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Research Article

Rats with Acute Gastritis Experimentally Induced with Ethanol: Distribution of Mast Cells and Eosinophils in the Liver, Lungs and Kidneys

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ABSTRACT

Mast cells are found in systems in contact with the external environment, such as blood vessels, nerves, respiration, and digestion. Mast cell proteases play important roles in regulating humoral and cellular events in the tissue. In rodents, mast cells are of two types: mucosal and connective tissue origin. This study aimed to examine mast cells histochemically and eosinophils in the possible damage that ethanol may cause in the liver, lung, and kidneys of rats with gastritis and also to determine whether pomegranate extract affects these cells. In this study, liver, lung, and kidney tissue samples taken from rats were stained with Alcian Blue/ Safranin O, Toluidine Blue to identify mast cells, and Congo Red to identify eosinophils. In the histological evaluation, it was determined that mast cells were located especially around blood vessels. It was determined that heparin-containing mast cells were dense in liver tissue, and histamine-containing mast cells were dense in lung tissue. It was determined that mast cells were less dense in the kidney than in the liver and lung tissues. When the locations and densities of mast cells in rats are considered, it can be said that they undertake similar tasks in functions such as vasopermeability and inflammatory cell response, as in other mammalian species.

Keywords: Heparin, histamine, mast cells, rat

INTRODUCTION

Mast cells and eosinophils are the main effector cells of innate immunity and play a fundamental role in defense mechanisms against bacterial, viral, and parasitic infections. These cells differ in their development, maturation, and location in tissues. Eosinophils mature completely in the bone marrow and enter the circulation as blood cells under physiological conditions. Mast cells leave the bone marrow as progenitor cells and mature in peripheral tissues. These cells are activated under the influence of chemical inflammatory stimuli and accumulate in inflamed tissues, participating in various pathological conditions together. Given their strategic location on major surfaces of the body, including the skin, kidneys, lungs, and the inner surface of the digestive system, mast cells are among the first cells to recognize danger signals from the external stimulating other inflammatory cells. Eosinophils accumulate in organs and tissues following inflammatory and chemotactic stimuli produced as a result of inflammation. Mast cells and eosinophils are, therefore, simultaneously active in many diseases, including infections, allergic and autoimmune disorders, and cancer. Therefore, it is important to investigate the existence, role, and function of these two cells in various conditions (diseases, chemical stimuli, etc.) (1).

Mast cells are found in systems that interact with the external environment, such as blood vessels, nerves, respiratory and digestive systems. In humans and mammals, mast cell granules contain vital factors such as heparin, histamine, prostaglandin, neutral protease, β-glucuronidase, aryl sulfatase, tryptase, eosinophil chemotactic factor of anaphylaxis (ECF-A), and slow

reaction substance of anaphylaxis (SRS-A). These factors and mast cell proteases play important roles in regulating humoral and cellular events in the tissue (2). Mast cells in rodents also contain a substance called serotonin, which is not present in human mast cells and has an effect on the respiratory and digestive tracts (3,4).

In rodents, mast cells are classified as mucosal or connective tissue, depending on where they are located (5). Mucosal mast cells are located in the small intestinal mucosa, while connective tissue mast cells are located in the small intestinal submucosa, skin, skeletal muscle, and serosa (5). Eosinophils, sometimes called acidophils, are white blood cells. They are one of the components of the immune system responsible for combating multicellular parasites and certain infections in vertebrates. Together with mast cells and basophils, they also control mechanisms associated with allergy and asthma. Recent studies have provided important information on the selective infiltration of eosinophils into diseased tissues along with its molecular mechanism (6). It has been reported that eosinophil granule proteins stimulate various cells, including rat mast cells, neutrophils, respiratory goblet cells, basophils, and platelets (7).

