Articles

Ultrastructural evaluation of the effects of cinnamon on the *nervus ischiadicus* in diabetic rats

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ABSTRACT

الأهداف: فحص آثار تناول القرفة عبر الفم على العصب الوركي عند مستوى الإلكترون لدى الجرذان.

الطريقة: أجريت هذه الدراسة في الفترة ما بين عام 2004م و عام 2006م – كلية الطب –جامعة دايكل – مدينة دياربكر – تركيا. وشملت الدراسة عدد 15 جرذ بالغ من نوع سبارجيو داولي. تم تقسيم الجرذان إلى 3 مجموعات. مجموعة التحكم (C) عدد=5 والمجموعة المصابة بداء السكري والتي لم تتلق القرفة (D) عدد=5 والمجموعة المصابة بداء السكري والتي تلقت القرفة (C–D) عدد=5. تم تحفيز السكري بواسطة تلقي عقار ألوكسان عبر الجلد. تمت معالجة جميع الجرذان المصابة بالسكري بالأنسولين البشري. تم تغذية جميع الجرذان بواسطة حبيبات الغذاء القياسية. تم تغذية مجموعة (C–D) بحبيبات الغذاء القياسية، بالإضافة إلى القرفة بجرعة مقدارها 400 ملجم. تم التضحية بجميع الجرذان بعد 3 أشهر وتم الحصول على العصب الوركي لجميع الجرذان. تم تقييم الصبغة بواسطة الميكروسكوب وتم الحصول على عينات الصور.

النتائج: لم يكن سكر الجلوكوز الرئيسي و (HBA1c) وشاكلة الدهون لكلتا المجموعتين مختلفاً من الناحية الإحصائية. تبين وجود انفصال ملحوظ في النخاعين وانحلال في المتيوكوندريا وتغيرات تنكسية وتبعثر في الجزيئات العضوية في الغمد الليفي العصبي ووذمات ملحوظة في الجرذان المصابة بالسكري (D). في المقابل كان الميسوكسون والنواة والبقعة النووية وورقة المايلين في الشكل الطبيعي عند مستوى البنية الفوقية لدى مجموعة (C–D).

خاتمة: قد يكون لمستخلصات القرفة آثار مفيدة في تطور اعتلال الشبكية الناجم عن داء السكري لدى الجرذان المصابة بداء السكري والمحرضة بعقار ألوكسان.

Objective: To investigate the effects of oral cinnamon supplementation on the *nervus ischiadicus* at the electron microscopical level in rats.

Methods: This study was performed between 2004-2006 in Dicle University School of Medicine, Diyarbakir, Turkey in 15 adult Sprague-Dawley rats. Rats were divided into 3 groups; control (C) (n=5), diabetic without cinnamon (D) (n=5), and diabetic with cinnamon (D-C) (n=5). Diabetes was induced with intraperitoneal alloxan administration. All diabetic rats were treated with human insulin. All rats were fed with standard pellet chow. The D-C group rats were fed with standard pellet chow plus *Cinnamomum cassia* at the dose of 400mg/kg. All rats were sacrificed after 3 months and we obtained the *nervus ischiadicus* of all rats. Contrast stained thin sections evaluated by Jeol-TEM-1010 electron microscope, were not statistically different in both groups and photo samples were obtained.

Results: Mean blood glucose, hemoglobin A1C, and lipid profile were not statistically different in both groups. Marked detachment of myelin lamellae at Schmidt-Lanterman clefts, lysis in cristae mitochondrialis and degenerative changes, severe dispersion of organelles in neurolemma, mesoaxon region, and remarkable edema at the endoneurium were found in diabetic rats. On the contrary, mesoaxon, nucleus, nucleolus and myelin sheet were almost of normal appearance at the ultra-structural level in the D-C group.

Conclusions: Cinnamon extracts may have beneficial effects on the development of diabetic neuropathy in alloxan induced diabetic rats.

