Review (Derleme)

Venoactive Drug Therapy On Treatment Of Venous Ulcer

Venöz Ülser Tedavisinde Venoaktif İlaçlar

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Özet

Venöz ülserler anlamlı morbiditeye sebep olan önemli tıbbi sorunlardır. Primer venöz yetmezliğin ileri evrelerinde veya venöz yetmezliğe bağlı oluşan posttrombotik sendrom sonucunda gelişir. Tedavisi kompresyon, bacak elevasyonu, local yara bakımı, ilaç tedavisi ve altta yatan venöz patolojinin cerrahi veya girişimsel olarak düzeltilmesidir. Diğer tedavi yöntemleri eğer kompresyon tedavisi ile birlikte uygulanırsa başarılı olabilir. Biz burada venöz ülser tedavisinde venoaktif ilaçların etkinliğini bilimsel kanıtlar eşliğinde tartıştık.

Anahtar Kelimeler: Venöz ülser; venoaktif ilaçlar; venöz yetersizli

Abstract

Venous ulcers are important medical problem that caused significant morbidity. They develop as a result of advanced chronic venous insufficiency, caused most frequently by primary venous incompetence or the post thrombotic syndrome. Treatment of venous ulcers include compression therapy, leg elevation, local ulcer and skin care, drug treatment, and correction of the underlying venous pathology with surgical and endovenous interventions. Primary and sine qua non method to cure venous ulcer is compression treatment. Other treatment modalities can be successful if only they have performed with compression treatment. In here we discuss the scientific evidence on efficacy of venoactive drug therapy on treatment of venous ulcer.

Key Words: Venous ulcer; venoactive drug; venous insufficiency

INTRODUCTION

Venous ulcers are the most common leg ulcers, and are important medical problem that caused significant morbidity. They develop as a result of advanced chronic venous insufficiency, caused most frequently by primary venous incompetence or the post thrombotic syndrome. Up to 0.5% of the adult Western population has active ulcers, and 0.6% to 1.4% has healed venous ulcers. The overall incidence of venous ulcers in patients older than 45 years of age is estimated at 3.5 per thousand per year; the incidence of venous ulcers has remained unchanged over the last 20 years. (1-3). "Varicose ulcer", "gravitational ulcer", "stasis ulcer" and "hypostatic ulcer" have been used as synonyms for venous ulcer. It is not recommended that these terms be used as the commonly agreed upon term is "venous ulcer" (4). Some risk factors described for CVI development and consequently venous ulcer include age, sex, family history, race, body weight, occupation and number of pregnancies. The influence of each risk factor is controversial. The prevalence of venous ulcer increases progressively with age regarding gender, most studies show a predominance of women affected. The female: male ratio is variable, ranging from 1.5 : 1 to 10 : 1. (4)

Primary venous valvular insufficiency or venous hypertension caused by venous obstruction is the essential pathology for venous ulcer development. Underlying mechanics can be summarized as : Venous hypertension has significant consequences on the dermal microcirculation and has a negative impact on capillary system. Erythrocytes and macromolecules extravasate from the dermal capillary into the interstitium, where the red cells are broken down and the hemosiderin is engulfed by macrophages. The breakdown products of red blood cells are chemoattractive to leukocytes by activating the endothelial intracellular adhesion molecules. These leukocytes are responsible from releasing of proteolytic enzymes and free oxygen radicals which trigger inflammatory response then Cytokine and proteinase activation causes endothelium and tissue damage. (2, 4-9)

Treatment of venous ulcers include compression therapy, leg elevation, local ulcer and skin care, drug treatment, and correction of the underlying venous pathology with surgical and endovenous interventions. Primary and sine qua non method to cure venous ulcer is compression treatment. Other treatment modalities can be successful if only they have performed with compression treatment. Previously in venous ulcer therapy venoactive medications such as pentoxifylline, prostaglandin, acetylsalicylic acid and sulodexide had been used. (10-17). In this document, we discuss the scientific evidence on efficacy of venoactive drug therapy on treatment of venous ulcer.

Venoactive drugs are safe and reliable drugs and have been used for a long time in all stages of the chronic venous disease. The venoactive drugs can be defined as a group of naturally occurring or synthetic drugs that act on capillary permeability or venous tone (18). They are listed Table 1 (19). Venoactive drugs have been shown to increase venous tone, increase capillary resistance and reduce capillary filtration, improve lymphatic flow and protection against inflammation (3, 18-20). Thus they relieve subjective symptoms such as pain and cramp

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with decreasing limb odema. However despite widely use of these medications there is not a consensus about their efficiency. In Siena consensus paper; a grade A was assigned to calcium dobesilat, MPFF, and O-(beta-hydroxyethyl) rutosides (HR)-oxerutins, a grade B to escin and ruscus extracts and a grade C to the remaining VADS (21). Recently published Society for Vascular Surgery and the American Venous Forum guideline suggested that diosmin, hesperidin, rutoside, micronized purified flavonoid fraction or horse chestnut seed extract (escin) must be used in addition to compression therapy for chronic venous disease (Grade 2, Evidence level B) (22).

