ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Effect of Cinnamon on Distribution and Number of Mast Cells in Diabetic Rat Liver

Diyabetik Sıçan Karaciğerinde Tarçının Mast Hücrelerinin Dağılımı ve Sayısı Üzerine Etkisi

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ABSTRACT Objective: In the present study, we investigated the effects of cinnamon on distribution and number of mast cells (MCs) in liver tissue of diabetic rats. **Material and Methods:** The rats used as the control group received no application. All rats in the diabetes and diabetes+cinnamon groups were made diabetic by a single dose intraperitoneal injection of streptozotocin. In our study, after establishment of diabetes, cinnamon extract was administered daily by oral gavage for 14 days to both cinnamon and diabetes+cinnamon groups. To determine the MCs, the tissue sections were stained both with toluidin blue (TB) and Alcian blue/Safranin O (AB/SO) methods. **Results:** It was determined that the number of MCs in the portal areas increased with diabetes. As a notable finding, it was observed in our study that cinnamon reduced the MC numerical increase that increases with diabetes in the diabetes+cinnamon group. As a result of the AB/SO combined staining method application, it was shown that there were 3 types of MC populations as blue AB(+), red SO(+) and mixed color AB/SO(+) in rat liver tissue. It was found that cinnamon application was effective on the SO(+) content in diabetes. **Conclusion:** In conclusion, the findings of this study show that diabetes alters the number of MC and cinnamon can reduced the increase in diabetes-related MC numbers. In addition, this study demonstrated the possible effects of diabetes and cinnamon on the histochemical and morphological differences of MCs in rat liver.

Keywords: Diabetes; cinnamon; mast cell; histochemistry

ÖZET Amaç: Bu çalışmada, tarçının diyabetik sıçanların karaciğer dokusundaki mast hücrelerinin (MH) dağılımı ve sayısı üzerindeki etkileri araştırıldı. **Gereç ve Yöntemler:** Kontrol grubu olarak kullanılan ratlara herhangi bir uygulama yapılmadı. Diyabet ve diyabet+tarçın gruplarındaki tüm ratlar tek doz intraperitoneal streptozotosin enjeksiyonu ile diyabetik hâle getirildi. Çalışmamızda, diyabet oluşturulduktan sonra tarçın ve diyabet+tarçın gruplarındaki tüm ratlara 14 gün boyunca günlük olarak oral gavaj yoluyla tarçın uygulandı. Mast hücrelerini belirlemek için doku kesitleri hem toluidin blue (TB) hem de Alcian blue/Safranin O (AB/SO) yöntemleri ile boyandı. **Bulgular:** Diyabetle birlikte portal alanlardaki MH sayısının arttığı belirlendi. Dikkate değer bir bulgu olarak çalışmamızda, tarçının diyabet+tarçın grubunda diyabetle birlikte artan MH sayısal artışını azalttığı gözlendi. AB/SO kombine boyama metodu uygulaması sonucunda, rat karaciğer dokusunda mavi AB(+), kırmızı SO(+) ve karışık renkli AB/SO(+) olmak üzere 3 tip mast hücre popülasyonunun olduğu saptandı. Diyabette tarçın uygulamasının, SO(+) içeriği üzerinde etkili olduğu bulundu. **Sonuç:** Sonuç olarak bu çalışmanın bulguları, diyabetin MH sayısını değiştirdiği ve tarçının diyabete bağlı MH sayılarındaki artışı engelleyebileceğini göstermektedir. Ayrıca bu çalışma, sıçan karaciğerindeki MH'lerin histokimyasal ve morfolojik farklılıkları üzerinde diyabet ve tarçının olası etkilerini göstermiştir.

