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Immunohistochemical Investigation of Trk-A Receptor Levels in Pancreatic Tissue of Cumin (*Cuminum cyminum*) Plant Essential Oil Treated-Mice

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Abstract

This study was conducted to immunohistochemically investigate Trk-A receptor levels in pancreas tissue of mice treated by cumin (*Cuminum cyminum*) plant essential oil. Mice were grouped into control group (n = 10) and trial group (n = 10). No application was performed to the mice in the control group. The mice in trial group were treated by 500 mg/kg of oral cumin essential oil every 24 hours for two days. At the end of study, the pancreatic tissues obtained were blocked in paraffin following routine histological processes. Triple staining was performed to the sections taken from these blocks to examine general histological structure of pancreas. Acinus, islets of Langerhans, pars initialis, pars excretory and ductus excretorius were determined in mice pancreas. Immunohistochemical studies showed that all mice had Trk-A immunoreactivity in pancreatic tissue. Moderate immunoreactivity in acini and weak immunoreactivity in islets of Langerhans and excretory ducts were detected in pancreas tissue of mice in control and trial groups. It was determined that there was no difference between the groups in terms of Trk-A immunoreactivity in acini and islets of Langerhans. Based on the immunohistochemical results, cumin was used in field of diuretic, degassing, digestion facilitator, antimicrobial and antidiabetic effects in field of traditional medicine; It was concluded that Trk-A receptor synthesized from pancreatic tissue does not change its levels.

Keywords: Cumin, pancreas, Trk-A.

1. INTRODUCTION

Plants have been used for various purposes such as treatment of diseases, defense and nutrition since the past. Today, people continue to benefit from these effects of plants but the use of plants is more consciously done. Plants are used in many fields such as food, pharmacy, agriculture and medicine (Göktaş and Gıdık, 2019). Cumin (*Cuminum cyminum*) is a plant also known as Persian cumin, Avcar or Kemmon. Its native soil is Egypt, but is raised in Mediterranean countries and Middle Anatolian Region of Turkey as well. In addition to its

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usage as a spice, it has been used as carminative and diuretic, reliever of stomach discomfort and exudative (Baytop, 1999). Cumin was reported to have beneficial effects on patients with obesity and metabolic syndrome (Fernando, Perera, Athauda, Sivakanesan, Kumar, Jayasinghe, 2019; Morovati, Pourghassem, Sarbakhsh, Azari and Lotfi-Dizaji, 2019).

Cumin has been reported to may have positive effects on cancer treatment disadvantages and antimicrobial resistance (Nirmala, Durai, Rao and Nagarajan, 2020). Trk-A receptor belongs to the family of thyrosine kinase which has a role in the development of peripheral nervous system (Kiriş, Wang, Yanpallewar, Dorsey, Becker, ….Tessarollo, 2014). It has been stated that Trk-A may have effects on insülin and glucose metabolism with its synthesis in the pancreas, other than the nervous system (Rosenbaum, Sánchez-Soto and Hiriart, 2001). NTRK gene fusions involving either NTRK1, NTRK2 or NTRK3 (encoding the neurotrophin receptors TRKA, TRKB and TRKC, respectively) have important roles in diagnostic evaluation of central nervous system tumors and pediatric mesenchymal tumors (Gambella, Senetta, Collemi, Vallero, Monticelli, …. Bertero, 2018; Rudzinski, Lockwood, Stohr, Vargas, Sheridan, ….Davis, 2020). In addition, It is also considered as a biomarker in digestive system cancers, including esophageal, stomach and pancreatic cancers (Blondy, Christou, David, Verdier, Jauberteau, Mathonnet and Perraud, 2019). It was reported that Trk-A immunolocalization in the pancreas different in islet cells and ductal cells throughout fetal life. Trk-A immunoreactivity was observed to be weaker in islet cells than in adult rats in the early postnatal period. In addition, that as the maturation of the pancreas progresses, the Trk-A immunoreactivity was reported in the ducts gradually decreases. Therefore, it was stated that the intensity and localization of Trk-A immunoreactivity in pancreatic tissue are regulated developmentally (Kanaka-Gantenbein, Tazi, Czernichow and Scharfmann, 1995). In pancreatic tissue, Trk-A immunoreactivity has been reported to be strong granular in the cytoplasm of acinar cells and weak cytoplasmic in langerhans islets (Yediel-Aras, 2016). This study was carried out to immunohistochemically investigate Trk-A receptor levels in the pancreas tissue of cumin (*Cuminum cyminum*) plant essential oil treated-mice.