Methyl alcohol and isopropyl alcohol are toxic and prohibited from being consumed. However, ethanol is an intoxicating substance and is found in beverages such as beer, wine, and raki (8). Excessive alcohol consumption can damage different organs, such as the brain, liver, heart, lungs, skeletal muscles, and bones. Alcohol taken orally is absorbed from all parts of the digestive tract, then quickly enters the bloodstream and spreads to all tissues (9). Studies have reported that even low doses of ethanol reduce several important functions of mast cells (10). It has been reported that ethanol inhibits histamine release in lung mast cells (10).

Alcohol consumption has harmful effects on the internal organs of the body, and rapid treatment is essential to prevent further deterioration of the situation in alcohol-induced gastritis. However, treatment methods have some negative effects. Therefore, it is important to determine the cellular and molecular pathogenic mechanisms that occur in various organs as a result of alcoholic gastritis. This study was planned to determine the regional localization, distribution, density, and quantitative distribution of mast cells and eosinophils in the liver, lungs, and kidneys damage that ethanol caused due to experimentally induced acute gastritis in rats. It was also planned to determine whether or not pomegranate extract given for prophylactic purposes affects the distribution and quantitative density of these cells.

MATERIAL AND METHODS

Tissue Procurement and Histochemical Procedure

In this study, liver, lung, and kidney tissues taken from the ethanol-induced gastritis in rats were used (2022/8). Twenty-four female Wistar albino rats (180-200 g) were used in the study. The animals were fed ad libitum with pellet feed and water in a constant temperature (22±3 °C) and humidity (50-55%) with 12 hours of light and 12 hours of darkness during the experiment. To create the experimental gastritis model with ethanol, the rats were fasted for 24 hours in advance but were only allowed access to water. Experimental groups include Control (1 mL saline oral gavage), Ethanol (2 mL ethanol oral gavage on the first day), and Ethanol+Pomegranate extract group (2 mL ethanol on the first day, then 0.5 mL (100 mg/kg) Pomegranate extract orally throughout the study (11). At the end of the study period, animals were sacrificed under xylazine-ketamine (10-90 mg/kg) anesthesia by taking blood from the heart, and tissue samples were taken. The tissue samples were fixed in 10% buffered formaldehyde. Tissue samples were embedded in paraffin blocks using the routine histological preparation method. Five µm thick sections were taken from each block and stained with Alcian Blue (AB, pH 0.3) / Safranin O (SO, pH 1.0), Toluidine Blue and Congo Red staining methods (12). Toluidine blue stained reveal mast cell granules, and Alcian Blue/Safranin O stained distinguish granules containing histamine and heparin. Preparations were examined and photographed under a research microscope (Nikon Eclipse E-400) with a digital camera (Nikon Coolpix4500) attachment.

Histochemical Evaluation

The numerical distribution of mast cells was evaluated by counting mast cells in 100 square unit areas with a magnification of 40 from each randomly selected area and converting them into the number of cells in 1 mm^2 unit area (2,13). Statistical analysis of the data was performed using the SPSS (IBM® Ver; 20.0 Windows, USA) package program. Parametric data were expressed as mean and standard deviation (M[SD]). Shapiro-Wilk test was used to check whether the data were normally distributed. ANOVA test was used to compare groups that did not show normal distribution. Kruskal Wallis H Test was used to compare groups that did not show normal distribution. The statistical significance level was defined as $P \leq 0.001$.

RESULTS

The numerical distribution of mast cells and eosinophils in rat tissues is given in Table 1.

Mast cells were found to be scattered, especially in the portal triad region in the liver, around the bronchi, bronchioles, and blood vessels in the lung, around the glomerulus in the kidney, and in the connective tissue between the tubules. Mast cells were observed to be round, spindle, or oval in shape in different sizes.