Anahtar Kelimeler: Diyabet; tarçın; mast hücre; histokimya

Diabetes mellitus (DM) is a severe metabolic disorder with numerous functional and structural consequences.[1] The chronic high glucose level in the blood is a disorder caused by the inability of the pancreas's beta cells (β cells) to produce enough insulin or the use of ineffective insulin by the cells in the body.[2]

Cinnamon is a spice that exhibits economic and medicinal value and finds use in traditional medicine. Antimicrobial, antitumor, antioxidant, anti-diabetic, anti-inflammatory, and analgesic properties are among its biological activities.[3] Cinnamon promotes the expression of insulin-sensitive glucose transporters, resulting in decreased insulin resistance.[4]

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Mast cells (MCs) are granular hematopoietic cells found throughout all tissues. MCs are recognized to play a role in allergic reactions, tissue remodeling, wound healing, and inflammation, among other biological processes. MCs have multiple electron-dense cytoplasmic granules carrying diverse biological mediators, distinguishing them morphologically.[5] Although all MCs have some features, they constitute a varied population phenotypically. In general, classification is based on the neutral protease content of their cytoplasmic granules.[6] In particular, MCs are divided into 2 subgroups considering the mediators they contain and their histochemical differences; they are classified as connective tissue mast cells (CTMC) and mucosal mast cells (MMC).[7] MC mediators such as chemokines, cytokines, growth factors, heparin, histamine, proteases, chymase, and tryptase are thought to play a role in the pathogenesis of metabolic diseases, including DM.[8]

While the role of mediators released by granules of MCs in tissue remodeling, wound healing, and allergic and inflammatory reactions are well known, there are few reports of their physiological role in diabetic liver tissue. Therefore, in this study, we aimed to evaluate the effects of cinnamon on the quantitative distribution and morphological and histochemical properties of MCs in diabetic rat liver.

MATERIAL AND METHODS

ANIMALS

The Ondokuz Mayıs University Animal Experiments Ethical Committee gave its approval to all procedures (date: March 11, 2020, no: 16). In this investigation, 32 male albino Wistar rats weighing 250-300 g were employed. All rats were housed in standard polycarbonate cages (4 rats/cage) with *ad libitum* access to chow and tap water and kept under a constant environment (light and dark cycles of 12 hours and a temperature of 22±2°C). The rats were randomly divided into 4 groups (n=8 per group): control, cinnamon, diabetes, and diabetes+cinnamon.

EXPERIMENTAL DESIGN

The rats used as the control group received no application. Daily 0.5 mg/kg of the cinnamon extract was dissolved in 0.2 mL distilled water and each rat in the cinnamon and diabetes+cinnamon groups was given by oral gavage daily at 13:00-14:00 h for 14 days.[9] All rats in the diabetes and diabetes+cinnamon groups were made diabetic by a single intraperitoneal injection of streptozotocin (STZ; Sigma, S0130-1G) 45 mg/kg as the dosage was freshly dissolved in 10 mL distilled water.[10]

STZ-induced diabetes was confirmed by measuring the elevated blood glucose level of rats. Briefly, all rats in the diabetes and diabetes+cinnamon groups were fasted for 8 hours after 3 days of STZ injection, and blood glucose level of each rat was measured from tail vein using a glucometer (PlusMED Accuro) after 8 hours of fasting. As a result, rats in diabetes and diabetes+cinnamon groups were classified as diabetic if their blood glucose level was greater than 300 mg/dL (Type I diabetes). In our study, after establishment of diabetes, all rats in the cinnamon and diabetes+cinnamon groups were daily treated with cinnamon (0.5 mg/kg/day by oral gavage) for 14 days. At the end of the 14-day treatment of cinnamon, all rats were sacrificed under deep anesthesia and their liver tissues were collected. To keep the animals in our study from feeling pain or discomfort, anesthesia [ketamine (60 mg/kg)-xylazine (6 mg/kg)] was administered to all groups. According to the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html), the animals were treated humanely.

HISTOLOGICAL PROCEDURE

For histological analysis, liver tissue samples were fixed in a 10% formalin solution for 12 hours. After standing in a water bath for 24 hours to remove formaldehyde, they were passed through graded alcohols and xylol and embedded in paraffin blocks.