T*able 1. Classification of the main venoactive drugs (19)*

Group	Substance	Origin
Alpha-benzopyrones	Coumarin	Melilot (Melilotus officinalis)
		Woodruff (Asperula odorata)
Gamma-benzopyrones (flavonoids)	Diosmin	Citruss spp. (Sophora japonica)
	Micronized purified flavonoid fraction	Rutaceae aurantiae
	Rutin and rutosides	Sophora japonica
	O-(β-hydroxyethyl)-rutosides (troxerutin, HR)	Eucalyptus spp. Fagopyrum spp.
Saponins	Escin	Horse chestnut seed extracts
	Ruscus extract	Butcher's broom (Ruscus aculeatus)
Other plant extracts	Anthocyans	Bilberry (Vaccinium myrtillus)
	Proanthocyanidins (oligomers)	Red wine leaves extracts, Maritime pine
	Extracts of Ginkgo, heptaminol and troxerutin	Ginkgo biloba
	Total triterpene fraction	Centella asiatica
Synthetic products	Calcium dobesilate	Synthetic
	Benzaron	Synthetic
	Naftazon	Synthetic

Nonetheless effectiveness of these drugs in treatment of venous ulcer still remains controversy. The first study about venoactive drugs in treatment of venous ulcer was reported in 1991 (23). 138 patients with healed venous ulcer were randomized to okserutin and placebo combined with compression sock. Okserutin had no superiority to placebo in terms of venous ulcer recurrence at the end of 3 months therapy. However a guideline which is published in 1995 reported that drug therapy is not beneficial in venous ulcer treatment (24), in 1997 Guilhou et al. reported that 2 months long Diosmin-Hesperidin therapy combined with compression sock decreased ulcer size significantly compared to placebo group in 107 patients who had venous ulcer (25). In a study that is published in 2001, 140 patients treated for 24 weeks by Diosmin-Hesperidin was of significantly well venous ulcer healing (26). Another subsequent study showed that Diosmin-Hesperidin therapy for 6 months accelerates venous ulcer healing (27). Coleridge-Smith et al. reported a meta-analyze including two more study in addition to studies mentioned above in 2005 (28). They have concluded in this meta-analyze that Diosmin-Hesperidin therapy accelerates venous ulcer healing and suggested that Diosmin-Hesperidin might be a useful adjunct to conventional therapy in large and long standing ulcers. In a guideline published in 2006 also reported that oral treatment with micronized purified flavonoid fraction (MPFF) may be a useful adjunct to conventional compression therapy in the treatment of leg ulcers-LEVEL I. (29)

Meanwhile, in a non-randomized study with 25 patients in 2003 with calcium dobesilate, subjective complaints, leg diameters, and ulcer sizes of the patients decreased (30). A study with horse chestnut seed extract used for 12 weeks in 2006 reported that no significant affect on venous ulcer healing (31).

In conclusion, Diosmin-Hesperidin combination is the only venotrop that effectiveness is shown in large randomized studies and meta-analyses. Therefore in light of these scientific investigations, American Venous Forum Guideline published in 2009 recommends only Diosmin-Hesperidin among venotrop agents in treatment of venous ulcer as Level of Evidence: A (32). However associate guideline of Society for Vascular Surgery and American Venous Forum published in 2011 reported that micronized purified flavonoid fraction in venous ulcer treatment as Grade 2 – Level of Evidence: B (22), large randomized studies with the other agents could change this knowledge.

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Original Article (Orijinal Araştırma)

In vitro Investigation of the Effect of Cinnamon on Protein Glycosylation, Na+-K+ ATPase, Ca++ ATPase and Lipid Peroxidation in Human Erythrocytes Exposed to High Glucose Concentration

Tarçının (Cinnamon) Yüksek Glukoz Konsantrasyonlarına Maruz Bırakılan İnsan Eritrositlerinde (in vitro) Protein Glikozilasyonu, Na+-K+ ATPaz, Ca++ ATPaz ve Lipid Peroksidasyonu Düzeylerine Etkisinin Araştırılması

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Özet

Amaç: Bu çalışmada, yüksek glukoz konsantrasyonuna maruz bırakılan insan eritrositlerinde tarçının protein glikozilasyonu, Na+-K+ ATPaz ve Ca++ ATPaz aktiviteleri ve lipid peroksidasyonu seviyelerine etkisi in vitro olarak araştırılmıştır.

Gereç ve Yöntem: Bu amaçla, sağlıklı bireylerden alınan kan örneklerini ya normal glikoz (kontrol grubu) ya da yüksek glikoz konsantrasyonlarına maruz bırakılmış ve daha sonra farklı konsantrasyonlarda tarçın ile inkübe edilmiştir.

