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Investigation the Effect of Capsaicin Protein Glycosylation, Na^+-K^+ ATPase, Ca^{+2} ATPase and Lipid Peroxidation Levels in Human Erythrocytes Which are Exposed to High Glucose Concentration (*in vitro*)

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ABSTRACT

In this study, the effects of capsaicin on protein glycosylation, Na^+-K^+ ATPase, Ca^{+2} ATPase activities and lipid peroxidation level in human erythrocytes which exposed to high glucose concentrations *in vitro* are investigated. The blood samples obtained from healthy individuals have been exposed to normal glucose and high glucose concentrations and then incubated with capsaicin at different concentrations. The samples which have only been exposed to normal glucose are used as a control group. In erythrocyte samples which have been exposed to high glucose concentration, Ca^{+2} ATPase and Na^+-K^+ ATPase activity are lower than those of control group and the differences between these two groups are statistically significant ($p<0.001$). In the group which has been exposed to capsaicin, the activity of the membrane enzyme is increased at a statistically significant level due to the increase of capsaicin concentrations. MDA and HbA_{1c} levels are increased more in high concentration glucose group than normal concentration glucose group, and in addition, these levels decrease proportionally with the increase of capsaicin concentration.

As a result, capsaicin increase the activity of Na^+-K^+ ATPase and Ca^{+2} ATPase, and decreased the level of lipid peroxidation in erythrocytes in high glucose concentration, as well as in normal glucose concentrations. It is concluded that the effect of capsaicin on these parameters have a special importance for diabetes mellitus, a disease known commonly all over the world, which is characterized by high blood glucose level.

Keywords: Ca^{+2} ATPase, Capsaicin, Lipid Peroxidation, Na^+-K^+ ATPase

Kapsaisinin Yüksek Glukoz Konsantrasyonlarına Maruz Bırakılan İnsan Eritrositlerinde (*in vitro*) Protein Glikozilasyonu, Na^+-K^+ ATPaz, Ca^{+2} ATPaz ve Lipid Peroksidasyonu Düzeylerine Etkisinin Araştırılması

ÖZ

Bu çalışmada, yüksek glukoz konsantrasyonuna maruz bırakılan insan eritrositlerinde *in vitro* ortamda kapsaisinin eritrosit membranı Na^+-K^+ ATPaz ve Ca^{+2} ATPaz aktiviteleri ile glikozillenmiş hemoglobin (HbA_{1c}) ve MDA düzeylerine etkisi araştırılmıştır. Sağlıklı bireylerden toplanan kan örneklerinden elde edilen eritrositler normal ve yüksek glukoz konsantrasyonlarına maruz bırakılarak farklı konsantrasyonlardaki kapsaisin ile bir saat süresince inkübe edilmiştir. Sadece normal glukoza maruz bırakılan eritrositler kontrol grubu olarak kullanılmıştır. Yüksek glukoza maruz bırakılan eritrositlerde Na^+-K^+ ATPaz ve Ca^{+2} ATPaz aktiviteleri normal glukoza maruz bırakılan kontrol grubuna göre istatistiksel olarak önemli düzeyde düşük bulunmuştur ($p<0.001$). Kapsaisin uygulanan gruplarda kapsaisin konsantrasyonlarına da bağlı olarak bu enzimlerin aktivitelerinin istatistiki olarak önemli düzeyde arttığı saptanmıştır. Yüksek konsantrasyondaki glukozun etkisi ile MDA ve HbA_{1c} değerlerinin normal konsantrasyonda glukoz uygulanan kontrol grubuna göre istatistiki olarak önemli düzeyde arttığı, kapsaisin etkisi ile kapsaisinin konsantrasyonlarına da bağlı olarak MDA ve HbA_{1c} değerlerinin azaldığı saptanmıştır. Sonuçta, kapsaisinin eritrositlerde normal glukoz konsantrasyonlarında olduğu gibi yüksek glukoz konsantrasyonlarında da Na^+-K^+ ATPaz ve Ca^{+2} ATPaz aktivitelerini artırıcı ve lipid peroksidasyonu ile protein glikozilasyonunu azaltıcı etkiye sahip olduğu görülmüş, bunun ise yüksek kan glukozu ile karakterize ve bütün dünyada yaygın bir hastalık olan diabetes mellitusta özel bir önem arz edebileceği düşünülmüştür.