MAST CELL HISTOCHEMISTRY

Toluidine Blue Staining Protocol

To show the distribution and count of metachromatic MCs, 10 serial sections of 5-µm cross-sections at 30 µm intervals of the prepared blocks were stained with toluidine blue (TB) (0.5%, pH 0.5, Sigma-Aldrich,

92-31-9) produced in McIlvaine's citric acid disodium phosphate buffer for 10 minutes.[11]

Combined Alcian Blue-Safranin O ProtocoL

In order to identify subtypes of MCs, slices from blocks 5-µm thick sections with 30-µm intervals were taken from each block on the same slide and stained with 0.2 M acetate solution with Alcian blue (AB; 0.5%, pH 0.2, Sigma-Aldrich, 22864-99-2) and Safranin O (SO; 0.25%, pH 1.42, Sigma161 Aldrich, 477-73-6) mixed dyes.[11]

Counting Of Mast Cells

Following histochemical staining, preparates were examined under an examination microscope (Nikon Eclipse 50i) in terms of staining properties. Metachromatic staining with TB, AB, SO, and AB/SO positive cell distribution was evaluated.

In the serial sections prepared to find out the numerical distribution of TB(+) MCs, AB(+), SO(+) MCs, and AB/SO(+) (mixed type), cell counts were performed with 100 squares ocular micrometer (eyepiece graticule). MCs were counted at 100 square units of the ocular micrometer under a magnification of X40. Cell count was performed at 10 randomly chosen different areas of the sections, and the arithmetic mean of the results was taken. Then, all the numerical data were converted into the number of MCs in a 1 mm^2 area.

STATISTICAL ANALYSIS

All statistical evaluations were performed utilizing IBM SPSS Statistics Version 22.0 (IBM United States Software Announcement 213-309). Depending on the normality of the data, the one-way analysis of variance (ANOVA) was used, followed by the Tukey's post-hoc test (confirmed by Shapiro-Wilk W test). Results were expressed as mean±SEM (standard error of the mean), and $p<0.05$ was considered statistically significant.

RESULTS

MCs of varying sizes and appearances were detected in sections stained with TB and AB/SO in all groups. As a whole, MCs' shapes ranged from oval-round to flat spindle-like (Figure 1). The number of the SO(+) MCs was more abundant than the AB(+) and AB/SO(+) subtypes in the rat liver. It was observed that MCs were predominantly located in the connective tissue near the arteria hepatica, vena interlobularis, and ductus biliferi structures forming the trias hepatis in the liver (Figure 2). It was remarkable that MCs were seen to be particularly near the vena interlobularis and surround the vena. Furthermore, MCs were found in the liver Glisson's capsule, centrilobular veins, and around the hepatocytes, which compose the remark cords. It was seen that MCs were mainly located in the portal tracts in all groups (Figure 3).

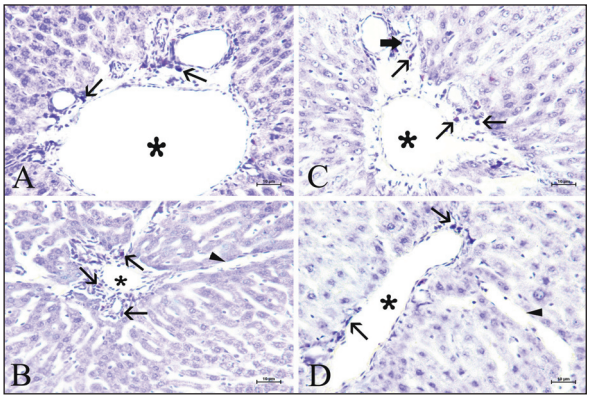

FIGURE 1: Control group (A), cinnamon group (B), diabetes group (C), diabetes+cinnamon (D). Toluidine blue staining. Thin arrow: mast cell, thick arrow: arteria hepatica, arrowhead: sinusoid, asterix: vena interlobularis, ×40; range bar, 10 µm.