Bulgular: Yüksek glukoz konsantrasyonuna maruz bırakılan eritrosit örneklerinde Na+-K+ ATPaz ve Ca++ ATPaz aktiviteleri kontrol grubuna göre daha düşük bulundu ve bu iki grup arasındaki fark istatistiksel olarak anlamlıydı ($p < 0.001$). Tarçına maruz bırakılan gruplarda tarçın konsantrasyonlarının artması nedeniyle bu iki zar enziminin aktiviteleri istatistiksel olarak önemli bir seviyede artmıştır. Ayrıca MDA ve HbA1c düzeylerinin yüksek glikoz grubunda normal glikoz grubuna göre daha fazla arttığı ve tarçının etkisi altında, tarçın konsantrasyonuna bağlı olarak azaldığı tespit edildi. Sonuç olarak, tarçın Na+-K+ ATPaz ve Ca++ ATPaz aktivitelerini arttırdı ve yüksek glikoz konsantrasyonuna maruz bırakılan eritrositlerdeki lipid peroksidasyonu seviyesini düşürdü, normal glikoz konsantrasyonlarında bunu yaptı.

Sonuç: Tarçının bu parametreler üzerindeki etkilerinin, yüksek kan şekeri seviyesi ile karakterize edilen ve dünyada yaygın olarak bilinen bir hastalık olan diabetes mellitus üzerinde özel bir öneme sahip olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Anahtar kelimeler: Tarçın, Na+-K+ ATPaz, Ca++ ATPaz, Lipid Peroksidasyonu, Eritrosit Membranı, Protein Glikozilasyonu.

Abstract

Objective: In this study, the effect of cinnamon on protein glycosylation, Na+-K+ ATPase and Ca++ ATPase activities and lipid peroxidation levels in human erythrocytes exposed to high glucose concentration was investigated in vitro.

Materials and methods: For this purpose, the blood samples obtained from healthy individuals were exposed to either normal glucose (control group) or high glucose concentrations and then incubated with cinnamon at different concentrations.

Results: In samples of erythrocyte, which have been exposed to high glucose concentration, Na+-K+ ATPase and Ca++ ATPase activities were found to be lower than those of control group and the differences between these two groups were statistically significant ($p<0.001$). In the groups exposed to cinnamon, the activities of these two membrane enzymes increased at a statistically significant level due to the increase of cinnamon concentrations. It was also detected that MDA and HbA1c levels increased more in high glucose group than normal glucose group, and they also decreased under the effect of cinnamon, directly depending on the concentration of applied cinnamon. As a result, cinnamon increased the activities of Na+-K+ ATPase and Ca++ ATPase and decreased the level of lipid peroxidation in erythrocytes subjected to high glucose concentration, did so in normal glucose concentrations.

Conclusion: It is concluded that the effects of cinnamon on these parameters have a special importance on diabetes mellitus, a disease known commonly all over the world, which is characterized by high blood glucose level.

Key Words: Key Words: Cinnamon, Na+-K+ ATPase, Ca++ ATPase, Lipid Peroxidation, Erythrocyte Membrane, Protein Glycosylation.

INTRODUCTION

There are many plants in the nature that are considerably valuable for the fields of medicine and chemistry. In recent years, taking advantage of these plants for the purpose of protection from diseases and finding cures have become more essential. This situation implies the necessity for conscientious studies on such plants. Diabetes Mellitus (DM) is an endocrinal and metabolic disease that arises from absolute or relative insulin deficiency or insulin resistance, and is characterized by alterations in carbohydrate, lipid and protein metabolisms (1). 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 (2). It has been reported

This study was published as a Master Degree Thesis in KSU in 2006 and as a poster in 2nd International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signalling and TRP Channels.

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that in patients with diabetes, there is an increase in the production of free oxygen radicals and lipid peroxidation by-products, and the antioxidant defense system becomes insufficient (3). Increases in the production of free radicals contribute to the emergence and advancement of diabetic complications (4). In diabetes, reactive oxidants that cause oxidative damage are created due to the high level of glucose (5). Non-enzymatic glycosylation, metabolic stress caused by the changes in the energy metabolism, sorbitol tract activity, levels of inflammatory mediators and localized tissue damage caused by changes in the antioxidant defense system are the mechanisms that increase oxidative stress in diabetic patients (4).