Anahtar Kelimeler: Ca^{+2} ATPaz, Lipid Peroksidasyonu, Kapsaisin, Na^+-K^+ ATPaz

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INTRODUCTION

There are many plants in nature that are considerably valuable for the fields of medicine, chemical, and recently, taking advantage of these plants for the purpose of protection against diseases and finding cures have become more essential. This situation implies the necessity for studies on such plants. Diabetes Mellitus is an endocrinal and metabolic disease that arises due to absolute or relative insulin deficiency or insulin resistance and is characterized by impaired in carbohydrate, lipid and protein metabolism. During the course of this disorder, specific complications such as retinopathy, nephropathy and atherosclerosis may develop, and each year, thousands of people around the world die due to these diabetic complications. It has been reported that in patients with diabetes, there is an increase in production of free oxygen radicals and lipid peroxidation and the antioxidant defense system becomes insufficient. Increase in the production of free radicals contributes to the emergence and advancement of diabetic complications. In diabetes, reactive oxidants that cause oxidative damage are created due to the high level of glucose. There are some mechanisms that increase oxidative stress in diabetes patients such as non-enzymatic glycosylation, metabolic stress, sorbitol tract activity, levels of inflammatory mediators and localized tissue damage [1-3].

Na^+ ions have an important role that in transportation of glucose within the cells. The glucose is transported into the cell by means of Na^+, through glucose-Na^+ symport system. Na^+-K^+ ATPase is an enzyme that catalyzes the hydrolysis of ATP and it is responsible for the Na^+ and K^+ ions; together with Ca^{+2} ATPase which is responsible for Ca^{+2} ions, these two enzymes have the role of keeping the intracellular and extracellular concentrations of these ions within the physiological levels [4, 5].

Increase in extracellular glucose concentration beyond normal rates and consumption of glucose after cellular ingestion cause changes in the intracellular and extracellular concentrations of Na^+ ions directly and Ca^{+2} ions indirectly, and hence it can be implied that the Na^+-K^+ ATPase and Ca^{+2} ATPase enzyme activities can be affected by these changes. In previous studies, it has been indicated that impaired sodium transport and Na^+-K^+ ATPase activity in the erythrocyte membrane possibly have prominent roles in the pathophysiology of chronic complications of diabetes mellitus; and it has been reported that there are changes in the Na^+-K^+ ATPase and Ca^{+2} ATPase activities in accordance with increase in glycaemia after several studies conducted in vivo and *in vitro* conditions [6-9]. On the other hand, it has been reported that various plants can improve the glucose metabolisms in diabetic patients due to their hypoglycemic effects. One of the important plant is Capsaicin (*Capsicum annuum* L.) [10].

Capsaicin the active substance red chili peppers which are called *Capsicum annuum* L. in botany is an extremely hot, white, odorless substance which is easily soluble in hot water, ethyl alcohol, methyl alcohol and acetone. The amount of capsaicin in the red chili pepper is in the 0.12-17% mg range. Red chili pepper essentially contains capsaicin which gives its hot taste, and in addition, some vitamins, red carotenoids, fat, minerals and aromatic compounds [11].

Red chili pepper is a type of plant that is cultivated in abundance in the South East Anatolia region of Turkey, and is widely consumed in the city of Kahramanmaraş. It is often used in pharmaceutical industry, chemical industry as well as among people for various purposes [12, 13]. In a study conducted *in vitro* with rat jejunum, it has been reported that capsaicin inhibits the absorption of glucose depending on the dosage [14]. Another study indicates that capsaicin has the property of reducing the plasma glucose levels in response to oral glucose intake [15]. Previous studies show that capsaicin affects the human erythrocyte osmotic fragility and acetylcholine esterase activity, and has antioxidant effects [16]. Moreover, it has also been reported that capsaicin induces the body temperature, increases energy consumption and blood flow, prevents oxidative stress and decreases lipid peroxidation [17]. The conducted studies indicate that capsaicin also affects the cholesterol level in the serum [18], and that capsicum reduces the risk of developing atherosclerosis by way of reducing the blood serum cholesterol and triglyceride values [19].

MATERIAL and METHOD

This study has been conducted in order to investigate the erythrocyte membrane ATPases activities and lipid peroxidation as well as changes in the glycosylated hemoglobin (HbA_{1c}) levels for the human erythrocytes subject to *in vitro* exposure of high and normal glucose concentrations. The erythrocytes used in the study are obtained from the blood samples collected from healthy and voluntary subjects. During the study, a portion of the samples have been exposed to 45 mM glucose and the rest have been exposed to 6 mM glucose. These samples have been incubated afterwards in different concentrations of capsaicin. Glucose applied to the erythrocyte samples are grouped according to the capsaicin concentrations as stated below.