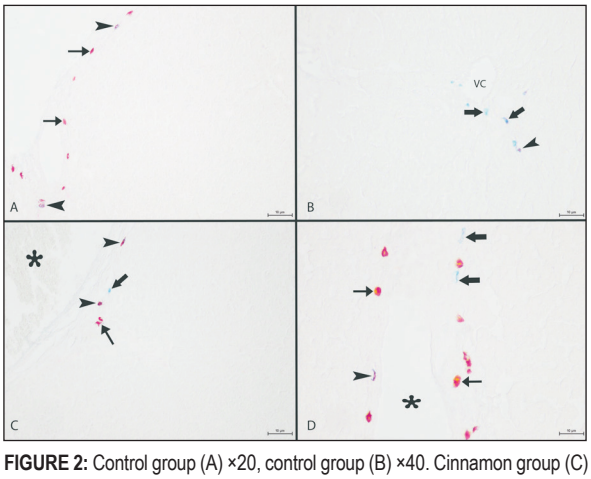

FIGURE 2: Control group (A) ×20, control group (B) ×40. Cinnamon group (C) ×20, cinnamon group (D), ×40; range bar, 10 µm. Thin arrow: SO (+) mast cell, thick arrow: AB (+) mast cell, arrowhead: AB/SO (mixt) mast cell, asterix: vena interlobularis, vc: vena centralis.

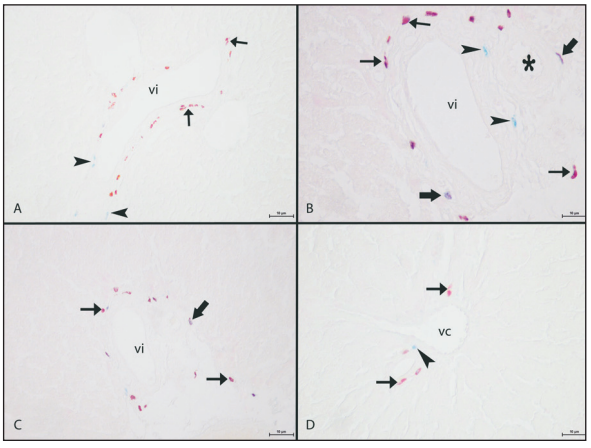

FIGURE 3: Diabetes group (A) ×20, diabetes group (B) ×40. Diabetes+cinnamon group (C) ×20, diabetes+cinnamon group (D), ×40; range bar, 10 µm. Thin arrow: SO (+) mast cell, thick arrow: AB/SO (mixt) mast cell, arrowhead: AB (+) mast cell, vi: vena interlobularis, vc: vena centralis, asterix: arteria hepatica.

The total numbers of MC distribution and histochemical properties of all experimental groups are summarized in Table 1. Compared to the control group, it was determined that the number of MCs did not change in the cinnamon group. On the other hand, there was an increase in the number of TB(+) ($p<0.001$), SO(+) ($p<0.001$), and AB/SO(+) ($p<0.05$) type MCs in the diabetes group. Notably, the increase in TB(+) and SO(+) was higher than in AB/SO(+). However, it was seen that the numerical increase in AB(+) was not statistically significant in the diabetes group. Markedly, our data also showed that the number of MCs was reduced considerably in TB(+) ($p<0.01$) and SO(+) ($p<0.01$) in the diabetes+ cinnamon group compared to diabetes group depending on cinnamon application.

DISCUSSION

The present study first demonstrated the effects of cinnamon on MC (as distribution, morphological, and histochemical properties) in rat liver in an STZ-induced in vivo diabetes model.

Chemical messengers released by Kupffer cells draw inflammatory cells like MCs to the surrounding area, where they mediate immunoregulatory activities.[12] Since hepatocytes are in contact with the blood supply, it is thought that MCs located near blood vessels contribute to regulating hepatic injury by cross-talk with hepatocytes.[13] It is known that MCs increase in diabetic rats, especially in the peripheral areas of the islets of Langerhans.[14] Batbayar et al reported that after STZ application, an increase in the number of MCs in all layers of the oral mucosa and tongue of rats, especially around the blood vessels and in the lamina propria, compared to the control tissue.[15] Another experimental study used insulin to investigate the activation of the MC in diabetes. This study reported that higher MC numbers were determined in diabetic groups before insulin was administered and decreased MC numbers after it was administered.[16] It has been reported that

TABLE 1: Effects of cinnamon and/or diabetes on the MC distribution, morphological, and histochemical properties in rat liver tissue.