2. MATERIALS AND METHOD

Approval for the research was received from Kafkas University Animal Experimentation Local Ethics Committee (KAÜ-HAYDEK No: 2017-071). Laboratory animals used in the research were supplied from Atatürk University Laboratory Animals unit.

2.1. Animals

Eight-week-old, twenty female mice (*Mus musculus*) weighing 20 ± 1 gr. were used in the study. Mice were grouped into control group (n = 10) and trial group (n = 10). Mice were fed ad libitum and were given free access to tap water. No intervention was performed to the mice in the control group. Oral gavage of cumin volatile oil per 24 hours for two days was applied to the trial group mice depending on their weight as 500 mg/kg (Rodrigues-Alves, Souza dos Santos, Calil, Niero, Lopes, …. Maistro, 2014). At the end of the study, mice were sacrificed under deep anesthesia, and obtained pancreas tissue samples.

2.2. Preparation of cumin extract

Cumin plant was with distilled water vapour with Non-Asbestos brand Clevenger device for 3,5- 4 hours by adding 40 grams of plant and 400 ml of water. At the end of 4 hours volatile oil was stored in drip pan and at +4˚C.

2.3. Histological examinations

Pancreas tissue samples were fixed within 10% formalin solution. Following routine procedures, they were embedded into paraffin blocks, and 5 μm sections were obtained. In order to demonstrate histological structure of pancreatic tissue, the sections were performed Crossman's Triple Staining (Luna, 1968) staining methods.

2.4. Immunohistochemical investigations

The Avidine-Biotin-Peroxidase technique was applied to the tissue samples to investigate Trk-A immunoreactivity (Hsu, Raine and Fanger, 1981). The slides were incubated in 3% H_20_2 (hydrogen peroxide) prepared in 0.1 M phosphate buffered saline (PBS) for 15 min, in order to inhibit endogenous peroxidase activity after deparaffinization and rehydration procedures. Then slides were washed in PBS solution and for releasing the antigenic receptors was boiled in microwave at 600 watts for 10 minutes in a tris- EDTA Buffer solution (pH: 6.0). Tissues were incubated with Blocking Solution A (Invitrogen-Histostatin Plus Bulk Kit) for 10 minutes and then Trk-A (Abcam-AB76291) (1/400 dilution) primary antibody at room

temperature for an hour. Biotinylated Secondary Antibody and Streptavidin-Peroxidase solutions were applied to the slides 30 minutes. DAB-H_2O_2 (Diamino benzidine hidrogen peroxide) (Shu, Ju and Fan, 1988) was applied to the PBS washed tissues as an encolouring substrate. After adding a chromogen solution on tissues, the reaction was terminated with PBS depending on the status of immunoreactivity by controlling under the light microscope. Hematoxylin was applied on tissues for counterstaining after washing with distilled water. Tissues were then dehydrated and covered with immunmount. Staining level was accepted as a criterion and scoring were performed by semi quantitative method. The evaluation was made by two independent observers. Depending on the staining properties, tissues were scored within the range of 0-3 during their evaluation: weak staining (1), intermediate staining (2), intense staining (3) (Zhu, 1989; Seidal, Balaton and Battifora, 2001). In order to test the specificity of immunohistochemical staining, the same procedures without adding antibodies (negative control) were applied to pancreatic tissue of mice in all groups. Tissues prepared for histological and immunohistochemical investigations were evaluated and photographed with a light microscope (Olympus BX51; Olympus Optical Co. Osaka, Japan). The number of Trk-A immunopositive cells were count using the image-j (vI. 50i) software. Numerical distribution of Trk-A positive cells were observed in ten different sections chosen from ten unit area of Langerhans islet and acinar cells of each animals (Eliş-Yıldız, Yediel-Aras, Dağ ve Karadağ-Sarı, 2019).

2.5. Statistical analysis

SPSS (20.0) package software was used to evaluate the data obtained in the study. T test was used to determine the difference between the groups on account of Trk-A immunoreactivity.