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Diabetes mellitus is the most common metabolic disease worldwide, with an estimated 1700 new cases diagnosed daily.[1] Of these, 85-90% of the patients have type 2 diabetes mellitus (T2DM), with insulin resistance playing a key role in the development of the disease.[2,3] Diabetes is often associated with neuropathic complications such as peripheral, autonomic, and cranial nerve disorders.[4] These conditions usually result from diabetic microvascular injury involving small blood vessels that supply nerves.[4] The classification of the diabetic neuropathies is not yet finalized (rapidly reversible phenomena, generalized polyneuropathies), focal and multifocal neuropathies, and superimposed chronic inflammatory demyelinating polyneuropathy, and has required successive modifications in the light of accumulating knowledge.[5] Compounds that augment the action of insulin may be beneficial in long-term treatments of T2DM. One such compound is the mineral chromium. Chromium plays a key role in the regulation of glucose metabolism, and chromium may decrease the risk of development of diabetes.[6] Spices such as cinnamon, cloves, bay leaves, and turmeric display insulin-enhancing activity in vitro.[7,8] Botanical products can improve glucose metabolism and the overall condition of individuals with diabetes not only by hypoglycemic effects, but also by improving lipid metabolism, antioxidant status, and capillary function.[9] It was reported that the cinnamon extract seems to have a moderate effect in reducing fasting plasma glucose concentrations in diabetic patients.[10] Kim et al[11] suggested that cinnamon extract had a regulatory role in blood glucose level and lipids, and it may also exert a blood glucose-suppressing effect by improving insulin sensitivity or slowing absorption of carbohydrates in the small intestine. Researchers have found that cinnamon can even help increase insulin sensitivity and therefore, may be a potential treatment for diabetes. The active ingredient in cinnamon, hydroxychalcone, affects insulin receptors to help promote glucose uptake into cells and promote glycogen synthesis.[11] In a study, cinnamon was found to improve glucose and lipid levels in people with diabetes.[12] However, the effects of cinnamon extracts on the development of diabetic neuropathy are still unclear. The aim of this study was to investigate the effects of oral cinnamon supplementation on the *nervus ischiadicus,* a nerve which originates in the lumbar and sacral spinal cord and supplies motor and sensory innervation to the lower extremity, at the ultrastructural level in alloxan-induced experimental diabetic rats.

Methods. All the experimental procedures were performed in accordance with the guidelines of the Experimental Research Institute of Dicle University (DUSAM), Diyarbakir, Turkey. Consent to conduct the study was obtained from the Local Ethics Committee of Dicle University School of Medicine (Grant #04-6) between 2004 and 2006. Fifteen adult female Sprague-Dawley rats, weighing 300-350 gr, obtained from the DUSAM were used in this study. Rats were divided into 3 groups; control (C) (n=5), diabetic without cinnamon (D) (n=5) and diabetic with cinnamon (D-C) (n=5). In the control group one ml normal saline (0.9% NaCl) was applied intraperitoneally, whereas 150 mg/kg Alloxan was applied intraperitoneally for inducing diabetes in D-C and D groups. After 24 hours, venous blood samples were obtained from the tail vein of rats and blood glucose levels were measured with the glucose oxidase method. Blood glucose levels higher than 250 mg/dl were accepted as DM. The diabetic rats were treated with 4 IU/d human insulin. All rats were housed at 22^{0}C and 45% humidity and were fed with standard rat pellet chow, which had 21% protein and free access to water. To determine whether cinnamon extracts prevent neuropathy or not in the cinnamon group (D-C) all rats were fed with standard pellet chow plus cinnamon (*Cinnamomum cassia*) at the dose of 400 mg/kg after the dividing process. The nutrient contents of feedstuffs and trial diets were analyzed according to the Association of Analytical Communities (AOAC) method in Dicle University, School of Veterinary, Department of Feed and Nutrition Laboratory. Crude fiber contents were analyzed according to the Crampton and Maynard method.[13] Daily blood glucose levels of all rats were determined at 08:00-08:30 am and were recorded. A diabetic rat without cinnamon died on the eighth day of intervention. All rats were followed for 3 months and anesthetized with Ketamine (50-200 mg/kg) after cardiac blood samples were obtained. The *nervus ischiadicus* were obtained and fixed in 2.5% glutaraldehyde. One day after fixation, nerve samples were kept in osmiumtetrahydroxide for 2 hours. After washing, they kept uranyl acetate solution and on the third day we turned it in a pure araldehyde for 2 hours. Semi-thin sections were taken from the slides and stained with toluidine blue. Thin sections were cut with Leica ultra-cut R ultra-microtome (Ontario, Canada) and the nerve samples were taken on the copper grids and they were waited for 24 hours for drying of the specimens. Two different pathologist observed and compared the results in a blinded fashion. Contrast stained thin sections were evaluated by JEOL-TEM-1010 electron microscope (Jeol Ltd, Tokyo, Japan) and photo samples were obtained. Results were shown as mean±standard deviation and independent t-test was used. Values of $p<0.05$ were accepted as statistically meaningful.

Results. *Laboratory findings.* Mean blood glucose levels and glycated hemoglobin of both groups were not statistically different. The serum total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, very-low-density lipoprotein-

Table 1 - Blood glucose, glycated hemoglobin, and lipid profile of rats.

