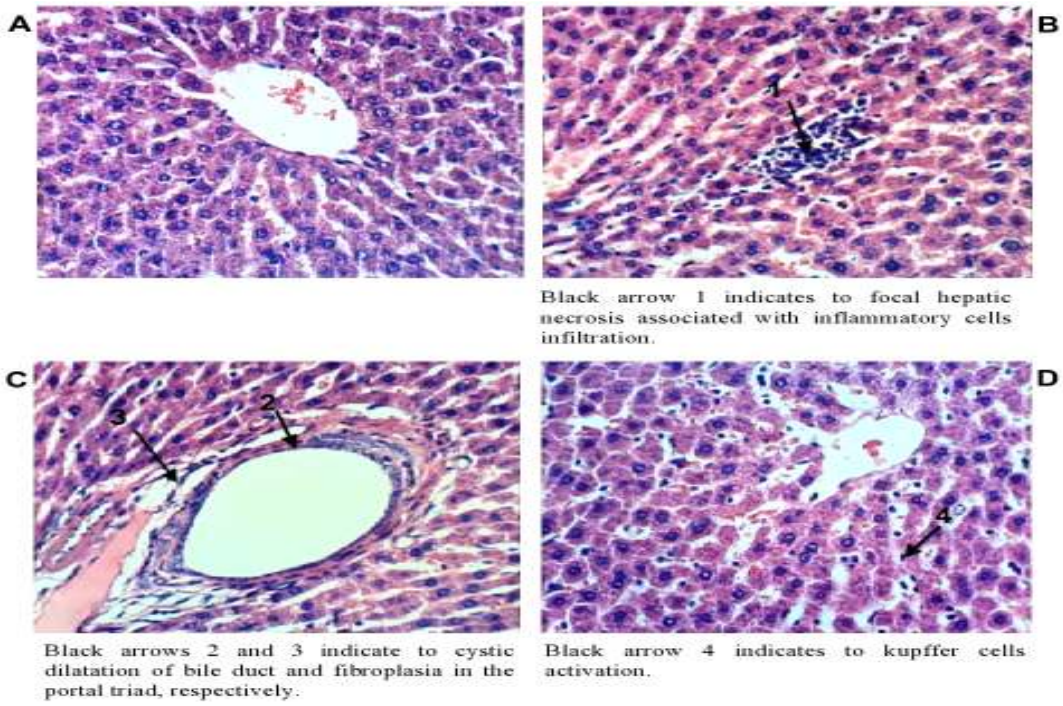

Fig. 3. Histological changes of liver tissue in hypertensive-induced rats (H&E X400).
A: groups 1 and 3 (normal liver); B and C: group 2; D: group 4.

Kidney tissues

Microscopical examination for kidney of rats of groups 1, 3 and 4 showed the normal histological structure of renal parenchyma (Fig. 4, A). In contrary, the kidney of rats administered captopril (positive control) appeared vacuolation of renal tubular epithelium, perivascular oedema, congestion of intertubular blood vessels, necrobiosis of renal tubular epithelium and congestion of the glomerular tuft (Fig. 4, B and C). In this respect, Moinuddin *et al*. (2011) reported that the kidney of captopril treated rats showed moderate edema, a moderate degree of hemorrhage and congested blood vessels.

