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Trabajo Original

Epidemiología y dietética

Antihypertensive effect of fermented skim camel (*Camelus dromedarius*) milk on spontaneously hypertensive rats

Efecto antihipertensivo de la leche de camello fermentada (*Camelus dromedarius*) en ratas hipertensas

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Abstract

Background: Hypertension is one of the most common diseases in worldwide, thus prevention of hypertension is important in reducing the risks of cardiovascular disease. Milk contains bioactive peptides released during milk fermentation which lead to exhibit angiotensin I converting enzyme (ACE) inhibitory.

Objective: The aim of this study was to investigate the antihypertensive effect of fermented skim camel milk on rats and compared with unfermented skim camel milk as control.

Methods: The antihypertensive effect of fermented skim camel milk on thirty six male spontaneously hypertensive rats (SHR) was carried out for (short-term) and (long-term) using different doses (80, 240 and 1200 mg/kg body weight). Angiotensin converting enzyme (ACE) activity was also measured using ACE Kit.

Results: The blood pressure (systolic and diastolic) of spontaneously hypertensive rats (SHR) in short term administration (24 hours) of 1200 mg/kg body weight fermented skim camel milk decreased significantly ($p < 0.05$) from 22 to 36 mmHg and 28 to 32 mmHg, respectively, at four and eight hour of post administration. On the other hand, the blood pressure of fermented skim camel milk for long-term (20 days) decreased and affected the heart rate (beats/min). The lowest record of systolic (41 mmHg) and diastolic blood pressure (19 mmHg) were at dose of 1200 mg/kg body weight of fermented skim camel milk at 15 days of administration. Likewise, ACE activity in plasma of SHR administered fermented skim camel milk decreased significantly ($p < 0.05$) compared with the control group.

Conclusion: The hypotensive effect of fermented skim camel milk by *L. helveticus* and *S. thermophilus* in SHR rats depends on the high dose of fermented skim camel milk in short and long-term. The ACE activity inhibitory was clear with fermented skim camel milk.

Key words:

Hypertension. Skim camel milk. Blood pressure. Angiotensin I converting enzyme activity.

Resumen

Introducción: la hipertensión es una de las enfermedades más frecuentes en el mundo, por lo que su prevención es importante en el objetivo de disminuir el riesgo de enfermedad cardiovascular. La leche contiene péptidos bioactivos que se liberan durante su fermentación con un efecto inhibitorio sobre el enzima convertidor de la angiotensina (ECA).

Objetivo: el objeto de este estudio fue investigar el efecto antihipertensivo de la leche de camello fermentada en un modelo experimental de ratas con hipertensión comparándolas con un grupo control alimentado con la misma leche sin fermentar.

Métodos: se valoró el efecto antihipertensivo de la leche de camello fermentada en 36 ratas macho hipertensas de forma espontánea a corto y a largo plazo usando diferentes dosis (80, 240 y 1.200 mg/kg de peso). También se midió la actividad del ECA.

Resultados: la presión arterial (sistólica y diastólica) disminuyó a corto plazo (24 horas) con la dosis de 1.200 mg/kg ($p < 0,05$), pasando de 36 a 22 mmHg y de 32 a 28 mmHg, respectivamente a las 4 y 8 horas postadministración. Por otra parte, la tensión arterial a largo plazo en el grupo que consumió la leche de camello fermentada afectó disminuyendo la frecuencia cardíaca. Las medidas inferiores de presión sistólica (41 mmHg) y diastólica (19 mmHg) aparecieron en el grupo que recibía 1.200 mg/kg a los 15 días del inicio de la administración de leche de camello fermentada. Por otra parte, la actividad del ECA disminuyó significativamente en el grupo con leche fermentada ($p < 0,05$).

Conclusiones: el efecto antihipertensivo de la leche de camello fermentada con *L. helveticus* y *S. Thermophilus* en ratas con hipertensión depende de la cantidad administrada, tanto a corto como a largo plazo. El efecto inhibitorio sobre el ECA fue manifiesto en el grupo que recibió leche de camello fermentada.

Palabras clave:

Hipertensión. Leche de camello desnatada. Presión arterial. Enzima convertidor de la angiotensina.