Variables	Control (n=5)	D group (n=4)	D-C group (n=5)	*P*-value
Blood glucose (mg/dL)	118.3 ± 19.7	123.5 ± 16.5	122.5 ± 25.7	NS
HbA1c	6.1 ± 1.4	6.7 ± 1.2	6.4 ± 1.1	NS
Triglyceride (mg/dL)	59.1 ± 10.4	71.5 ± 26.8	67.3 ± 19.4	NS
Total cholesterol (mg/dL)	63.7 ± 15.4	66.3 ± 9.1	68.6 ± 5.7	NS
HDL-cholesterol (mg/dL)	28.4 ± 10.2	25.6 ± 3.2	22.5 ± 3.7	NS
LDL-cholesterol (mg/dL)	23.5 ± 3.8	25.4 ± 6.5	23.2 ± 5.6	NS
VLDL-cholesterol (mg/dL)	11.4 ± 2.6	14.3 ± 5.1	13.3 ± 3.7	NS

HbA1c - hemoglobin A1c, high-density lipoprotein, HDL - high-density lipoprotein, LDL - low-density lipoprotein, VLDL - very low density lipoprotein, NS - not significant

cholesterol and triglyceride levels of D-C group was also not statistically different from group D. All laboratory results of rats are shown in **Table 1**.

Ultrastructural evaluations. In group C, the axons and myelin sheets appear to be normal on ultra-structural evaluation. Endoneurium, collagen fibers and neurilemma were also normal (**Figure 1**). In group D, we determined a marked detachment of myelin lamellae at Schmidt-Lanterman clefts. Mitochondrial cristae and degenerative changes were found in some of the axons in this group (**Figure 2a**). Lysis in cristae mitochondrialis in axon, and edema at endoneurium were also determined (**Figure 2a**). Mild dispersion of organelles in neurolemma and mesoaxon region were fixed. We also observed deterioration in euchromatin and heterochromatin in nucleus, and remarkable edema at endoneurium in Schwann cell (**Figures 2b**). In addition detachment of myelin lamella at Schmidt-Lanterman clefts, lysis in cristae mitochondrialis at motor neurons and withdrawing in axons were remarkable (**Figures 2c & 2d**). In the D-C group, the myelin sheet was normal at the ultra-structural level. Although there were some degenerative changes in the axon, we determined a decrement in mitochondrial cristae and edema in the endoneurium compared with the diabetic group (D). Mesoaxon was closely normal (**Figure 3a**). In addition, deterioration at neurolemma in the Schwann cell and absence of nucleolus in nucleus were observed in this group. Euchromatin and heterochromatin in the nucleus was seen close to normal appearance (**Figure 3b**). In this group, the nucleolus and nucleus were almost normal in appearance. Decrements in edema at the neurolemma in the Schwann cell and endoneurium were marked (**Figure 3b**). The Schwann cell was almost normal in appearance with nucleolus and nucleus (**Figures 3c & 3d**).

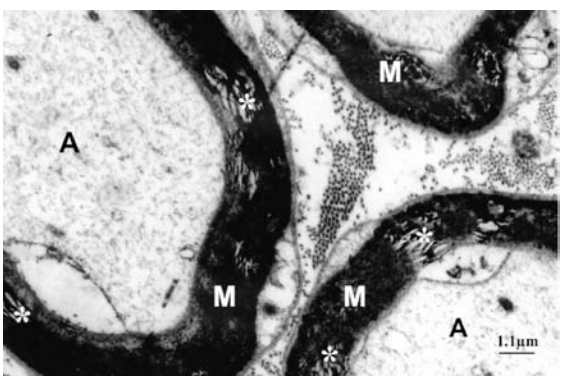
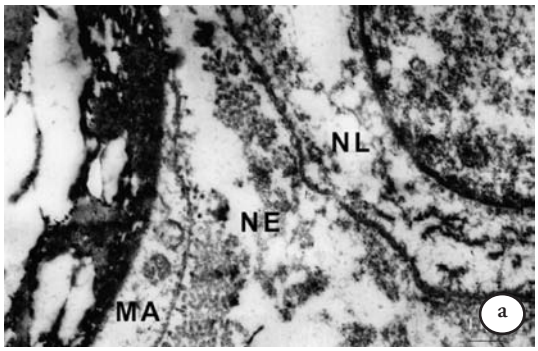
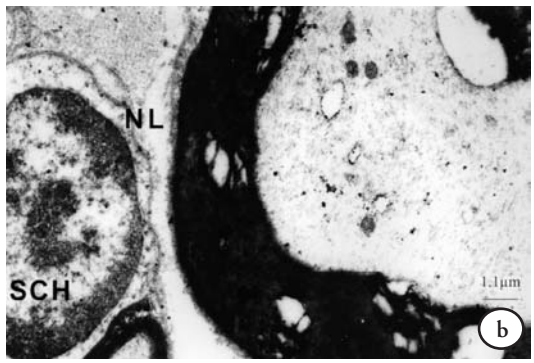
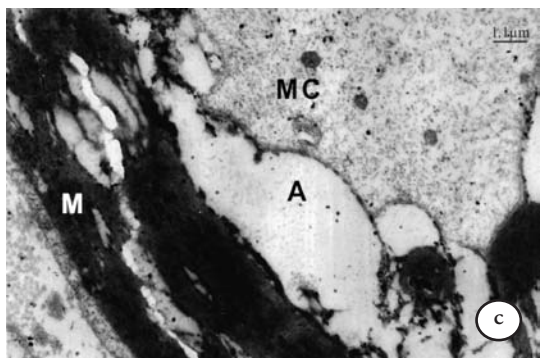