General Procedure

Group I: High concentration glucose medium (45 mM glucose)
Group II: 45 mM glucose + 100 μM Capsaicin
Group II.A: 45 mM glucose + 100 μM Capsaicin (24 h incubation)

Group II.B: 45 mM glucose + 100 μM Capsaicin (48 h incubation)
Group III: 45 mM glucose+ 1 μM Capsaicin
Group IV: 45 mM glucose + 0.1 μM Capsaicin
Group V: Normal concentration glucose medium (6 mM glucose) (Control Group)
Group VI: 6 mM glucose + 100 μM Capsaicin
Group VI.A: 6 mM glucose + 100 μM Capsaicin (24 h incubation)
Group VI.B: 6 mM glucose + 100 μM Capsaicin (48 h incubation)

Incubation of Erythrocytes in Glucose and Capsaicin

Heparinized blood samples have been isolated from the plasma by means of centrifugation (4500 x g). Erythrocytes have been brought to 10% hematocrit with the saline solution buffered with phosphate [4]. Then, same amounts (100 μL) of these erythrocytes have been added glucose solutions and slowly stirred so that the final concentration is 6 mM for the normal concentration glucose group and 45 mM for the high concentration group. 10 μL of antibiotics (sefazol) has been added to the incubation medium in order to prevent the reproduction of microorganisms [5]. Following this procedure, different concentrations of capsaicin have been added to the samples, and incubated for 1 hour, 24 hours and 48 hours at 37˚ C temperature. The 1 hour incubated samples are used in order to determine the enzyme activity, and 1, 24 and 48 hour incubated samples are used for the MDA and HbA_{1c} measurements.

Separating the Erythrocyte Membrane

The erythrocyte membrane is separated by using Moretti method [20]. The obtained erythrocyte membranes have been used within the same day in order to determine the Na^+-K^+ ATPase and Ca^{+2} ATPase activities.

Measurement of Na^+-K^+ ATPase Enzyme Activity

Na^+-K^+ ATPase enzyme activity is measured based on the Mazzanti method. 1 mL incubation medium is used in order to determine the enzyme activity. The experimental procedure is then repeated without the ouabain in the medium. The activity is calculated by subtracting the Pi value measured with the oubaine from the Pi value measured without the oubaine. The enzyme activity is given as the amount of inorganic phosphate produced from ATP by 1 mg protein in 1 hour (μmol Pi/mg protein hour) [21].

Measurement of Ca^{+2} ATPase Enzyme Activity

Ca^{+2} ATPase enzyme activity is measured based on the Flecha method. 1 mL incubation medium is used in order to determine the enzyme activity. The enzyme activity is given as the amount of inorganic phosphate produced from ATP by 1 mg protein in 1 hour (μmol Pi/mg protein hour) [22].

Determination of the Amount of Inorganic Phosphate

The amount of inorganic phosphate is measured by modifying the Ames method. This method is essentially based on the principle of reduction of molybdate complex by ascorbic acid [23].

Determination of Lipid Peroxidation

Determination of lipid peroxidation is based on the principle of spectrophotometric evaluation of the absorbance of the pink-red color resulting from reaction between the products (mainly MDA) and TBA [24; 25].
The net absorbance is calculated by subtracting the baseline absorbance from the absorbance of the sample. The MDA concentration is calculated in terms of nmol/mL by making use of the molar extinction coefficient of MDA-TBA complex in 532 nm ($1.56x10^5$ M^{-1} cm^{-1}) and taking dilution factor into consideration.

Determination of Total Protein

Copper-protein complex is created in alkali solution. This complex reduces the phosphomolybdate-phosphotunstate reactive and it gives a dark blue color. The intensity of this dark blue is directly proportional to the concentration of protein in the medium. One important aspect to watch out during the addition of folinic reactive is that this reactive is durable only in acidic medium. The described reduction process takes place in pH
10. Therefore the folinic reactive should be immediately added to the copper-protein solution and be immediately stirred strongly. Thus, the reduction process can take place before the decomposition of phosphomolybdate-phosphotunstate reactive [26]. Albumin is used as the standard solution.