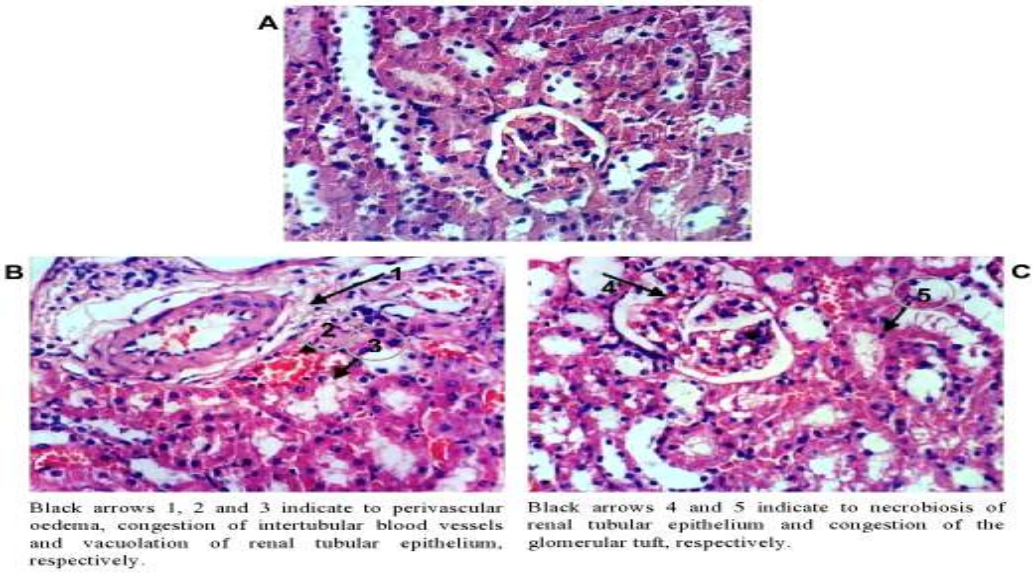

Fig. 4. Histological changes of kidney tissue in hypertensive-induced rats (H&E X400).
A: groups 1, 3 and 4 (normal kidney); B and C: group 2.

Heart tissues

Histologically, the heart of negative control rats and rats administered traditional yogurt or functional fermented milk revealed normal cardiac myocytes (Fig. 5, A). Our findings was propped by Shenana *et al.* (2006) who found that the heart of control rats and treated rats with kumbu-yogurt (yogurt made with yogurt starter, *Acetobacterxylium*, *Zygosacharomycisrouxii*, *Candida* spp. and *Bifidobacteria*) showed normal structure comparing with hypercholesterlemic rats. Also, Fig. (5, B and C) showed that the heart of rats administered captopril (positive control) appeared intermuscular mononuclear inflammatory cells infiltration

and focal necrosis of cardiac myocytes associated with inflammatory cells infiltration.

Aorta tissues

Microscopically, the aorta of negative control rats and rats administered traditional yogurt or functional fermented milk revealed no histopathological changes (Fig. 6, A). Our results are in accordance with those of Shenana *et al.* (2006) who noticed that the aorta of treated rats with kumbu-yogurt exhibited normal structure. On the other hand, the aorta of rats administered captopril showed vacuolation of cells of tunica media (Fig. 6, B). These results are disagreement with Moinuddin *et al*. (2011) who found that the thoracic aorta of treated rats with captopril showed no histopathological changes.

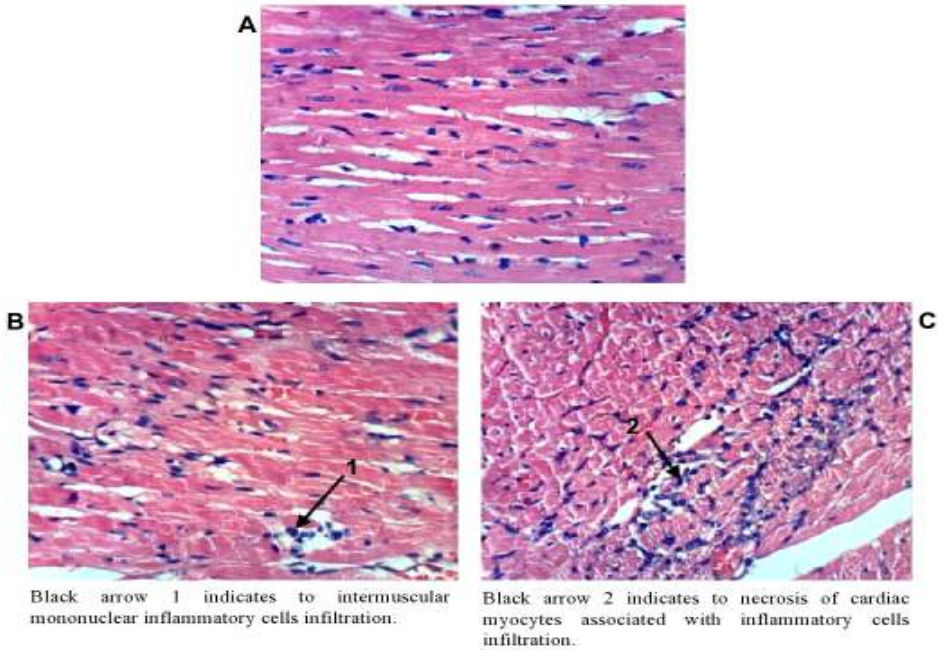

Fig. 5. Histological changes of heart tissue in hypertensive-induced rats (H&E X400).
A: groups 1, 3 and 4 (normal heart); B and C: group 2.