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INTRODUCTION

Hypertension disease is an important public health problem around the world. Unprocessed hypertension leads to cardiovascular and renal diseases such as coronary heart disease, stroke and kidney dysfunction (WHO, 2015). Dietary factors influence the development of hypertension (McCarron et al., 1984), on the other hand, the use of dietary approaches to stop hypertension using a diet rich in vegetables, fruits and low fat dairy products, was associated with useful decrease in blood pressure (Harsha et al., 1999). Food protein is now being consumed as a source of biologically active components that might have health benefits when ingested (Roberts et al., 1999). Milk is a rich source of dietary proteins and could play an important role in promotion of health and prevention of diseases (Meisel, 2005). Camel milk contains all essential nutrients found in bovine milk (El-Agamy et al., 1998) and is consumed in Saudi Arabia as fresh or sour (Abu-Taraboush et al., 1998). Milk protein derived biologically active peptides prevented the rise of blood pressure in SHR (Sipola et al., 2001) and decreased blood pressure (BP) in mildly hypertensive subjects (Sipola et al., 2002). Moreover, many studies have reported the ACE inhibitor activity of various bioactive peptides isolated by milk proteins fermentation (Gobbetti et al., 2000; FitzGerald & Murray (2006). Milk protein contain bioactive peptides could be angiotensin I converting enzyme (ACE) inhibitors, this peptides can be release during milk fermentation with *Lactobacillus helveticus* strains (Yamamoto et al., 1999; Sipola et al., 2002) or combined with *Streptococcus thermophilus* (Donkor et al., 2007). It has been reported that milk fermentation by two or more different types of strain might contain wide variety of functional substances than milk cultured with a single strain (Kuwabara et al., 1995). Few studies reported the ACE inhibitor activity of fermented camel milk proteins. The elevated ACE-inhibitory activity was observed in cultured camel milk peptide fractions compared to bovine milk. This is due to the presence of higher proline content in the primary structure of camel milk caseins than in bovine milk (Moslehishada et al., 2013). Moreover, whole casein and β -CN of camel milk found to have significant ACE-inhibitory activity after hydrolysis with pepsin alone or after pepsinolysis followed by trypsinolysis and chymotrypsinolysis (Salami et al., 2011). Therefore, the aim of this study was to investigate the antihypertensive effect of fermented and unfermented skim camel milk using rats.

MATERIALS AND METHODS

MATERIALS

Lactobacillus helveticus (LMG11445) strain was from Belgian coordinated collection of microorganisms (BCCM-LMG) and *Streptococcus thermophilus* (ATCC 19258) was from American type culture collection (ATCC) in freeze dried form. Lactase enzyme (*Aspergillus oryzae* 9000 FCC lactase units) supplied by Webber Naturals Co. Canada. Captopril was from Sigma, St. Louis, MO, USA) ACE Kit was from Buhlmann Laboratories AG, Switzerland.

PREPARATION OF FERMENTED AND UNFERMENTED SKIM CAMEL MILK

Camel milk was obtained from private farm located in central region of Saudi Arabia. Fat was removed using centrifugal separation at temperature of 45 °C and lactose was hydrolyzed using lactase enzyme. Skimmed milk was pasteurized at 85 °C for 30 minutes. Then, skimmed camel milk (lactose hydrolyzed) was divided into two parts (unfermented and fermented skim camel milk). Then, one part of the milk was inoculated with 3% of active cultures (*L. helveticus* + *S. thermophilus*) and incubated at 40 °C until reaching pH reached 4.3. Later, fermented skim milk samples were frozen at -80 °C, and then freeze-dried and kept in the refrigerator until used as a diet for rats to determine the antihypertensive effect of fermented skim camel milk on rats. In this study, captopril (a proven hypotensive drug, 50 mg/kg of body weight) was used to make sure that rat's response to ACE- inhibitors.

ANIMALS GROUPING AND FEEDING

Thirty six male spontaneously hypertensive rats (SHR) were supplied from Harlan Laboratory co, USA, at age of 11 week-old and weighting 272 ± 3 g. Rats were housed in stainless steel cages at 22 ± 2 °C with 12 h light/dark cycles and $50 \pm 5\%$ relative humidity. The rats were given free access to water during the experimental period. Animals handling, treatment, euthanasia and other experimental procedures were in agreement with the National Institute of Health Guide for the care and use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publications No. 80-23; 1996) as well as the obtained approval (108-EACC-2015) from the Ethical committee of Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Rats were divided into six groups after a 1-week period of adaptation after arrival, each consisting of six rats: a negative control group were only fed distilled water orally, a positive control group were orally fed captopril (50 mg/kg b.w./day), one group of rats fed unfermented skim camel milk (UFCM) 1200 mg/kg b.w./day orally and three groups of rats were orally fed with different doses of fermented skim camel milk (FCM) at 80, 240 or 1200 mg/kg b.w./day, respectively (Wang et al., 2012). Diet for maintenance of rats was prepared according to the American Institute of Nutrition (AIN). Rats were orally fed between 10-11 a.m. for 20 d (long-term).