Statistical Analysis

In order to carry out the statistical evaluation of the obtained data, arithmetic averages (X) and standard deviations (SD) have been calculated first. ANOVA (one way variance analysis) test is carried out in order to determine whether there is significant variance between the groups, and TUKEY HSD test which is a POST HOC test has been utilized in order to compare the groups in pairs between themselves. Limit of significance is taken as $p<0.05$. These statistical procedures have been carried out by use of SPSS package program.

RESULT and DISCUSSION

In this study, the effect of capsaicin on the levels of glycosylated hemoglobin (HbA_{1c}) and MDA, and activities of erythrocyte membrane Na^+-K^+ ATPase and Ca^{+2} ATPase in human erythrocytes which have been exposed to normal and high concentration of glucose; and the obtained results are statistically analyzed.

Erythrocyte Membrane Na^+-K^+ ATPase and Ca^{+2} ATPase Activities, MDA and HbA_{1c} Values

Erythrocyte membrane ATPases activities, MDA and HbA_{1c} values in human erythrocytes which have been exposed to normal and high concentration of glucose and then applied different concentrations of capsaicin are shown in Table 1.

Table 1. Erythrocyte membrane ATPases, erythrocyte MDA and HbA_{1c} values in mediums with normal and high glucose concentration (X±SD; Average±Standard Deviation)

CAPSAICIN	Na^+-K^+ ATPase (µmol Pi/mg protein. hour)	Ca^{+2} ATPase (µmol Pi/mg protein. hour)	MDA (nmol/gr hemoglobin)	HbA_{1c} (%)
45 mM Glucose (n = 5)	0.0406±0.0002	0.0681±0.0005	1.138±0.0228	7.2800±0.1923
45 mM Glucose + 100µM capsaicin (n = 5)	0.0543±0.0003	0.0784±0.0003	0.0824±0.0167	6.8200±0.0836
45 mM Glucose + 1 µM capsaicin (n = 5)	0.0501±0.0007	0.0740±0.0002	0.978±0.0334	7.2000±0.1581
45 mM Glucose + 0.1 µM capsaicin (n = 5)	0.0436±0.0003	0.0725±0.0003	1.010±0.1224	7.2200±0.1643
6 mM Glucose (n = 5)	0.0528±0.0002	0.0791±0.0002	0.8700±0.0441	5.7000±0.1581
6 mM Glucose + 100 µM capsaicin (n = 5)	0.0563±0.0004	0.0858±0.0006	0.752±0.0414	5.4800±0.1923

As seen in Table 1, for the human erythrocytes used in this study that are *in vitro* exposed to high concentrations of glucose, ATPases activities are found to be lower, and MDA and HbA_{1c} values are found to be higher than those of the control group (Group V) which are exposed to glucose at normal concentration. In groups where capsaicin is applied, it has been detected that these enzyme activities have increased and MDA and HbA_{1c} values have decreased depending on the capsaicin concentration. In the study, with the trials carried out for the capsaicin incubation of erythrocyte samples in mediums with normal or high glucose concentration, it has been determined that the ideal incubation duration is 1 hour, and hence the erythrocytes have been incubated with capsaicin for a duration of 1 hour. In the trials performed for 0-120 minutes and additional 24 hour duration, the maximum activity values have been obtained after 60 minutes from the start of incubation, and the values decreased near 120^{th} minute mark. In a similar study conducted on this topic, as a result of comparing the ATPases activities obtained from the erythrocyte samples that have been incubated for 0-120 minutes, it has also been reported that the enzyme activities has peaked at the 60 minute mark and decreased towards 120^{th} minute [16].

Erythrocyte Membrane Na^+-K^+ ATPase and Ca^{+2} ATPase Activities, MDA and HbA_{1c} Values under Capsaicin Application

Erythrocyte membrane ATPases activities, MDA and HbA_{1c} values in human erythrocytes which have been exposed to normal and high concentration of glucose and then applied different concentrations of capsaicin are shown in Table 2 and Table 3, Figure 1, Figure 2, Figure 3 and Figure 4 the statistical comparison of the obtained values are shown in Table 4

Table 2. Erythrocyte membrane ATPase activities values of groups on which capsaicin is applied (X ± SD; Average± Standard Deviation)

Groups	Na^+-K^+ ATPase (µmol Pi/mg protein. hour)	Ca^{+2} ATPase (µmol Pi/mg protein. hour)
Group I (45 mM Glucose)	0.0406±0.0002	0.0681±0.0005
Group II (45 mM Glucose + 100 µM capsaicin)	0.0543±0.0003	0.0784±0.0003
Group III (45 mM Glucose + 1 µM capsaicin)	0.0501±0.0007	0.0740±0.0002
Group IV (45 mM Glucose + 0.1 µM capsaicin)	0.0436±0.0003	0.0725±0.0003
Group V (6 mM Glucose)	0.0528±0.0002	0.0791±0.0002
Group VI (6 mM Glucose + 100 µM capsaicin)	0.0563±0.0004	0.0858±0.0006