3. RESULTS

It was observed that the acinus, islets of Langerhans, pars initialis, pars excretory and ductus excretorius of the pancreatic tissues of control and trial groups in normal histological structure (Figure 1 A-B). Trk-A expression was observed both in the endocrine cells (islets of Langerhans) and in the exocrine cells in mice pancreas of two groups. Trk-A immunoreactivity was observed in acinus, islets of Langerhans and ductus excretorius in mice pancreatic tissues of control and trial groups. Intermediate immunoreactivity was detected in the cytoplasm of acinar cells in pancreatic tissues of control group mice. Weak diffuse cytoplasmic Trk-A immunoreactivity was determined in islets of Langerhans and ductus excretorius (Figure 2-A).

Regions that show Trk-A immunoreactivity findings in pancreatic tissues of the mice revealed similarities with regards to control and trial groups. Intermediate diffuse cytoplasmic Trk-A immunoreactivity was detected in acinar cells whereas islets of Langerhans and ductus excretorius showed weak diffuse immunoreactivity (Figure 2-B).

Count of Trk-A immunoreactivity positive cells acini and Langerhans island in among groups were summarized in Table 1-2. There was no difference between the groups in terms of Trk-A immunoreactivity in acinus and Langerhans islets (p>0.05).

Table 1. Count of Trk-A immunoreactivity positive cells acini in among groups.

Group	N	Mean	Std. Deviation	P
Control	10	400.5	18.02	0.979
Trial	10	400.7	15.98	

There was no difference between the groups in terms of Trk-A immunoreactivity in acinus (p>0.05).

Table 2. Count of Trk-A immunoreactivity positive cells Langerhans island in among groups.

Group	N	Mean	Std. Deviation	P
Control	10	88.6	16.243	0.974
Trial	10	88.4	9.845	

There was no difference between the groups in terms of Trk-A immunoreactivity in Langerhans islets (p>0.05).