The role of Na^+ ions in the transportation of glucose within the cells is well documented and glucose uptake is mediated by glucose-Na^+ symport system (6). In connection with the latter, Na^+-K^+ ATPase is an enzyme that is responsible for the efflux and influx of Na^+ and K^+ ions, respectively (6). In addition, Ca^{+2} ATPase, which is responsible for the efflux of Ca^{+2} ions, together with Na^+-K^+ ATPase plays a central role in keeping the intracellular and extracellular concentrations of these ions within the physiological levels (7). Hyperglycaemia and consumption of glucose within the cell cause changes in intracellular and extracellular concentrations of Na^+ ions directly and Ca^{++} ions indirectly (7), and hence it may be implied that the Na^+-K^+ ATPase and Ca^{++} ATPase enzyme activities could 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 (8-11). Furthermore, it has been reported in several studies conducted *in vivo* and *in vitro* conditions that there are changes in the Na^+-K^+ ATPase and Ca^{++} ATPase activities in accordance with the increase in glycaemia (9, 10). Moreover, it has been reported that various plants, including Cinnamon, may improve the glucose metabolisms in diabetic patients due to their hypoglycemic effects (12).-

Cinnamon is a fragrant evergreen tree belonging in the lauraceae family which is native to the South and South East Asia (13). Its leaves are leathery and have usually three veins (13). It has a strong and special scent, and a sweet and hot taste (13). Its bark has been used as spice and medicine for thousands of years (13). Especially in the Far East and Indian cultures, it has been used for the treatment of indigestion, loss of appetite, bloating, and stomach aches (14). After so many years of traditional usage, there have been various scientific studies on animals in order to utilize this plant in modern therapy methods (14). Cinnamon is obtained from the bark of *Cinnamomi cassia* tree (15). It contains cinnamic aldehyde, cinnamic acid, tannin and methyl hydroxyl chalcone polymers (MHCP) (15). There are two main species found in nature: *Cinnamomum Zeylanicum N.* and *Cinnamomum Verum* (16). Additionally, there is 1-4% aromatic volatile oil in the composition of cinnamon (17). This oil contains ethyl cinnamate, eugenol, cinnamaldehyde, beta-caryophyllene, linalool, and methyl chavicol (17). It is a plant which is frequently mentioned in studies on diabetes with its hypoglycemic effects; it has been reported that the cinnamon extracts increase the *in vitro* ingestion of glucose and glycogen synthesis, and it has positive effect on the glucose metabolism by increasing the phosphorylation of insulin receptors (12). Moreover, it is also reported that cinnamon has antioxidant effects (18). It has been shown that cinnamon decreases the glucose level in blood and this effect is related to the methyl hydroxyl chalcone polymer (MHCP) that is found in cinnamon. It has been indicated that this substance shows effects similar to that of insulin. MHCP is an important biological compound which has anti-bacterial, anti-fungal and fever reducing properties, as well as having the effect of reducing the cholesterol in the blood (12, 19). Especially in United States and Germany, cinnamon is used as the active ingredient in anti-flatulent herbal drugs. It also has a role in the treatment of cold and inflammatory diseases (20). A significant benefit of phytochemicals, vitamins, minerals, and fruits and vegetables in foods is thought to be capable of scavenging free radicals, lowering the incidence of chronic diseases (2).

The aims of this study was determined the effect of cinnamon on protein glycosylation, Na^+-K^+ ATPase and Ca^{++} ATPase activities and lipid peroxidation levels in human erythrocytes exposed to high glucose concentration.

MATERIAL AND METHODS

Sampling and Study groups

Erythrocytes used in the study were obtained from blood samples collected from healthy and voluntary subjects. During the study, a portion of the samples was exposed to 45 mM glucose and the rest was exposed to 6 mM glucose. Afterwards, these samples were incubated in different concentrations of cinnamon. Glucose applied to the erythrocyte samples were grouped according to the cinnamon concentrations as stated below. Study groups are shown in Table I.

Incubation of Erythrocytes in Glucose and Cinnamon

Heparinized blood samples were isolated from plasma by means of centrifugation (4500 x g). Erythrocytes were brought to 10% hematocrit with the saline solution buffered with phosphate (6). Then, same amounts (100 µL) of these erythrocytes were added glucose solutions and slowly stirred so that the final concentration is 6mM for the normal concentration glucose group and 45 mM for the high concentration group. 10 µL of antibiotics (sefazol) were added to the incubation medium in order to prevent the reproduction of microorganisms (7). Following this procedure, different concentrations of cinnamon were added to the samples, and incubated for 1 hour, 24 hours and 48 hours at 37° C temperature. The 1 hour incubated samples were used in order to determine the enzyme activity, and 1, 24 and 48 hour incubated samples were used for the MDA and HbA_{1c} measurements.

Separating the Erythrocyte Membrane

The erythrocyte membrane was separated by

Table I. Study groups of the present study

Erythrocytes exposed to 45 mM glucose (Experimental groups)	Erythrocytes exposed to 6 mM glucose (Control groups)
Group IA: High concentration glucose medium (45 mM glucose)	Group IB: Normal concentration glucose medium (6 mM glucose)
Group IIA: 45 mM glucose + 50 µM Cinnamon	Group IIB: 6 mM glucose + 50 µM Cinnamon
Group IIIA: 45 mM glucose + 100 µM Cinnamon	Group IIIB: 6 mM glucose + 100 µM Cinnamon
Group IVA: 45 mM glucose + 100 µM Cinnamon (24 h incubation)	Group IVB: 6 mM glucose + 100 µM Cinnamon (24 h incubation)
Group VA: 45 mM glucose + 100 µM Cinnamon (48 h incubation)	Group VB: 6 mM glucose + 100 µM Cinnamon (48 h incubation)
Group VIA: 45 mM glucose + 200 µM Cinnamon	Group VIB: 6 mM glucose + 200 µM Cinnamon

using Moretti method (21). The obtained erythrocyte membranes were used within the same day in order to determine the Na^+-K^+ ATPase and Ca^{++} ATPase activities.