In this study, for the human erythrocytes used that are *in vitro* exposed to high concentrations of glucose, ATPases activities are found to be lower at a statistically significant level ($p<0.001$) than those of the control group (Group V) which are exposed to glucose at normal concentration (Table 2 and Table 3). For the groups on which capsaicin has been applied, it has been detected that the activities of these enzymes have increased at a statistically significant level, depending on the capsaicin concentration. In the group that is exposed to 45 mM glucose, under the effect of 100 µM capsaicin, it has been observed that the ATPases activities have increased to the level that of the control group. In addition, for the erythrocytes on which 6 mM glucose have been applied under the effect of 100 µM capsaicin, an increase in both enzyme activities has been detected at a statistically significant level ($p<0.001$). In this study, it has been determined that capsaicin increases ATPases activities for erythrocytes in mediums with high and normal glucose concentrations. It is observed that the differences between groups are statistically significant ($p<0.001$) for both Na^+-K^+ ATPase and Ca^{+2} ATPase activities, except for the case of group II-V.

Table 3. Statistical results (p values) of erythrocyte membrane Na^+-K^+ ATPase and Ca^{+2} ATPase activities in groups on which capsaicin is applied

Compared Groups	p Values	
	Na^+-K^+ ATPase	**Ca^{+2} ATPase**
I-II	0.000	0.000
I-III	0.000	0.000
I-IV	0.000	0.000
I-V	0.000	0.000
I-VI	0.000	0.000
II-III	0.000	0.000
II-IV	0.000	0.000
II-V	0.648	0.112
II-VI	0.000	0.000
III-IV	0.000	0.000
III-V	0.000	0.000
III-VI	0.000	0.000
IV-V	0.000	0.000
IV-VI	0.000	0.000
V-VI	0.000	0.000

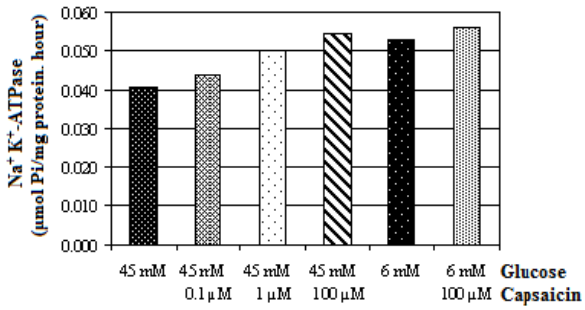

Figure 1. The graphical representation of the effect of capsaicin on Na^+-K^+ ATPase activity

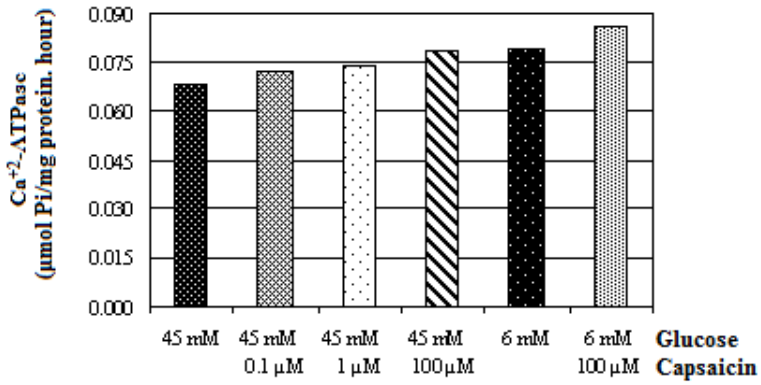

Figure 2. The graphical representation of the effect of capsaicin on Ca^{+2} ATPase activity

Table 4. Erythrocyte MDA and HbA_{1c}values in groups on which capsaicin is applied (X ± SD; Average ± Standard Deviation)