ANTIHYPERTENSIVE EFFECT MEASUREMENTS

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate value (HR) were measured by tail-cuff instrument noninvasive blood pressure method using CODA 20830 (Kent Scientific, USA). The measurements were taken before administration and continued for every five day in long-term study (21 days) and 0, 2, 4, 8 and 24 hours after single oral administration

(short-term study). Rats were kept at 38 °C for 10 min before taking the measurements to calm down and make the pulsations of the tail artery readable. To avoid the influence of the circadian, blood pressure measurements were conducted during day at time between 10 a.m. and 14 p.m. and the SBP and DBP values were obtained by calculating the average reading of 15 measurements.

DETERMINATION OF ACE ACTIVITY

Rats were allowed to fast for 12 h before anesthetization with diethyl ether and sacrificed at the end of experiment period. The blood samples were collected in EDTA tubes by orbital venipuncture and centrifuged for 10 min at $4000 \times g$ at 4 °C to obtain blood plasma (Tuck et al., 2009). The plasma samples were immediately stored at -80 °C until day of analysis. Angiotensin Converting Enzyme activity was measured using ACE Kit (Buhlmann Laboratories AG, Switzerland).

STATISTICAL ANALYSIS

Data were expressed as means \pm standard deviation (SD). Group's differences were analyzed using one-way ANOVA, followed by Duncan's Multiple Range (DMR) test using SPSS 21 software (SPSS Inc., Chicago, IL, USA). Difference was considered statistically significant when P value is less than 0.05 ($p < 0.05$).

RESULTS

THE EFFECT OF SHORT-TERM ADMINISTRATION OF FERMENTED SKIM CAMEL MILK ON BLOOD PRESSURE OF SHR

Figure 1 shows the effect of the different treatments (PC, UFCM1200, FCM80, FCM240 and FCM1200) on SBP of SHR. All treatments except unfermented skim camel milk and the low dose (80 mg) of fermented skim camel milk decreased significantly ($p < 0.05$) the SBP of SHR in four and eight hours of the administration compared with NC group. The high dose (1200 mg) of fermented skim camel milk significantly ($p < 0.05$) reduced the SBP by 22 and 36 mmHg at four and eight hours, respectively, followed by (240 mg) of fermented skim camel milk which significantly ($p < 0.05$) decreased the SBP by 16 and 21 at four and eight hours, respectively. The effect of high dose (1200 mg) of fermented skim camel milk in SBP was similar to the captopril at these two time intervals. Furthermore, figure 2 shows that fermented skim camel milk (1200 mg and 240 mg) significantly ($p < 0.05$) reduced the DBP by 28 and 12 mmHg at four hours and 32 and 22 mmHg at eight hours, respectively compared with negative control (NC). Generally, this effect decreased the SBP and DBP after eight hours of the administration and the milk had no effect after this time (24 h).

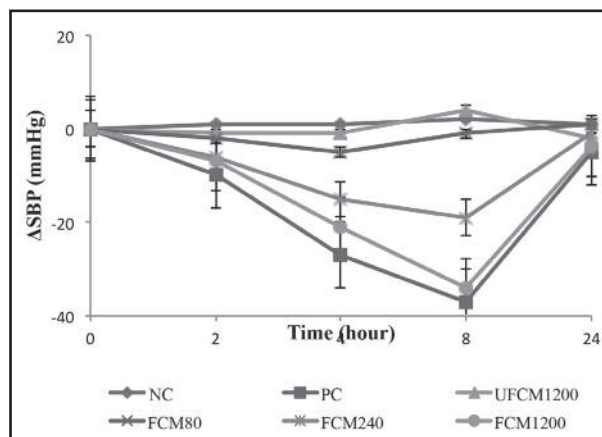


Figure 1.