When the liver and kidneys were compared with the control group, it was determined that the number of mast cells was statistically significant in the groups given ethanol and pomegranate extract and in the lungs in the ethanol group (Figure 2-3, Table 1) ($P<0.05$). It was determined that heparin-containing mast cells were common in the liver, and histamine-containing mast cells were common in the lung tissue. It was

Table 1. Numerical distributions of mast cells and eosinophils between groups in rat liver, lung, and kidneys (M/SD)

	Groups	Mast cells	Eosinophils
Liver	Control	2,42±0,78[a]	1,57±0,53
	Ethanol	2,71±0,75[b]	1,71±0,48
	Ethanol+*P.granatum*	2,85±0,90[b]	1,85±0,37
Lung	Control	2,85±0,90[a]	2,57±0,53
	Ethanol	2,42±0,78[b]	2,57±0,53
	Ethanol+*P.granatum*	2,85±0,90[a]	2,57±0,53
Kidney	Control	1,42±0,53[a]	1,28±0,48[c]
	Ethanol	2,42±0,53[b]	1,28±0,48[c]
	Ethanol+*P.granatum*	2,96±0,78[b]	2,00±0,57[d]

a, b, c, d: Difference between group means with different letters in the same column, p<0.05

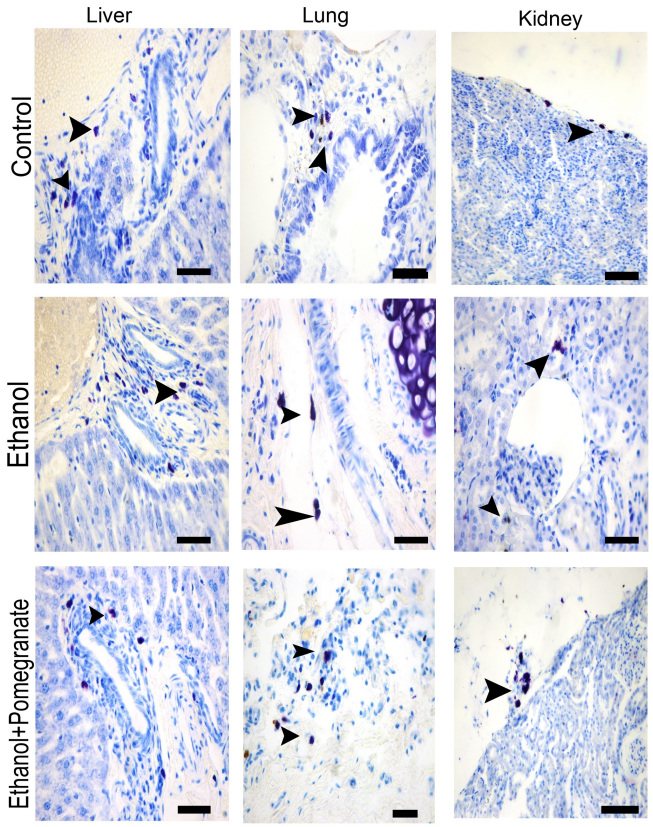

Figure 1: Distribution of mast cells in liver, lung and kidney in control, ethanol and pomegranate groups. Arrow head: mast cells containing heparin. Toluidine blue X40

determined that mast cells were less common in the kidney compared to the liver and lung tissues (Figure 2-3). In addition, histamine-containing mast cells were more common in the liver and lung groups compared to the control group.

It was observed that the localization of eosinophils in the liver, lung, and kidney was similar to mast cells. It was determined that eosinophils were widely distributed in the liver. The number of mast cells in the kidneys was found to be significant in the pomegranate extract group (Figure 3, Table 1) (P<0.05).