Groups (n=8)		TB	AB	SO	AB/SO
Control	Glisson's capsule	5.8 ± 0.93	1.93 ± 0.25	3.54 ± 0.27	0.89 ± 0.38
	Sinusoidal region	3.45 ± 0.2	1.64 ± 0.23	2.65 ± 0.35	0.75 ± 0.46
	Portal tract	11.96 ± 0.66	3.01 ± 0.12	4.96 ± 0.25	2.03 ± 0.42
Cinnamon	Glisson's capsule	6.01 ± 0.58	2.05 ± 0.43	4.2 ± 0.3	1.02 ± 0.63
	Sinusoidal region	3.95 ± 0.72	1.54 ± 0.42	3.01 ± 0.43	0.83 ± 0.32
	Portal tract	12.37 ± 0.83	3.82 ± 0.33	5.03 ± 0.95	2.25 ± 0.72
Diabetes	Glisson's capsule	9.75 ± 0.71*	3.32 ± 0.22	6.73 ± 0.83*	2.93 ± 0.25&
	Sinusoidal region	5.85 ± 0.32*	2.51 ± 0.21	5.7 ± 0.53*	2.08 ± 0.25&
	Portal tract	18.23 ± 0.75*	4.42 ± 0.25	8.83 ± 0.79*	4.01 ± 0.32&
Diabetes+cinnamon	Glisson's capsule	6.93 ± 0.44$	2.13 ± 0.83	4.98 ± 0.72$	1.22 ± 0.33
	Sinusoidal region	4.03 ± 0.83$	1.63 ± 0.25	3.42 ± 0.23$	1.03 ± 0.72
	Portal tract	13.03 ± 0.25$	3.93 ± 0.28	6.22 ± 0.27$	3.83 ± 0.41

&p<0.05 and *p<0.001 when compared to the control group; $p<0.01 when compared to the diabetes group; TB: Toludin blue; AB: Alcian blue; SO: Safrain O.

conditional MCs deletion in nonobese diabetic mice, which results in MCs selective depletion at an early stage, protects the mice against autoimmune DM.[17] In a study in the heart tissues of diabetic mice, the number of chymase-positive MCs was found to be significantly higher than in normal mice.[18] Parallel to the previous studies, an increase in the numbers of MCs was observed in the liver tissue of the rats whose diabetes induced by the STZ model was treated in this study.

Several natural chemicals, including cinnamon, have powerful effects on biological activities. Cinnamon may reduce the release of stored and *de novo* synthesized MC mediators such as β-hexosaminidase and cysteinyl leukotrienes, as well as the production of various proinflammatory cytokines.[19] Studies have demonstrated that the membrane-membrane fusion proteins known as SNAREs regulate MC degranulation. Using SNAREs, secretory granules can attach to the plasma membrane, releasing previously held chemicals like histamine and -hexosaminidase into the extracellular space. Therefore, the release of β-hexosaminidase is widely used as an indicator of the degranulation of MCs.[20] For this reason, experimental evidence has postulated that cinnamon reduced the degranulation of MCs, thereby regulating histamine synthesis.[21] It is thought that cinnamon plays an essential role in the intracellular mobilization of Ca^{++} ions in mucosal MCs with its inhibitory effects on the phospholipase C signaling pathway.[18] Consistent with these studies, our findings showed that cinnamon extract reduced the increase in liver MCs number induced by STZ. This result is in accordance with other studies on the effects of cinnamon. Our study's data suggests that cinnamon may be the main biological mediator of the inhibitory effects on MC activation.

The structural and functional characteristics of different subtypes, particularly the neutral proteases expressed within intracellular granules, can be used to distinguish MCs from one another.[22] CTMC granules contain a highly sulfated mucopolysaccharide and a high affinity for safranin. On the other hand, the MMC granules contain weakly sulfated mucopolysaccharide, chondroitin sulfate, which has a strong affinity for AB in their composition.[23] AB/safranin dye has been widely used for decades to distinguish CTMC from MMC concerning its staining properties.[24] Throughout the literature, in this staining method, it was stated that MMC contained granules stained blue with AB(+), while CTMC contained granules stained red with SO (+).[25] Safranin causes the heparin-containing CTMC to turn red. In contrast, MMC lacks heparin and looks blue when stained with alcian blue.[26] Additionally, a rat brain investigation revealed that the number of high heparin-containing MC cells stained red with safranin was significantly higher than that of heparin-free MC cells stained with alcian blue.[27] In a study on MC heterogeneity in the liver, 2 subgroups of MCs were reported to stain positively with SO and AB.[28] In our study, it was determined that the 3 phenotypes of MCs, AB(+), SO(+), and AB/SO(+), differed numerically between the groups. Based on the results of our study and in light of published data, we hypothesize that both SO(+) and AB/SO(+) MCs are more abundant in the liver and so these subtypes may play an active role in diabetes-like metabolism disorders.