The effect of fermented skim camel milk on systolic blood pressure (SBP) of spontaneously hypertensive rats (SHR), distilled water (●), captopril (■), 1200 mg unfermented camel milk/kg b.w. (▲), 80 mg fermented camel milk/kg b.w. (×), 240 mg fermented camel milk/kg b.w. (*), 1200 mg fermented camel milk/kg b.w. (●). Data are expressed as mean \pm SD ($n = 6$).

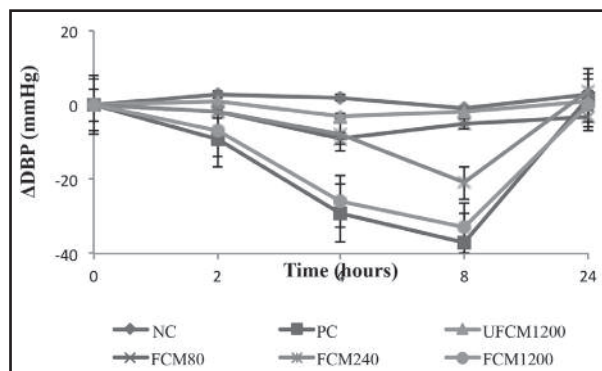


Figure 2.

The effect of fermented skim camel milk on diastolic blood pressure (DBP) of spontaneously hypertensive rats (SHR), distilled water (●), captopril (■), 1200 mg unfermented camel milk/kg b.w. (▲), 80 mg fermented camel milk/kg b.w. (×), 240 mg fermented camel milk/kg b.w. (*), 1200 mg fermented camel milk/kg b.w. (●). Data are expressed as mean \pm SD ($n = 6$).

THE EFFECT OF LONG-TERM ADMINISTRATION OF FERMENTED SKIM CAMEL MILK ON BODY WEIGHT, HEART RATE AND BLOOD PRESSURE OF SHR

Body weight of SHR

The body weight increased throughout the experimental period in all groups: NC, PC, UFCM1200, FCM80, FCM240 and FCM1200 from 273 ± 2 to 326 ± 2 , 271 ± 4 to 293 ± 4 , 275 ± 2 to 321 ± 2 , 269 ± 2 to 308 ± 3 , 273 ± 4 to 320 ± 3 and 271 ± 2 to 302 ± 3 , respectively. The PC group had significantly ($p < 0.05$) less body weight than that of other groups at the end of the experiment (20 d).

The heart rate value of SHR

Generally, the heart rates of rats fed fermented skim camel milk treated groups 1200, 240 mg and captopril group (PC) were significantly ($p < 0.05$) lower than that of the negative control group (NC) throughout the experimental period as shown in figure 3. Moreover, the heart rate of rats fed 1200 and 240 mg fermented skim camel milk generally significantly lower than captopril group (PC).

The systolic and diastolic blood pressure

Figure 4 shows the effect of the different treatments on the blood pressure of SHR. All treatments except unfermented skim camel milk and the low dose (80 mg) of fermented skim camel

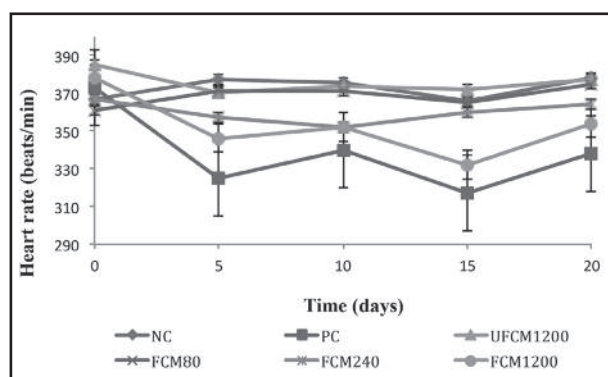


Figure 3.

The effect of fermented skim camel milk on heart rate of spontaneously hypertensive rats (SHR), distilled water (♦), captopril (■), 1200 mg unfermented camel milk/kg b.w. (▲), 80 mg fermented camel milk/kg b.w. (×), 240 mg fermented camel milk/kg b.w. (*), 1200 mg fermented camel milk/kg b.w. (●). Data are expressed as mean \pm SD ($n = 6$).