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

Na^+-K^+ ATPase enzyme activity was measured based on the Mazzanti method. 1 mL incubation medium was used in order to determine the enzyme activity. The experimental procedure was then repeated without the ouabain in the medium. The activity was 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) (22).

Measurement of Ca^{++} ATPase Enzyme Activity

Ca^{++} ATPase enzyme activity was measured based on the Flecha method. 1 mL incubation medium was 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) (23).

Determination of the Amount of Inorganic Phosphate

The amount of inorganic phosphate was measured by modifying the Ames method. This method was essentially based on the principle of reduction of molybdate complex by ascorbic acid (24).

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 (25, 26). 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 (27). Albumin is used as the standard solution.

Statistical Analysis

All values are expressed as mean ± standard deviation (standard error of mean (S.E.M.) is better!). One way analysis of variance (ANOVA) was carried out in order to determine whether there had been significant difference between the groups, and TUKEY HSD test, which is a POST HOC test, was utilized in order to compare the groups in pairs between themselves. Limit of significance is taken as $p<0.05$. These statistical procedures were carried out by the use of SPSS package program (version 13.0).

RESULTS

In this study, the effect of cinnamon on the levels of glycosylated hemoglobin (HbA_{1c}) and MDA, and activities of erythrocyte membrane Na^+-K^+ ATPase and Ca^{++} 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^{++} 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 cinnamon are shown in Table II.

As shown in Table II, in this study, in the human

erythrocytes that are *in vitro* exposed to 1 hour of high concentration of glucose, the ATPases activities are

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

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

CINNAMON	Na^+ K^+ ATPase (µmol Pi/mg protein. hour)	Ca^{++} ATPase (µmol Pi/mg protein. hour)	MDA (nmol/ gr hemoglobin)	HbA_{1c} (%)
45 mM Glucose (n = 5)	0.0408±0.0003	0.0679±0.0003	1.1836±0.0211	7.4200±0.1303
45 mM Glucose + 50 µM cinnamon (n = 5)	0.0422±0.0002	0.0734±0.0002	0.9836±0.0198	7.0400±0.1140
45 mM Glucose + 100 µM cinnamon (n = 5)	0.0439±0.0003	0.0748±0.0002	0.9096±0.0176	6.7400±0.1516
45 mM Glucose + 200 µM cinnamon (n = 5)	0.0470±0.0002	0.0784±0.0001	0.7666±0.0237	6.4400±0.2073
6 mM Glucose (n =5)	0.0524±0.0003	0.0788±0.0003	0.8830±0.0209	5.7000±0.1581
6 mM Glucose + 100 µM cinnamon (n = 5)	0.0575±0.0002	0.0809±0.0005	0.8068±0.0167	5.5600±0.1816

Table III. Erythrocyte membrane ATPase activities of groups on which cinnamon is applied (X ± SD; Mean± Standard Deviation)

Groups	Na^+-K^+ATPase (µmol Pi/mg protein. hour)	Ca^{++}ATPase (µmol Pi/mg protein. hour)
Group I (45 mM Glucose)	0.0408±0.0003	0.0679±0.0003
Group II (45 mM Glucose + 50 µM cinnamon)	0.0422±0.0002	0.0734±0.0002
Group III (45 mM Glucose + 100 µM cinnamon)	0.0439±0.0003	0.0748±0.0002
Group IV (45 mM Glucose + 200 µM cinnamon)	0.0470±0.0002	0.0784±0.0001
Group V (6 mM Glucose)	0.0524±0.0003	0.0788±0.0003
Group VI (6 mM Glucose + 100 µM cinnamon)	0.0575±0.0002	0.0809±0.0005

lower than that of the control group (Group V) which is exposed to normal concentration of glucose; and the MDA and HbA_{1c} values are found to be higher than the control group. In groups where cinnamon has been applied, it has been detected that there is an increase in the activity of these enzymes and decrease in the levels of MDA and HbA_{1c}, depending on the concentration of the applied cinnamon. In the study, it has been determined from the trials that the most ideal cinnamon incubation duration for the erythrocyte samples in normal and high concentration glucose medium is 1 hour; and hence the erythrocytes have been incubated with cinnamon for 1 hour.

Cinnamon 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 cinnamon are shown in Table IV and Table V, Figure I, Figure II, Figure III and IV and the statistical comparison of the obtained values are shown in Table IV.