Figure 1 - A section of a sample from control group. Axons (A) and myelin sheet (M) appeared with normal ultra-structural features. Endoneurium with collagen fibers and neurilemma (Schwann cell coat) also appeared as normal. *Schmidt-Lanterman clefts. (Uranyl acetate-lead citrate, X7000, Scale bars=1.1 µm).

Discussion. In this study we determined a marked detachment of myelin lamellae at Schmidt-Lanterman clefts that indicates demyelinization, lysis in cristae mitochondrial, and degenerative changes that reflects a defect in energy expenditure, mitochondrial cristae in axon and mesoaxon, edema at endoneurium, deterioration in euchromatin and heterochromatin in nucleus, and remarkable edema at the endoneurium in Schwann cell of *nervus ischiadicus* in diabetic rats without cinnamon extracts. These findings may imply that DM leads to ultrastructural changes (peripheral neuropathy) in the *nervus ischiadicus* at the electronmicroscopic level. It is well known that diabetic peripheral polyneuropathy is characterized by axonal degeneration and regeneration involving interactions with Schwann cell. Sima et al[14] evaluated fascicular sural nerve morphometry in patients with neuropathy complicating type 1 diabetes that revealed a pattern of interrelated structural changes strikingly similar to that of the diabetic rats. These changes have been described at the light microscopic level and electron microscope has not been applied to large clinical trials. On the contrary to D group, the myelin sheet was almost normal at the ultra-structural level in diabetic rats in group D-C. We also observed that euchromatin and heterochromatin







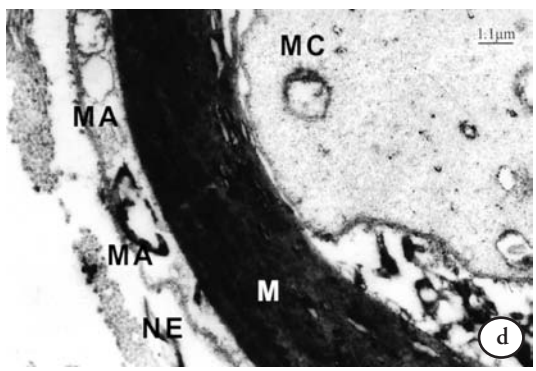
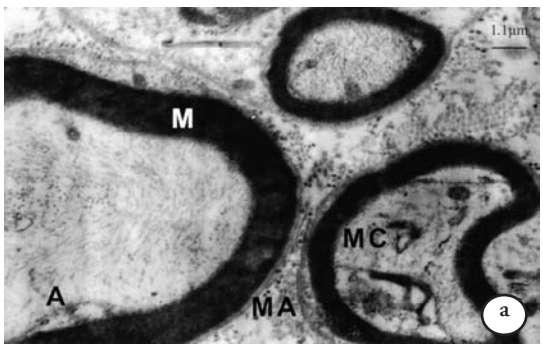
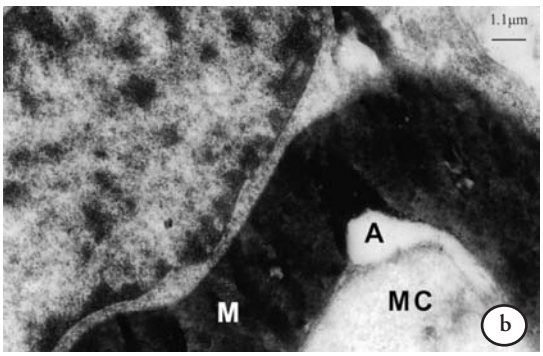
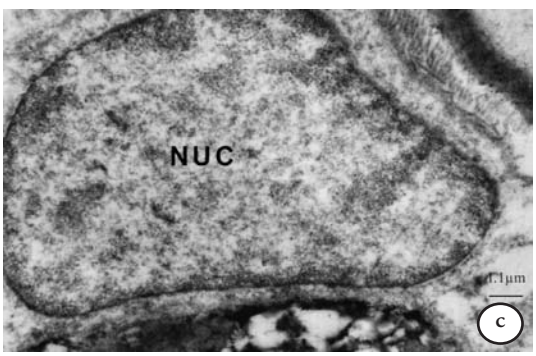