Groups	**MDA (nmol/gr hemoglobin)**	**HbA_{1c} (%)**
Group I (45 mM Glucose)	1.1380±0.0228	7.2800±0.1923
Group II (45 mM Glucose + 100 µM capsaicin)	0.8240±0.0167	6.8200±0.0836
Group II-A (45 mM Glucose + 100 µM capsaicin) (24 h incubation)	0.7600±0.0141	6.3600±0.3049
Group II-B (45 mM Glucose + 100 µM capsaicin) (48 h incubation)	0.7140±0.0296	6.1600±0.1140
Group III (45 mM Glucose + 1 µM capsaicin)	0.9780±0.0334	7.2000±0.1581
Group IV (45 mM Glucose + 0.1 µM capsaicin)	1.0100±0.1224	7.2200±0.1643
Group V (6 mM Glucose)	0.8700±0.0441	5.7000±0.1581
Group VI (6 mM Glucose + 100 µM capsaicin)	0.7520±0.0414	5.4800±0.1923
Group VI-A (6 mM Glucose + 100 µM capsaicin) (24 h incubation)	0.6020±0.0130	5.3600±0.1140
Group VI-B (6 mM Glucose + 100 µM capsaicin) (48 h incubation)	0.5380±0.0334	5.1600±0.1341

Table 5. Statistical results (p values) of erythrocyte MDA and HbA_{1c} in groups on which capsaicin is applied

Compared Groups	**p Values**	
	MDA	**HbA_{1c}**
I-II	0.000	0.005
I-II.A	0.000	0.005
I-II.B	0.000	0.000
I-III	0.000	0.999
I-IV	0.000	1.000
I-V	0.000	0.000
I-VI	0.000	0.000
I-VI.A	0.000	0.000
I-VI.B	0.000	0.000
II-II.A	0.030	0.000
II-II.B	0.000	0.000
II-III	0.000	0.034
II-IV	0.000	0.021
II-V	0.272	0.000
II-VI	0.009	0.000
II-VI.A	0.000	0.000
II-VI.B	0.000	0.000
II.A-II.B	0.000	0.706
II.A-III	0.000	0.000
II.A-IV	0.000	0.000
II.A-V	0.000	0.000
II.A-VI	1.000	0.000
II.A-VI.A	0.000	0.000
II.A-VI.B	0.000	0.000
II.B-III	0.000	0.000
II.B-IV	0.000	0.000
II.B-V	0.000	0.005
II.B-VI	0.532	0.000
II.B-VI.A	0.000	0.000
II.B-VI.B	0.000	0.000
III-IV	0.745	1.000
III-V	0.000	0.000
III-VI	0.000	0.000
III-VI.A	0.000	0.000
III-VI.B	0.000	0.000
IV-V	0.000	0.000
IV-VI	0.000	0.000
IV-VI.A	0.000	0.000
IV-VI.B	0.000	0.000
V-VI	0.000	0.587
V-VI.A	0.000	0.084
V-VI.B	0.000	0.004
VI-VI.A	0.000	0.981
VI-VI.B	0.000	0.126
VI.A-VI.B	0.030	0.706

It is observed that the differences between the groups on which capsaicin is applied, are statistically significant ($p<0.001$) for MDA levels, except for the cases of group II-V, group II.A-VI, group II.B-VI and group III-IV. Moreover, statistically significant differences between groups ($p<0.05$) in terms of HbA_{1c} values are also found between groups, except for the cases of group I-III, group II.A-II.B, group III-IV, group V-VI, group V-VI.A, group VI-VI.A, group VI-VI.B and group VI.A-VI.B.