In this study, in human erythrocytes, the Na^+-K^+ ATPase and Ca^{++} ATPase activity values are found that are exposed *in vitro* to high glucose for 1 hour, to be lower than those of the control group (Group V) with a

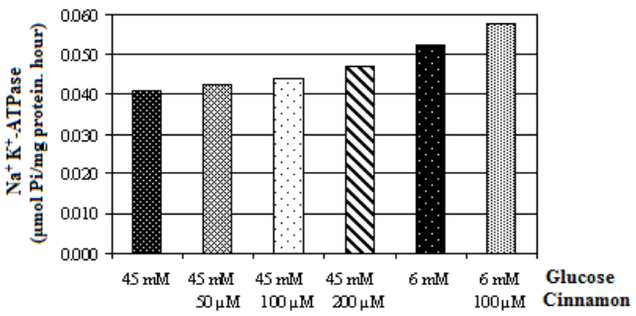

Figure I. The graphical representation of the effect of cinnamon on Na^{+}-K^{+} ATPase activity

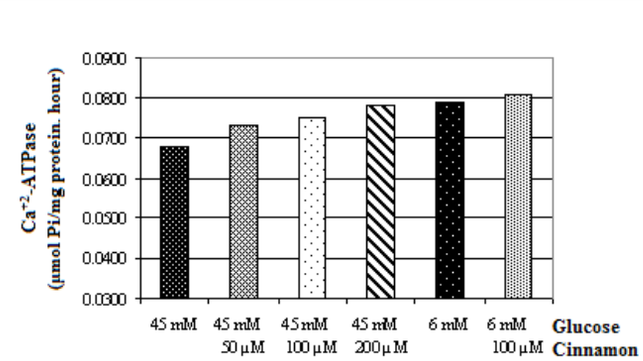

Figure II. The graphical representation of the effect of cinnamon on Ca^{++} ATPase activity

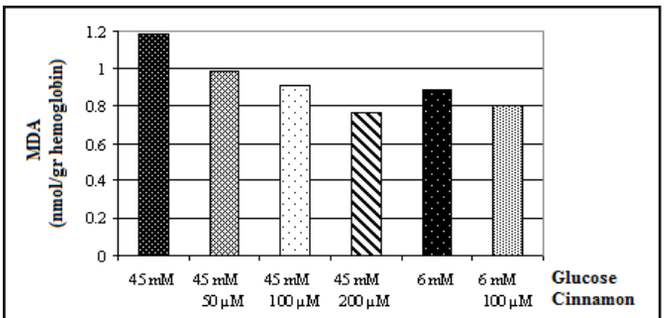

Figure III. Graphical representation of the effect of cinnamon on MDA level

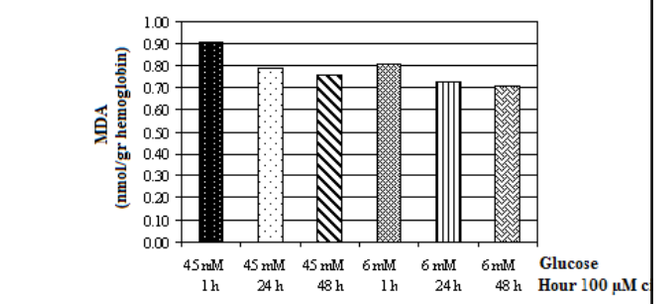

Figure IV. The variation of the effect of cinnamon on MDA levels in erythrocyte samples over time (1 h, 24 h and 48 h)

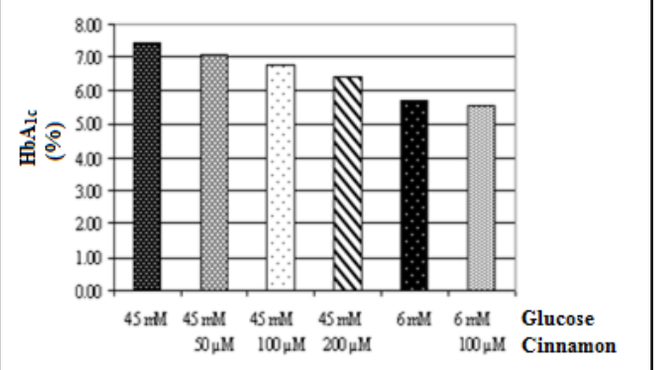

Figure V. Graphical representation of the effect of cinnamon on HbA_{1c} level

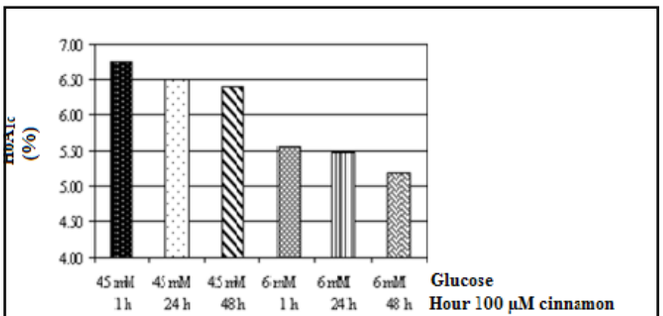

Figure VI. The variation of the effect of cinnamon on HbA_{1c} levels in erythrocyte samples over time (1 h, 24 h and 48 h)