Figure 2 - A selected sample of *Nervus ischiadicus* from D group. **a** & **b**) Evident organelle loss in neurolemma (NL) and mesoaxon region (MA), marked deterioration in euchromatin and heterochromatin structure in nucleus, remarkable edema at endoneurium (NE) attracting attention in Schwann cell. **c** & **d**) Detachment of myelin lamellae at Schmidt-Lanterman clefts (M), lysis in cristae mitochondrialis at motor neurons (MC) and withdrawing in axons (A) were remarkable. (Uranyl acetate-lead citrate, x7000, scale bars=1.1 µm).







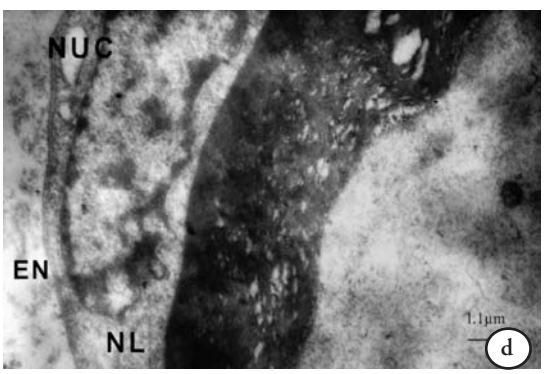


Figure 3 - A selected sample of *Nervus ischiadicus* from D-C group. **a** & **b**) Integrity of myelin coat was protected (M). Scarce axonal shrinkage (A), inconsiderable lysis in cristae mitochondrialis (MC) and mild endoneurial edema in endoneurium (NE) were observed. Organelle loss in mesoaxon region (MA) was indistinct. **c** & **d**) Even if there were some decrease in neurolemma (NL) and mild edema at endoneurium (NE), the Schwann cell was almost normal in appearance with nucleolus (NUC) and nucleus. (Uranyl acetate-lead citrate, x7000, scale bars=1.1 µm).

in the nucleus presented an appearance close to normal structure. These findings may indicate that consumption of cinnamon extracts may have beneficial effects on peripheral neuropathy in diabetic rats. Although there are numerous studies on the effect of cinnamon extracts on DM in both humans and rats, its effects on diabetic complications such as diabetic peripheral neuropathy has not been comprehensively evaluated yet. The exact mechanism of cinnamon extract on the peripheral nerve is not clear. The cinnamon extract seems to have a moderate effect in reducing fasting plasma glucose concentrations in diabetic patients with poor glycemic control.[10] Kim et al[11] showed that cinnamon extract had a regulatory role in the blood glucose level and lipids, and it may also exert a blood glucose-suppressing effect by improving insulin sensitivity or slowing absorption of carbohydrates in the small intestine in db/db mice.[11] Verspohl et al[15] evaluated the effect of different cinnamon species (*Cinnamomum cassia* bark or extracts from *Cinnamomum cassia* and *zeylanicum*) on blood glucose and plasma insulin levels in rats under various conditions, and they found that the *cassia* extract was superior to the *zeylanicum* extract.[15] It was also demonstrated that Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rats.[16] Whereas mean blood glucose levels and percent of glycated hemoglobin of both groups were not statistically different. The serum lipid profile of the D-C group was not statistically different compared with group D. For these reasons, the small anti-diabetic effects of cinnamon extracts may be responsible for these results, and further studies are needed to clearly explain these findings.

Limitation of this study was the small number of animals.

We conclude that diabetes may lead to ultrastructural defects in the *nervus ischiadicus*. Cinnamon extracts may have beneficial effects on the development of diabetic neuropathy in alloxan induced diabetic rats independent of plasma glucose concentrations, glycated hemoglobin and lipid profile. Further studies are needed to explain this issue.

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