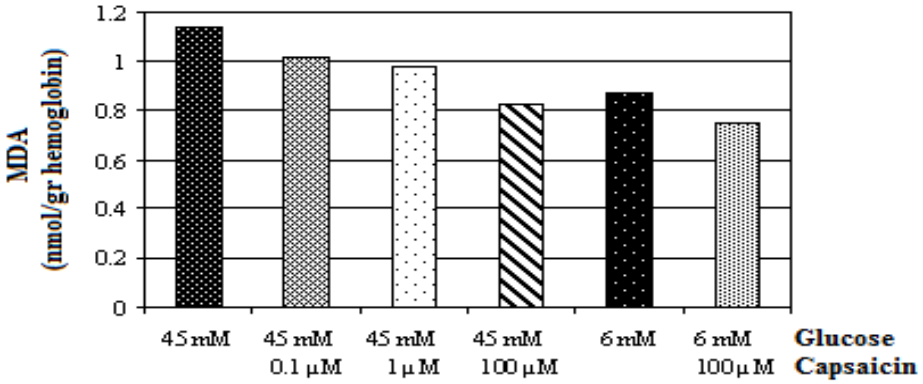

Figure3. Graphical representation of the effect of capsaicin on MDA level

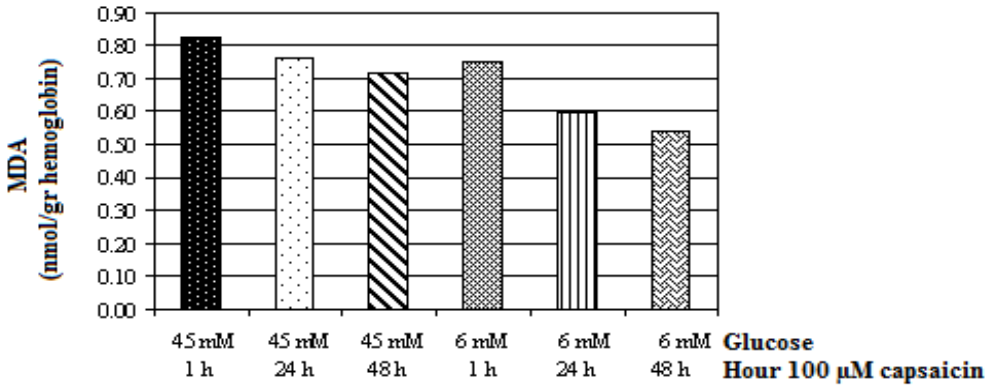

Figure4. The variation of the effect of capsaicin on MDA levels in erythrocyte samples over time (1 h, 24 h and 48 h)

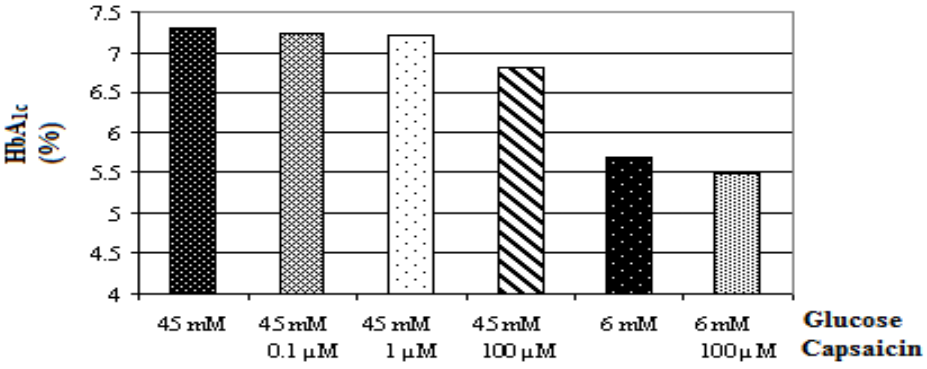

Figure5. Graphical representation of the effect of capsaicin on HbA_{1c} level

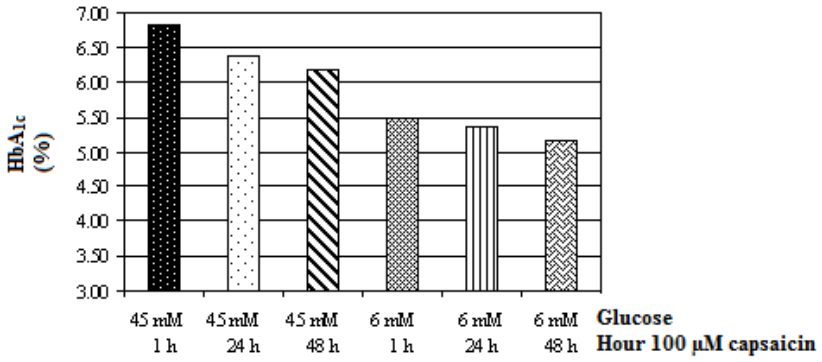

Figure 6. The variation of the effect of capsaicin on HbA_{1c} levels in erythrocyte samples over time (1 h, 24 h and 48 h)

In this study, under the effect of high concentrations of glucose, MDA and HbA_{1c} values are found to be increased at a statistically significant level than those of the control group (Group V) which is exposed to glucose at normal concentration, and for the groups on which capsaicin has been applied, it has been detected that these values have increased at a statistically significant level, depending on the capsaicin concentration (Table 4, Table 5). For the erythrocytes in normal and high concentration of glucose, in order to determine the long term effects of capsaicin on lipid peroxidation and glycosylation, MDA and HbA_{1c} levels are also measured for the next 24 hour and 48 hour durations following the incubation with capsaicin. Comparison of the MDA and HbA_{1c} values obtained from 1 hour (group II), 24 hour (group II-A) and 48 hour (group II-B) incubation of 100 µM capsaicin under high glucose concentration reveals that lipid peroxidation and hemoglobin glycosylation decrease as the capsaicin application duration increases. In addition, comparison of the MDA values obtained from 1 hour (group II), 24 hour (group II-A) and 48 hour (group II-B) incubation of 100 µM capsaicin under normal glucose concentration also indicates that lipid peroxidation and glycosylation decrease with increased capsaicin application duration.