statistical significance ($p<0.001$) (Table III). In groups which cinnamon has been applied, it has been observed that the levels of activities of these enzymes are higher with statistical significance. In the group that is exposed to 45mM glucose, the Ca^{++} ATPase activity levels are observed to rise to the levels of the control group under the application of 200 µM cinnamon. In addition, a statistically significant ($p<0.001$) increase has been detected in both enzyme activities in the 6 mM glucose medium under the effect of 100 µM cinnamon. The study indicates that in high and normal glucose concentrations, the cinnamon has the effect of increasing the erythrocyte membrane ATPases activities. It has been noted that there is statistically significant ($p<0.001$) variance among groups except for groups IV-V for Na^{+}-K^{+} ATPase and Ca^{++} ATPase.

In this work, it has been detected that there is a statistically significant increase in MDA and HbA_{1c} values due to the effect of the high concentration glucose compared to the control group (Group V) on which the normal concentration of glucose is applied; and with the effect of cinnamon, MDA and HbA_{1c} values have been lowered depending on the concentration of the applied cinnamon (Table IV, V). In order to determine the effect of cinnamon on the lipid peroxidation and glycosylation in the erythrocytes in normal and high concentration glucose mediums, MDA and HbA_{1c} levels have been measured during the 24 and 48 hours following the cinnamon incubation. Comparison of MDA and HbA_{1c}

Table IV. Erythrocyte membrane MDA values in groups on which cinnamon is applied (X ± SD; Mean ± Standard Deviation)

Groups	MDA (nmol/gr hemoglobin)	Compared Groups	*p
Group I (45 mM Glucose)	1.1836±0.0211	p<0.001	
Group II (45 mM Glucose + 50 µM cinnamon)	0.9836±0.0198	p<0.001	
Group III (45 mM Glucose + 100 µM cinnamon)	0.9096±0.0176	III-V	0.526
Group III-A (45 mM Glucose + 100 µM cinnamon) (24 h incubation)	0.7860±0.0114	III.A-III.B	0.578
		III.A-IV	0.865
		III.A-VI	0.812
		III.A-VI.A	0.002
Group III-B (45 mM Glucose + 100 µM cinnamon) (48 h incubation)	0.7604±0.0312	III.B-IV	1.000
		III.B-VI	0.020
		III.B-VI.A	0.298
		III.B-VI.B	0.003
Group IV (45 mM Glucose + 200 µM cinnamon)	0.7666±0.0237	IV-VI	0.071
		IV-VI.A	0.113
		IV-VI.B	0.001
Group V (6 mM Glucose)	0.8830±0.0209	p<0.001	
Group VI (6 mM Glucose +100 µM cinnamon)	0.8068±0.0167	p<0.001	
Group VI-A (6 mM Glucose + 100 µM cinnamon) (24 h incubation)	0.7290±0.0110	VI.A-VI.B	0.651
Group VI-B (6 mM Glucose + 100 µM cinnamon) (48 h incubation)	0.7048±0.01658	p<0.001	

values obtained from 1 hour (group III), 24 hours (group III-A) and 48 hours (group III-B) incubations of 100 µM cinnamon under high glucose concentration, indicate that lipid peroxidation and hemoglobin glycosylation have been reduced with the duration of application of cinnamon. Similarly, comparison of MDA and HbA_{1c} values obtained from 1 hour (group III), 24 hours (group III-A) and 48 hours (group III-B) incubations of 100 µM cinnamon under normal glucose concentration, indicate that lipid peroxidation and hemoglobin glycosylation have been reduced with the duration of application of cinnamon. In the following tables, each group was compared with the other groups and their subgroups.

It has been detected that in terms of MDA values, the variance among groups on which cinnamon is applied are statistically significant (p<0.001) except for group III-V, group III.A-III.B, group III.A-IV, group III.A-VI, group III.B-IV, group III.B-VI.A, group IV-VI, group IV-VI.A and group VI.A-VI.B. Moreover, in terms of HbA_{1c} values, it is also statistically significant (p<0.05) except for group II-III, group III-III.A, group III-III.B, group III-IV, group III.A-III.B, group III.A-IV, group

Table V. Erythrocyte membrane HbA_{1c} values in groups on which cinnamon is applied (X ± SD; Mean ± Standard Deviation)