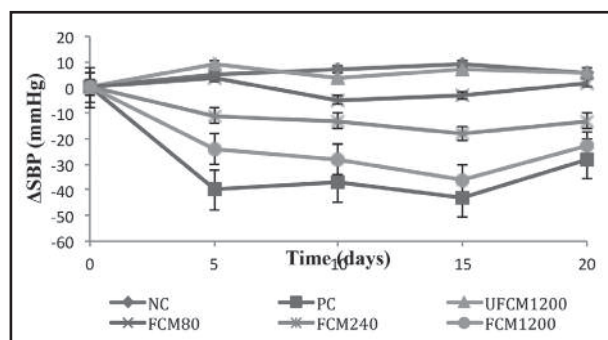


Figure 4.

The effect of fermented skim camel milk on systolic blood pressure (SBP) of spontaneously hypertensive rats (SHR), distilled water (♦), captopril (■), 1200 mg unfermented camel milk/kg b.w. (▲), 80 mg fermented camel milk/kg b.w. (×), 240 mg fermented camel milk/kg b.w. (*), 1200 mg fermented camel milk/kg b.w. (●). Data are expressed as mean \pm SD ($n = 6$).

milk reduced significantly ($p < 0.05$) the SBP in the fifth day of the experiment compared to the control group. SHR received the high dose (1200 mg) of fermented skim camel milk had significantly ($p < 0.05$) lower SBP than those received the low dose (240 mg). However, the effect of fermented skim camel milk on SBP was generally lower than that exerted by captopril. The significant lowering effect of the two doses (240 and 1200 mg) of fermented skim camel milk in the SBP of SHR generally continued until the end of the experiment. On the other hand, the high dose (1200 mg) only significantly ($p < 0.05$) decreased the DBP compared with the control group and the reduction of DBP in SHR rats continued to the last day of the treatment (Fig. 5). Moreover, the effect of the high dose (1200 mg) of fermented skim camel milk in DBP of SHR was generally not significantly ($p > 0.05$) different from the captopril.

ACE ACTIVITY IN PLASMA OF SHR

The effect of fermented skim camel milk (after oral administration) on ACE activity in plasma of SHR is shown in figure 6. Fermented skim camel milk (240 and 1200 mg) and captopril (PC) groups had significantly ($p < 0.05$) lower ACE activity in plasma of rats than negative control group.

DISCUSSION

In short-term study, the antihypertensive effect of fermented skim camel milk on SBP and DBP depended on dose of fermented milk (Figs. 1 and 2). The lowest reading of SBP and DBP was noticed with the highest concentration (1200 mg) of fermented skim camel milk, this reading was close to that recorded by captopril. the results are in agreement with Nakamura, et al. (1995) who

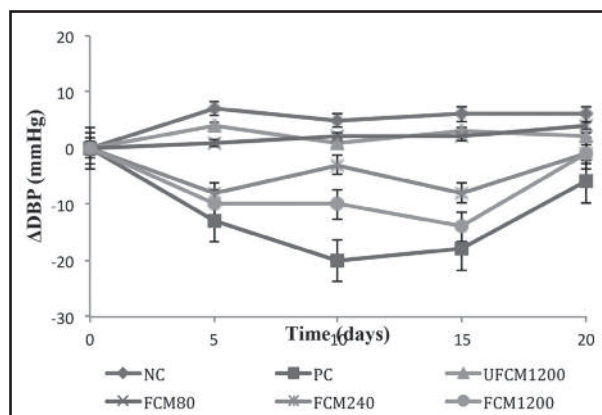


Figure 5.

The effect of fermented skim camel milk on diastolic blood pressure (DBP) of spontaneously hypertensive rats (SHR), distilled water (♦), captopril (■), 1200 mg unfermented camel milk/kg b.w. (▲), 80 mg fermented camel milk/kg b.w. (×), 240 mg fermented camel milk/kg b.w. (*), 1200 mg fermented camel milk/kg b.w. (●). Data are expressed as mean \pm SD ($n = 6$).

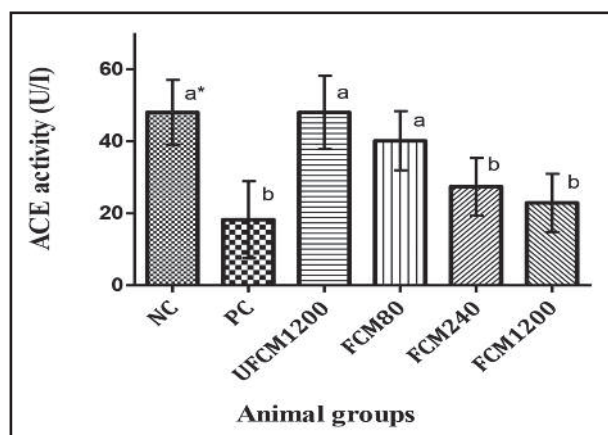


Figure 6.