MCs produce heparin as the proteoglycan serglycin, which has a core protein that has about 15 long heparin chains (about 100 kDa) on a recurrent serine-glycine stretch.[29] Granules with low sulphated glycosaminoglycans (AB-positive) are distinguished from those with high sulphated glycosaminoglycans by the AB/safranin reaction (safranin-positive). The presence of highly sulphated glycosaminoglycans, such as heparin, was indicated by safranin positivity.[30] Long-term low molecular weight heparin treatment in experimental diabetes has been demonstrated to avoid pathological reactions and maintain kidney function.[31] Moreover, it has been reported that low molecular weight heparin treatment prevents albuminuria and the increase in urinary glucosaminoglycan excretion, which is one of the indicators of diabetic nephropathy.[32] Heparin has been shown in both human and animal studies to accelerate wound healing and reduce the risk of thrombosis in diabetics.[33] Endogenous heparin is known to promote the activity of insulin-like growth factor-1, and it has also been shown that blood levels of heparin are higher than normal in diabetic

patients.[34] Consistent with this relationship, 1 study showed that insulin function was reduced in the presence of heparin.[35] In our study, among the 3 subtypes, the diabetic group had significantly more SO(+) MCs; this suggests that heparin containing SO(+) MC may have a key role in glucose homeostasis in diabetes.

CONCLUSION

The findings of this study indicate that diabetes induces an increase in the number of MCs in the liver tissue. On the other hand, it was observed that cinnamon application might reduce the increase in MC numbers due to diabetes. Therefore, cinnamon may be considered an anti-diabetic plant-derived component for preventing or treating diabetes-associated disorders in liver tissue. Additionally, this study was the first to examine how cinnamon affected three distinct MC subtypes in diabetic rats. Moreover, it was shown that the SO(+) and AB/SO(+) numbers were higher in the diabetes group. When the effect of cinnamon was investigated, it was found that cinnamon application was solely effective on the MC of SO(+) in diabetes. As a result, the present results indicate that by changing their number and granular content of MCs, cinnamon play an active role in preventing the adverse metabolic effects of diabetes in liver tissue.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Tuğrul Ertuğrul;* ***Design:*** *Tuğrul Ertuğrul;* ***Control/Supervision:*** *Tuğrul Ertuğrul, Nazife Ülker Ertuğrul;* ***Data Collection and/or Processing:*** *Tuğrul Ertuğrul;* ***Analysis and/or Interpretation:*** *Tuğrul Ertuğrul;* ***Literature Review:*** *Tuğrul Ertuğrul, Nazife Ülker Ertuğrul;* ***Writing the Article:*** *Tuğrul Ertuğrul;* ***Critical Review:*** *Nazife Ülker Ertuğrul;* ***References and Fundings:*** *Nazife Ülker Ertuğrul;* ***Materials:*** *Tuğrul Ertuğrul;* ***Other:*** *Nazife Ülker Ertuğrul.*