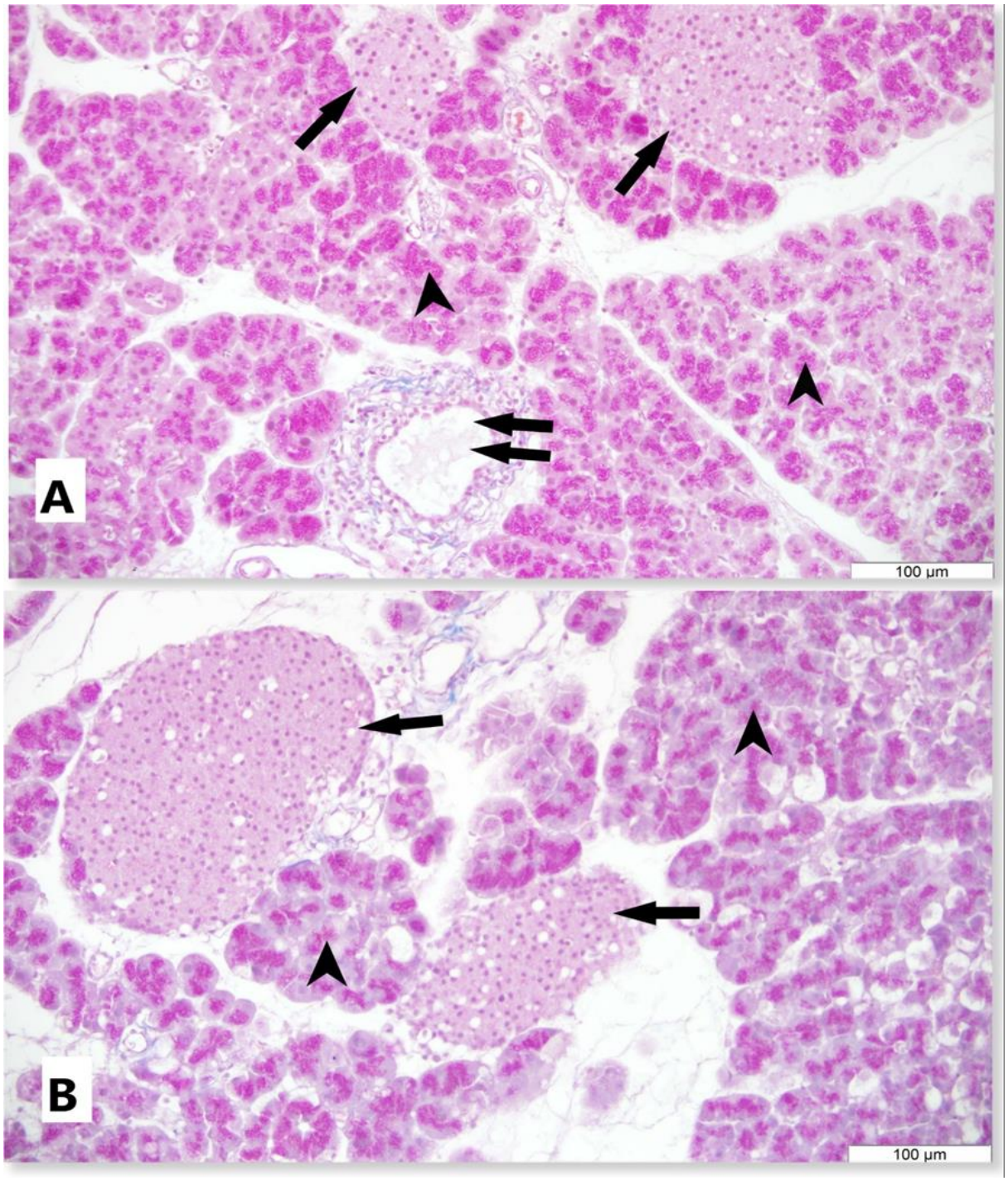

Figure 1. Mice pancreatic tissue. A: Control group, B: Trial group. Arrow: Langerhans island, arrowhead: acini, double arrow: pars excretoria. Triple staining.