The effect of fermented skim camel milk on ACE activity in plasma of SHR (NC: negative control; PC: captopril; UFCM1200: unfermented skim camel milk; FCM80: fermented skim camel milk; FCM240: fermented skim camel milk; FCM1200: fermented skim camel milk). *Mean \pm SD (n = 5). Bars with unlike letters differ significantly (p < 0.05).

noticed that SBP significantly reduced (p < 0.05) after four, six and eight hours of administration of bovine sour milk by 20.0 ± 5.2 , 21.8 ± 4.2 and 17.7 ± 3.5 , respectively. The effect of fermented milk administration to SHR was also observed by Muguerza, et al. (2006) who reported that the decrease in SBP was at maximum after four hours of oral feeding (34.81 ± 4.48 mmHg) milk fermented with *E. faecalis* CECT 5727 and CECT 5728, the reading was returned to the baseline after 24 hour.

Generally, fermented skim camel milk by *L. helveticus* and *S. thermophilus* had reducing effect on SBP and DBP in the long-term administration (Figs. 4 and 5). The high doses of 1200 and 240 mg of fermented skim camel milk decreased SBP throughout the treatment period, but high dose (1200 mg) only reduced the DBP and its effect was similar to that exerted by captopril. No effect was noticed on SBP and DBP of SHR rats by the use of low dose of 80 mg of fermented skim camel milk. These results are agreement with Sipola et al. (2002) who found that milk fermented by *L. helveticus* LBK16H was able to decrease SBP by 21 mmHg in hypertensive rats. In addition, Rodriguez-Figueroa et al. (2013) stated that the SBP and DBP of SHR decreased significantly (p < 0.05) in the second and third week when these rats received milk fermented with *L. Lactis* NRRL B-50571. This study suggests that antihypertensive effect of fermented skim camel milk (1200 and 240 mg) could be due to presence of bioactive peptides in the protein of the camel milk generated by the active bacterial cultures. The trend of heart rate decrease in FCM1200 and PC groups was also reported by Wang et al. (2012), who noticed that heart rate was decreased after oral feeding of captopril and two different doses of whey protein hydrolysate (1200 mg/kg b.w. and 240 mg/kg b.w.). Bioactive peptides derived from fermented skim camel milk may improve the oxygen consumption of myocardial which reduces the cardiac stress; this was obvious after orally taking of fermented skim camel milk and drug (captopril). Fluctuations

in heart rate could be due to the unrest of rats which not given relaxed period during blood pressure measurement by CODA tail-cuff method system. In current study, all groups except PC group showed increase in body weight of SHR rats. This result suggest that the slight increase in body weight of PC group compared to the other groups could be attributed to the side effects of captopril drug which cause weight loss according to the leaflet of the drug.

The rennin angiotensin system is a key factor in the protection of blood pressure. ACE (EC 3.4.15.1) prevents formation of angiotensin II. In the current study, the high ACE-inhibitory activity was noticed with the high doses (1200 and 240 mg) of fermented skim camel milk. This effect was similar to that noticed by captopril (PC group) which is proven as hypotensive drug. No effect was noticed with unfermented skim camel milk and the low dose (80 mg) of fermented skim camel milk. Thus, ACE-inhibitory activity in the current study could be attributed to the ACE-inhibitory peptides derived from fermented skim camel milk by *L. helveticus* and *S. thermophilus*. These result are in agreement with Wang et al. (2012) who showed decrease in ACE activity in plasma of SHR fed whey protein hydrolysate and captopril drug. Fermented milk containing IPP and VPP reduced ACE activity in the aorta and elevated plasma rennin activity in a long-term treatment (14 weeks) of SHR (Nakamura et al., 1996). Sipola et al. (2002) stated that lack of negative feedback for angiotensin II, lead to ACE inhibition.

CONCLUSION

Antihypertensive effect of fermented skim camel milk by *L. helveticus* and *S. thermophilus* in SHR rats depends on the fermentation and dose. In short-term, hypotensive effect on SBP and DBP was clear with high concentration of fermented skim camel milk but it did not continue until the end of the experimental period (24 h). The same effect was noticed in the long-term administration; however, the cessation of effect was not evaluated in this study. The decrease in ACE activity was noticed using fermented skim camel milk.

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