DISCUSSION

Considering the frequency of gastric ulcers due to various chemicals and the side effects and costs of some existing synthetic drugs, the use of natural products is an important alternative for many people. In this sense, it has been stated that pomegranate extract is advantageous in the treatment of various disorders in laboratory animals and patients. In addition, it has been shown in short- and long-term studies that such plant-based extracts do not contain toxicity (14). As a

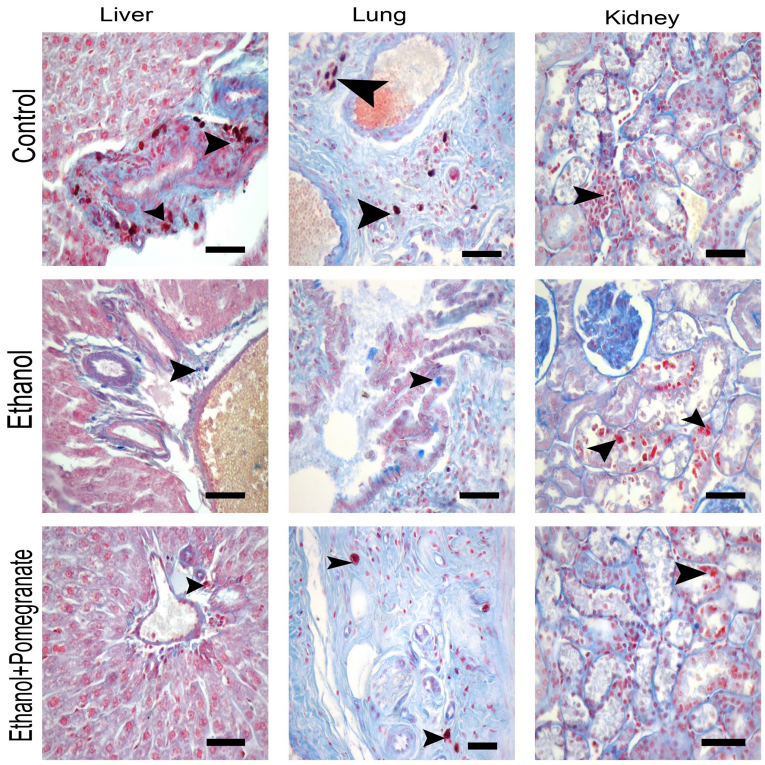

Figure 2: Distribution of mast cells in liver, lung and kidney in control, ethanol and pomegranate groups. Arrowhead: mast cells containing mixed pigment and histamine. Alcian blue/Safranin O X40