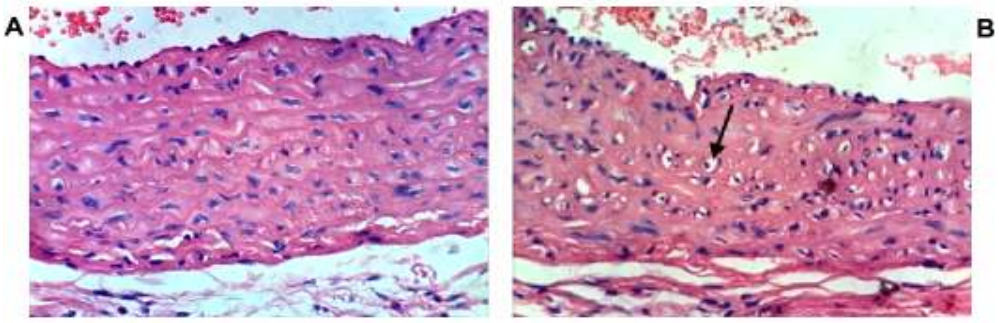

Fig. 6. Histological changes of aorta tissue in hypertensive-induces rats (H&E X400).
A: groups 1, 3 and 4 (normal aorta); B: group 2.
Black arrow indicates to vacuolation of the tunica media.

CONCLUSION

Skim milk supplemented with whey protein concentrate (WPC) and hydrolyzed partially by trypsin prior to fermentation with mixed starter of *Lb. rhamnosus* B-1445, O-114, *Lb. helveticus* Lh-B02 and YC-X11 can be used to manufacture functional fermented milk rich in bioactive peptides. This product had positive effect on the hypertension and lipid profile in hypertensive-induced rats, where it decreased blood pressure, triglycerides, cholesterol and did not exhibit any adverse histopathological effect on the tissues of liver, kidney, heart and aorta.

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التأثير الخافض للضغط للبن متخمر وظيفي جديد في الفئران المستحثة لضغط الدم المرتفع

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إستهدفت هذه الدراسة إنتاج لبن متخمر وظيفي غني بالبيتيدات الحيوية التي تحافظ على صحة القلب والأوعية الدموية وذلك عن طريق تدعيم اللبن منزوع الدهن بمركز بروتين الشرش والتحلل الجزئي للبروتين بإنزيم التربسين والتخمير ببادىء مختلط مكون من السلالات التجارية وسلالات جنس اللاكتوباسيلس المنتقاه لإنتاجها العالي من البيتيدات الحيوية و دراسة تأثير هذا المنتج علي كل من ضغط الدم و اللبيبدات و التأثير المضاد للأكسده، و أيضا التغيرات الحادثة في بعض الأعضاء و ذ لك في فئران التجارب المستحثة لضغط الدم المرتفع، حيث تم أولا إنتاج لبن وظيفي متخمر باستخدام اللبن الفرز المحلل جزئيا بالتربسين (٢.٥ مجم/١٠٠جم لبن/٢٠ دقيقة) و المدعم بـ ٢% من اللبن الفرز المجفف:مركز بروتينات الشرش (١:١) و ذلك قبل تخميرة بواسطة مزرعة من البادئ المختلط المكون من Lb. rhamnosus B-14454 و O-114 و YC-X11 و Lb. LhelveticusLh 02 B بنسبة ١:١:٢:٣ علي التوالي، و في التجربة الحيوية تم رفع ضغط الدم لعدد ٣٢ فأر بحقنهم مادة ديكساميثازون dexamethasone لمدة ١٤ يوم ثم تقسيم هذه الفئران إلى ٤ مجاميع كل منها يحتوي على ٨ فئران، تم تجريع فئران المجموعة الأولى بمحلول فسيولوجي (١.٥ مل/١٠٠ جم من وزن الجسم)، المجموعة الثانية بالـ captopril (٢ ملجم/١٠٠ جم من وزن الجسم)، المجموعة الثالثة بالزبادي (١.٥ مل/١٠٠ جم من وزن الجسم) والمجموعة الرابعة باللبن المتخمر الوظيفي (١.٥ مل/١٠٠جم من وزن الجسم) يوميا لمدة ٤ أسابيع مع التغذية على العليقة الأساسية لكل فئران التجربة، تم تسجيل كل من وزن الفئران وضغط الدم أسبوعيا خلال التجربة، كما تم تقدير اللبيبيدات ومضادات الأكسدة في بلازما الدم وكذلك الفحص الهستولوجي لأنسجة الكبد و الكلى و القلب والأورطى في نهاية التجربة، أظهرت النتائج المتحصل عليها من إجراء تجربة بيولوجية على هذا المنتج أن تجريعه للفئران المستحثة لضغط الدم المرتفع أدى إلى إنخفاض كل من ضغط الدم، الجليسريدات الثلاثية والكوليسترول، ولم يكن له تأثير مرضي من الناحية الهستولوجية على كل من أنسجة الكبد، الكلى، القلب والأورطى.