Jain et al. have investigated the effects of lipoic acid which is known to have positive effects on the human body, on the ATPases activities in the glycosylated human erythrocytes. For this purpose, erythrocyte membranes prepared from the blood samples obtained from healthy individuals have been incubated in 0.2 mM lipoic acid under normal (6 mM) and high (45 mM) concentrations of glucose. As a result, it has been detected that lipoic acid increases the activities of both enzymes for membranes in high concentration of glucose, and there is no change in the level of activities of these enzymes for the membranes with normal concentrations of glucose. The same study also reports that under high glucose concentrations, lipoic acid prevents lipid peroxidation and protein glycosylation [4].

Similar results have also been obtained by Nandhini et al. for erythrocytes exposed to high concentration of glucose with taurine [27].

CONCLUSION and SUGGESTION

In this study, where the effects of capsaicin *in vitro* conditions on the erythrocyte membrane Na^+- K^+ ATPase, Ca^{+2} ATPase activities and glycosylated hemoglobin (HbA_{1c}) and MDA levels for the human erythrocytes which have been exposed to high and normal glucose concentrations are investigated, the following results are obtained:

1. For the human erythrocytes which have *in vitro* been exposed to high concentration (45 mM) of glucose, ATPases activities are found to be lower at statistically significant levels ($p<0.001$) than those of the control group which has been exposed to normal concentration (6 mM) of glucose.
2. It has been detected that for groups on which 0.1, 1 and 100 µM of capsaicin has been applied for 1 hour, the activities of these enzymes have increased at a statistically significant level, depending on the concentration of capsaicin.
3. For the group which has been exposed to 45 mM of glucose, it has been observed that ATPases activities have increased to the level of the control group, under the effect of 100 µM of capsaicin.
4. In the erythrocytes on which 6 mM of glucose has been applied, a statistically significant ($p<0.001$) increase in the activities of both enzymes has been detected under the effect of 100 µM of capsaicin.
5. It has been determined from this study that capsaicin increases erythrocyte membrane ATPases activities under high and normal concentrations of glucose.
6. In the study, it has been detected that under the effect of high concentration of glucose, MDA and HbA_{1c} values are higher at statistically significant levels than those of the control group on which normal concentration of glucose has been applied, and MDA and HbA_{1c} values decrease under the

effect of capsaicin, depending on the concentration of capsaicin.

7. In order to determine the long term effects of capsaicin on lipid peroxidation and glycosylation in erythrocytes in mediums with normal and high concentrations of glucose, MDA and HbA_{1c} values have also been monitored within the 24 hour and 48 hour durations following the incubation with capsaicin. Under normal and high concentrations of glucose, as a result of incubation with 100 µM capsaicin for 1, 24 and 48 hour periods, it has been detected that the obtained MDA and HbA_{1c} values decrease as the duration of incubation increases.

In conclusion, this study indicates that at the end of 1 hour period *in vitro* application, capsaicin increases the human erythrocyte membrane ATPases activities and decreases the levels of MDA and HbA_{1c}. It has also been detected that these effects of capsaicin are also valid for the erythrocytes exposed to high glucose concentration, and the effects increase depending on the concentration of capsaicin.

In the modern world where the living conditions keep getting harder, diabetes mellitus which needs constant monitoring and treatment affects patients with both acute and chronic complications and these complications cause physical as well as mental and social problems for the patients. Increase in life span due to modern treatment methods causes increase in frequency and variety of diabetes complications, and early diagnosis and treatment become more and more essential for a healthy living. In diabetes treatment, natural and herbal remedies draw attention in addition to diet, oral antidiabetics and insulin. Red chili pepper, which is grown in abundance in the regions near Kahramanmaraş, Gaziantep, Şanlıurfa provinces of Turkey, is a widely consumed plant all over the world, and the results obtained from the study indicates that it may have positive effects on treatment of diabetes.

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