REFERENCES

1. Meyer K, Deutscher J, Anil M, Berthold A, Bartsch M, Kiess W. Serum androgen levels in adolescents with type 1 diabetes: relationship to pubertal stage and metabolic control. J Endocrinol Invest. 2000;23(6):362-8. [Crossref] [PubMed]
2. Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 diabetes and its impact on the immune system. Curr Diabetes Rev. 2020;16(5):442-9. [Crossref] [PubMed] [PMC]
3. Wang J, Su B, Jiang H, Cui N, Yu Z, Yang Y, et al. Traditional uses, phytochemistry and pharmacological activities of the genus Cinnamomum (Lauraceae): a review. Fitoterapia. 2020;146:104675. [Crossref] [PubMed]
4. Jitomir J, Willoughby DS. Cassia cinnamon for the attenuation of glucose intolerance and insulin resistance resulting from sleep loss. J Med Food. 2009;12(3):467-72. [Crossref] [PubMed]
5. Norström A, Vukas Radulovic N, Bullarbo M, Ekerhovd E. Mast cell involvement in human cervical ripening. Eur J Obstet Gynecol Reprod Biol. 2019;238:157-63. [Crossref] [PubMed]
6. Krishnaswamy G, Ajitawi O, Chi DS. Mast cells methods and protocols. In: Krishnaswamy G, Chi DS, eds. Methods in Molecular Biology. 1st ed. New York: Humana Press Inc; 2006. p.3-15.
7. Dimitriadou V, Rouleau A, Trung Tuong MD, Newlands GJ, Miller HR, Luffau G, et al. Functional relationships between sensory nerve fibers and mast cells of dura mater in normal and inflammatory conditions. Neuroscience. 1997;77(3):829-39. [Crossref] [PubMed]
8. Kempuraj D, Caraffa A, Ronconi G, Lessiani G, Conti P. Are mast cells important in diabetes? Pol J Pathol. 2016;67(3):199-206. [Crossref] [PubMed]
9. Hemayatkhah Jahromi V, Baqerzadeh P. The effect of cinnamon extract on ovary in adult rats treated lead acetate. Journal of Animal Biology. 2017;9(2):1-10. [Link]
10. Çetin A, Vardı N, Orman D. Deneysel diyabetin sıçan kalp dokusunda meydana getirdiği histolojik değişiklikler üzerine aminoguanidinin iyileştirici etkileri [Ameliorating effects of aminoguanidine against in the hıstological alterations of rat heart in experimental diabetes]. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksek Okulu Dergisi. 2013;1(1):17-26. [Link]
11. Enerbäck L. Mast cells in rat gastrointestinal mucosa. 2. Dye-binding and metachromatic properties. Acta Pathol Microbiol Scand. 1966;66(3):303-12. [Crossref] [PubMed]
12. Francis H, Meininger CJ. A review of mast cells and liver disease: what have we learned? Dig Liver Dis. 2010;42(8):529-36. [Crossref] [PubMed]
13. Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology. 2006;43(2 Suppl 1):S54-62. [Crossref] [PubMed]