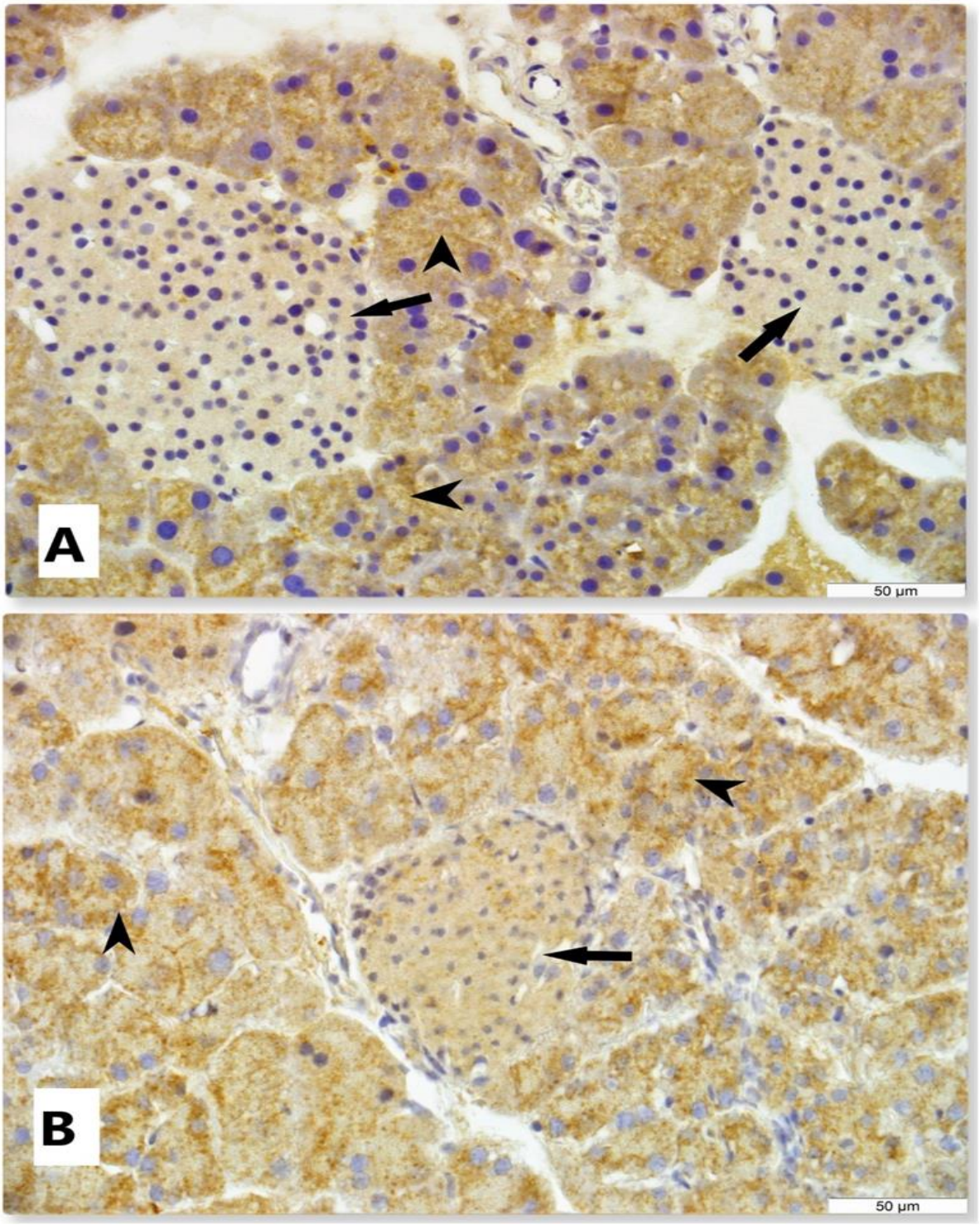

Figure 2. Mice pancreatic tissue Trk-A immunoreactivity. A: Control group, B: Trial group. Arrow: Langerhans island, arrowhead: acini.

4. DISCUSSION AND CONCLUSION

Based on the investigation of cumin on weight loss of overweighed people, high dose cumin application for 8 weeks has positive effects on hight weight, body mass index, total cholesterol and LDL levels (Taghizadeh, Memarzadeh, Abedi, Sharifi, Karamali, Keşan and Asemi, 2016). Cumin was reported to have a preventive effect as a natural antioxidant against experimental hepatotoxicity in mice (Sheweita, El-Hosseiny and Nashashibi, 2016). In a research investigating the antioxidant effects of plants, it was stated that cumin might be used as a natural antioxidant resource in food and it might be effective in diminishing the complications of diabetes (El-Ghorab, Nauman, Anjum, Hussain and Nadeem, 2010; Keihan, Gharib, Momeni, Hemati and Sedighin, 2016). Cumin was also reported to have roles on improvement of immune system in diabetes patients, to diminish IgE to a level close to normal and to decrease cytokine and total blood count levels (Moubarz, Embaby, Doleib and Taha, 2016) Cumin was reported to have hypolipidemic effect on obese rats (Haque and Ansari, 2018).

It was stated that the cumin has antimicrobial and antioxidant activities (Haşimi, Tolan, Kızıl and Kılınç, 2014) and at a result of the investigation on the effect of cumin on pancreatic digestion enzymes, it was notified that cumin used with diet have stimulative effect on amylase, trypsin and chymotrypsin and it might have stimulating effect on digestion (Platel and Srinivasan, 2000). Trk-A immunoreactivity was detected in insulin secreting cells of Langerhans islets and pancreatic duct, and decreasing of Trk-A levels in pancreatic tissue after the occurrence of diabetes was reported (Miralles, Philippe, Czernichow and Scharfmann, 1998; Sposato, Manni, Chaldakov and Aloe, 2007). However Shibayama and Koizumi (1996) did not encounter Trk-A immunoreactivity in acinar cells and Langerhans islets and reported weak immunoreactivity in pancreatic duct. In our research, we detected Trk-A immunoreactivity in acinar cells, Langerhans islets and excretory duct of pancreatic tissue in mice pancreas on both control and trial groups. Trk-A immunoreactivity levels of pancreatic tissue of mice in control and trial groups was the same. In our study, the determination of Trk-A immunoreactivity in Langerhans islets was parallel with Miralles et al., 1998 and Sposato et al., 2007 data. However, Trk-A immunoreactivity was also detected in asinus of pancreas in our study. These results set us think that Trk-A may have roles on the development of pancreatic tissue and synthesis and secretion of various pancreatic hormones. It was also thought that the application dose and duration of cumin may cause similar Trk-A immunoreactivity in the control and trial groups.

In conclusion, cumin is a plant that benefits from diuretic, degassing, digestive facilitating, antimicrobial and antidiyabetic effects in the field of traditional medicine. In our

study, Trk-A receptor secretion was close to each other in the control and trial groups that has important roles in the regulation on the neural development and insulin metabolism of the pancreas. We think that gathered data will contribute to other researches that investigate the effects of cumin on pancreatic tissue related disease.

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