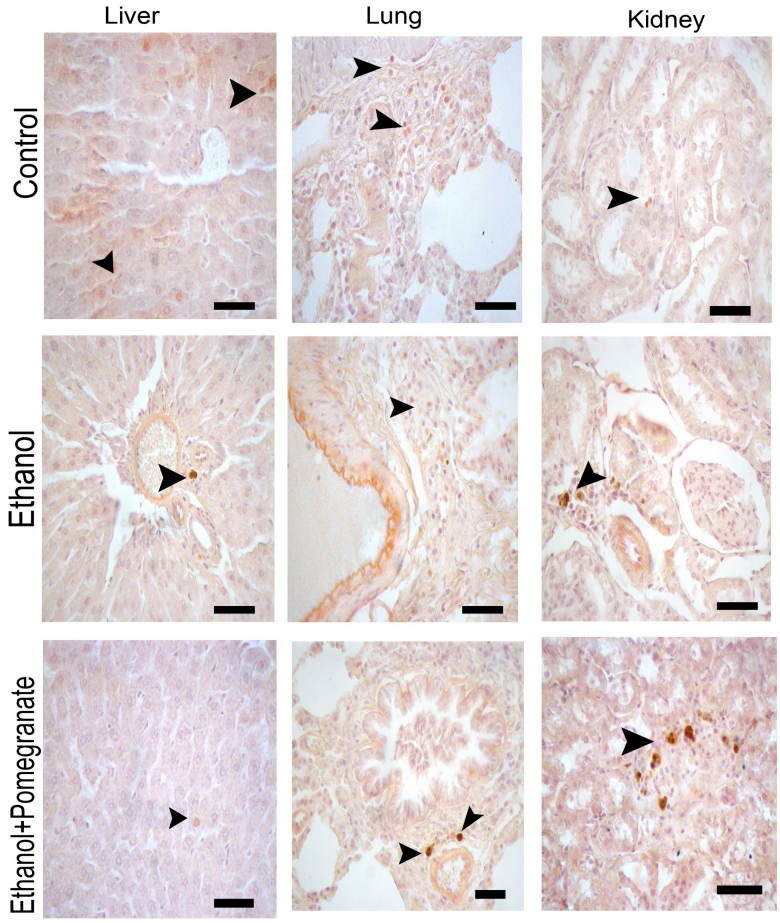

Figure 3: Distribution of eosinophils in liver, lung and kidney in control, ethanol and pomegranate groups. Arrowhead: eosinophils. Congo red X40

result of pomegranate extract given as a preservative revealed that the density of mast cells and eosinophils in the liver, lungs, and kidneys showed differences.

Although mast cells have been discovered for a long time, their functions have been associated with allergic diseases, and they were thought to have very few roles in other diseases and health. However, recent studies have reported that mast cells have very wide and diverse roles in both physiology and diseases (15). Mast cells act as a cellular interface between the external and internal environments and initiate and coordinate innate and adaptive immune responses by interacting with various cell types (16).

In normal rodent livers, there are few mast cells in the portal areas. After the liver injury, the number of hepatic mast cells increases, and they degranulate, releasing numerous growth mediators such as histamine, heparin, tryptase, TGF-1, TNF, ILs, cytokines, and basic fibroblast growth factor (bFGF) (16). Studies on mast cells in the liver have reported that ethanol-induced liver injury affects the function of mast cells (17). In the presented study, the finding of a significant difference in mast cells in the groups given ethanol and pomegranate extract compared to the control group supports the literature data.

In a study conducted on mice that were acutely poisoned with ethanol, it was reported that there was an increase in the number of mast cells due to damage in the lungs (18). In a study conducted on guinea pigs, it was reported that ethanol treatment of ethanol-induced gastritis in rats, it has been inhibited histamine release in lung mast cells (19). Studies have shown that ethanol has an effect on mast cells in the lungs (18,20). In the presented study, the significant number of mast cells in the ethanol group compared to the other groups supports the effect of ethanol on mast cells.

It is reported that mast cells are structurally found in small numbers in the kidneys, but their numbers increase in kidney diseases (21). Mast cells are thought to be related to the development of interstitial fibrosis in the kidneys (22). Ethanol has a direct effect on the kidneys, resulting in the diffusion of cell content into the intercellular space due to the increase in membrane fluidity (23). Studies have shown that the number of mast cells increases in the acute phase of renal diseases (21,22). In the presented study, the number of mast cells was found to be significant in the ethanol group compared to the control group. The number of mast cells was found to be significant in the pomegranate extract group compared to the ethanol group. It can be said that ethanol has a greater negative effect on the kidneys.

Recent studies have also provided important information about the selective infiltration of eosinophils into diseased tissues (24). It is known that mast cell-derived TNF-α and IL-1α stimulate the production of eotaxin, a CC chemokine subfamily of eosinophil chemotactic proteins in epithelial and endothelial cells (24,25). In the presented study, it can be said that eosinophils were not affected by the systemic effect of acute ethanol, but they affected the number of mast cells.

In conclusion, it has been determined that the locations and shapes of mast cells and eosinophils in rat lung, kidney, and liver tissues are similar to those in other mammalian species. It is thought that alcohol affects tissues systemically, that mast cells and eosinophils undergo changes in the affected tissues, and that pomegranate extract may be healing in tissues during acute alcohol consumption due to its antioxidant properties. More studies are needed to determine the effects of tissue damage on mast cells and eosinophils in alcohol consumption.

DECLARATIONS

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author (Z.K.).

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Authors' Contributions: Literature review related to the study (M.K.A., T.B., N.B.), application of experimental procedure (T.B., N.B.), histochemistry laboratory studies (Z.K., M.K.A., T.B.), evaluation of the data obtained and making statistics (Z.K., M.A.K.)

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