14. Abdel-Hamid AAM, Firgany AEL. Increased mast cell number is associated with a decrease in beta-cell mass and regeneration in type 2 diabetic rats. Acta Histochem. 2019;121(4):508-15. [Crossref] [PubMed]
15. Batbayar B, Somogyi J, Zelles T, Fehér E. Immunohistochemical analysis of substance P containing nerve fibres and their contacts with mast cells in the diabetic rat's tongue. Acta Biol Hung. 2003;54(3-4):275-83. [Crossref] [PubMed]
16. Cavalher-Machado SC, de Lima WT, Damazo AS, de Frias Carvalho V, Martins MA, e Silva PM, et al. Down-regulation of mast cell activation and airway reactivity in diabetic rats: role of insulin. Eur Respir J. 2004;24(4):552-8. [Crossref] [PubMed]
17. Betto E, Usuelli V, Mandelli A, Badami E, Sorini C, Capolla S, et al. Mast cells contribute to autoimmune diabetes by releasing interleukin-6 and failing to acquire a tolerogenic IL-10+ phenotype. Clin Immunol. 2017;178:29-38. [Crossref] [PubMed]
18. Zaidi SF, Aziz M, Muhammad JS, Kadowaki M. Review: Diverse pharmacological properties of Cinnamomum cassia: a review. Pak J Pharm Sci. 2015;28(4):1433-8. [PubMed]
19. Hagenlocher Y, Kiessling K, Schäffer M, Bischoff SC, Lorentz A. Cinnamaldehyde is the main mediator of cinnamon extract in mast cell inhibition. Eur J Nutr. 2015;54(8):1297-309. [Crossref] [PubMed]
20. Ahn S, Kim E, Lee K, Lee DC. Cinnamaldehyde derivatives inhibit degranulation and inflammatory mediator production in rat basophilic leukemia cells. Int Immunopharmacol. 2016;38:342-8. [Crossref] [PubMed]
21. Badger-Emeka LI, Emeka PM, Thirugnanasambantham K, Ibrahim HIM. Anti-allergic potential of cinnamaldehyde via the ınhibitory effect of histidine decarboxylase (HDC) producing Klebsiella pneumonia. Molecules. 2020;25(23):5580. [Crossref] [PubMed] [PMC]
22. Gurish MF, Austen KF. Developmental origin and functional specialization of mast cell subsets. Immunity. 2012;37(1):25-33. [Crossref] [PubMed]
23. Combs JW, Lagunoff D, Benditt EP. Differentiation and proliferation of embryonic mast cells of the rat. J Cell Biol. 1965;25(3):577-92. [Crossref] [PubMed] [PMC]
24. Enerbäck L. Mast cells in rat gastrointestinal mucosa. I. Effects of fixation. Acta Pathol Microbiol Scand. 1966;66(3):289-302. [Crossref] [PubMed]
25. Uslu S, Yörük M. Hindilerde sindirim sisteminde mast hücrelerinin dağılımı ve heterojenitesi üzerine morfolojik ve histometrik araştırmalar [Morphological and histometrical studies on distribution and heterogeneity of mast cells in turkey digestive system]. Y.Y.Ü. Veteriner Fakültesi Dergisi. 2008;19(2):47-51. [Link]
26. Galli SJ. New insights into "the riddle of the mast cells": microenvironmental regulation of mast cell development and phenotypic heterogeneity. Lab Invest. 1990;62(1):5-33. [PubMed]
27. Tunçel N, Sener E, Cerit C, Karasu U, Gürer F, Sahintürk V, et al. Brain mast cells and therapeutic potential of vasoactive intestinal peptide in a Parkinson's disease model in rats: brain microdialysis, behavior, and microscopy. Peptides. 2005;26(5):827-36. [Crossref] [PubMed]
28. Chan A, Cooley MA, Collins AM. Mast cells in the rat liver are phenotypically heterogeneous and exhibit features of immaturity. Immunol Cell Biol. 2001;79(1):35-40. [Crossref] [PubMed]
29. Wang A, Sankaranarayanan NV, Yanagishita M, Templeton DM, Desai UR, Sugahara K, et al. Heparin interaction with a receptor on hyperglycemic dividing cells prevents intracellular hyaluronan synthesis and autophagy responses in models of type 1 diabetes. Matrix Biol. 201548:36-41. [Crossref] [PubMed]
30. Chieffi Baccari G, Falvo S, Lanni A, Di Fiore MM, Cioffi F, Santillo A. Mast cell population and histamine content in hypothyroid rat tissues. Animals (Basel). 2022;12(14):1840. [Crossref] [PubMed] [PMC]
31. Gambaro G, Venturini AP, Noonan DM, Fries W, Re G, Garbisa S, et al. Treatment with a glycosaminoglycan formulation ameliorates experimental diabetic nephropathy. Kidney Int. 1994;46(3):797-806. [Crossref] [PubMed]
32. Gogas D, Budak Y, Ersöz Ö, Özener Ç, Deyneli O, Emerk K, et al. Diabetik rat modelinde kısa süreli düşük molekül ağırlıklı heparin tedavisinin renal etkileri [Renal effects of short term low molecular weight heparin administration in diabetic rat model]. Türk Nefrotoji Diyaliz ve Transplantasyon Dergisi. 1995;2:70-4.
33. Senturk B, Demircan BM, Ozkan AD, Tohumeken S, Delibasi T, Guler MO, et al. Diabetic wound regeneration using heparin-mimetic peptide amphiphile gel in db/db mice. Biomater Sci. 2017;5(7):1293-303. [Crossref] [PubMed]
34. Arai T, Parker A, Busby W Jr, Clemmons DR. Heparin, heparan sulfate, and dermatan sulfate regulate formation of the insulin-like growth factor-I and insulin-like growth factor-binding protein complexes. J Biol Chem. 1994;269(32):20388-93. [Crossref] [PubMed]
35. Kriauciunas KM, Grigorescu F, Kahn CR. Effects of heparin on insulin binding and biological activity. Diabetes. 1987;36(2):163-8. [PubMed]