Groups	HbA_{1c} (%)	Compared Groups	*p
Group I (45 mM Glucose)	7.4200±0.1303	I-II	0.021
Group II (45 mM Glucose + 50 µM cinnamon)	7.0400±0.1140	II-III	0.137
Group III (45 mM Glucose + 100 µM cinnamon)	6.7400±0.1516	III-III.A	0.395
		III-III.B	0.057
		III-IV	0.137
Group III-A (45 mM Glucose + 100 µM cinnamon) (24 h incubation)	6.5000±0.1581	III.A-III.B	0.992
		III.A-IV	1.000
Group III-B (45 mM Glucose + 100 µM cinnamon) (48 h incubation)	6.4000±0.0707	III.B-IV	1.000
Group IV (45 mM Glucose + 200 µM cinnamon)	6.4400±0.2073	p<0.05	
Group V (6 mM Glucose)	5.7000±0.1581	V-VI	0.933
		V-VI.A	0.517
		V-VI.B	0.001
Group VI (6 mM Glucose +100 µM cinnamon)	5.5600±0.1816	VI-VI.A	0.999
		VI-VI.B	0.035
Group VI-A (6 mM Glucose + 100 µM cinnamon) (24 h incubation)	5.4800±0.2387	VI.A-VI.B	0.203
Group VI-B (6 mM Glucose + 100 µM cinnamon) (48 h incubation)	5.2000±0.1581	p<0.05	

III.B-IV.B, group V-VI, group V-VI.A, group VI-VI.A and group VI.A-VI.B.

DISCUSSION

Cinnamon is recently a popular plant in diabetes which is known for its hypoglycemic effects, and it has been reported that the cinnamon extracts increase *in vitro* glucose inhibition and glycogen synthesis, and have positive effects on the glucose metabolism by increasing the phosphorilization of insulin receptors (12). It has also been stated that cinnamon has antioxidant effects (18).

Some researchers indicate that cinnamon may have antidiabetic effect by way of reducing the glucose level in the blood (15, 28). This study shows that cinnamon has beneficial effects in high glucose mediums.

Jain et al. have investigated the effects of lipoic acid which beneficial to the human body, on the ATPases activities in glycosylated human erythrocytes. For this purpose, erythrocyte membranes prepared from the blood samples of healthy individuals have been incubated with 0.2 mM lipoic acid under normal (6 mM) and high (45 mM) concentrations of glucose. As a result, it has been observed that the lipoic acid has increased the activity of both enzymes in high glucose membranes, and has no effect on enzyme in normal glucose membranes. In the same work, it has also been stated that under high glucose concentrations, lipoic acid prevents lipid peroxidation and protein glycosylation (6).

Similar finding have also been obtained by

Nandhini et al. in erythrocytes subjected to high concentrations of glucose with taurine (29).

CONCLUSIONS AND SUGGESTIONS

The following conclusions are drawn from this work where effects of cinnamon on the erythrocyte membrane Na^+-K^+ ATPase, Ca^{++} ATPase activities and glycosylated hemoglobin (HbA_{1c}) and MDA levels in the human erythrocytes that are subjected to *in vitro* high and normal glucose concentrations:

1. The levels of activities of Na^+-K^+ ATPase and Ca^{++} ATPase have been found to be lower in the human erythrocytes that are subjected to *in vitro* high glucose concentrations (45mM) than those of control group which is subjected to normal glucose concentration (6mM) at a statistically significant level ($p<0.001$).

2. It has been detected that for groups which have been exposed to 50, 100 and 200 µM of cinnamon for 1 hour, the activities of these enzymes have shown a statistically significant increase depending on the concentration of the cinnamon used.

3. In the group that is exposed to 45 mM glucose, it has been observed that under the effect of 200 µM cinnamon, ATPases activities have increased to the level of the control group.

4. In the presence of 6 mM of glucose, a statistically significant ($p<0.001$) increase in the activities of both enzymes have been detected under the effect of 100 µM cinnamon.

5. The study indicates cinnamon increases erythrocyte membrane ATPases activities under normal and high glucose concentrations.

6. The study shows that the due to the high glucose concentration, MDA and HbA_{1c} values have significantly increased compared to the control group on which normal concentration glucose is applied, and in addition, these values decreased under the effect of cinnamon, depending on the concentration of the applied cinnamon.

7. In order to determine the long term effect of cinnamon on lipid peroxidation and glycosylation in erythrocytes in normal and high glucose mediums, the MDA and HbA_{1c} levels have been measured in 24 and 48 hours following the cinnamon incubation. It has been detected that for high and normal glucose concentrations, as a result of 1 h, 24 h and 48h 100 µM cinnamon incubations, the MDA and HbA_{1c} values have decreased as the duration of incubation increases.

In conclusion, this study indicates that at the end of a 1 hour in vitro duration, cinnamon increases the Na^+-K^+ ATPase and Ca^{++} ATPase activity in human erythrocyte membrane and reduces the MDA and HbA_{1c} levels. It has also been detected that these effects of cinnamon are also valid for erythrocytes that are exposed to high concentration of glucose, and these effects increase depending on the concentration of the applied cinnamon.

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 the frequency and variety of diabetes complications and early diagnosis and treatment becomes 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.

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