Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Yılmaz HC, Yörük M (2022).** The Effects of Bisphenol A on the Distribution and Heterogeneity of Mast Cells in Rat Digestive Tract. *Van Vet J*, 33 (3), 122-129. DOI: https://doi.org/10.36483/vanvetj.1173575

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

The Effects of Bisphenol A on the Distribution and Heterogeneity of Mast Cells in Rat Digestive Tract

Halil Cumhur YILMAZ[1, *] Mecit YÖRÜK[1]

[1] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Histology, 65080, Van, Turkey*

Received: 10.09.2022 Accepted: 06.11.2022

ABSTRACT Bisphenol A (BPA) is a chemical component used in plastic products around the world. This study aims to examine the effects of these chemical compounds to which humans are frequently exposed in everyday life, on the heterogeneity and distribution of mast cells in the gastrointestinal system. For the study, 24 male Wistar albino rats were divided into 4 groups (control, sham, 25 mg/kg, and 50 mg/kg BPA treated). BPA was dissolved in corn oil and administration was done by oral gavage for thirty days. Gastrointestinal tissue samples taken from animals anesthetized with inhalation anesthesia were fixed using BLA (Basic Lead Acetate) and Carnoy fixation. Then, following routine tissue follow-up, they were blocked with Paraplast. Sections (6 µm) taken from the blocks were stained using Toluidine blue (TB) and Alcian blue-safranine O 8GX (AB-SO) combined dyes. Counting and statistical analysis of the mast cells in the TB-stained sections were performed. According to the results of the analysis, a higher number of mast cells was observed in the BLA fixation solution, while the increase in the number of mast cells was statistically significant in the groups treated with BPA ($p<0.05$). In the combined AB-SO 8GX staining results, AB (+), S (+), and Mix (+) mastocytes were found in almost all sections of the digestive tract. As a result of this staining process, again, no difference was found in the semi-quantitative evaluations performed including the groups treated with BPA. Although BPA does not affect the heterogeneity of mast cells, it does affect their distribution. Therefore, it is assumed that further studies will need to be carried out.

Keywords: *Bisphenol A, Gastrointestinal tract, Heterogeneity, Mast cell.*

ÖZ

Bisphenol A'nın Rat Sindirim Kanalındaki Mast Hücrelerinin Dağılımı ve Heterojenitesi Üzerine Etkileri

Bisphenol A (BPA) dünya genelinde plastik ürünlerinde çok yaygın olarak kullanılan kimyasal bir bileşendir. Bu çalışmanın amacı insanların gündelik hayatlarında oldukça sık olarak maruz kaldığı BPA'nın mast hücrelerinin heterojenite ve dağılımı üzerine etkilerinin araştırılmasıdır. Çalışma için 24 adet erkek Wistar Albino sıçan 4 gruba (Kontrol, Sham, 25mg/kg ve 50 mg/kg) ayrıldı. Bu grupların hepsine günlük standart diyet uygulanırken sham grubuna mısır yağı, diğer iki gruba ise (25mg/kg ve 50mg/kg) mısır yağı içerisinde çözündürülmüş olarak BPA 30 gün süre ile gavaj yoluyla verildi. İnhalasyon anestezik ile uyutulan deney hayvanlarından alınan sindirim kanalı doku örnekleri Basic Lead Acetate (BLA) ve Carnoy tespit solüsyonları kullanılarak tespit edildi. Ardından rutin doku takibi yapılarak paraplast ile bloklandılar. Bloklardan alınan 6 µm kalınlığındaki kesitler toluidine blue (TB) ve alcian blue-safranin O 8GX (AB/SO) kombine boyaları kullanılarak boyandı. TB ile boyanan kesitlerde mast hücrelerinin sayımı ve istatistiksel analizleri yapıldı. Analiz sonuçlarına göre BLA tespit solüsyonunda Carnoy tespitine oranla daha fazla sayıda mast hücresi gözlemlenirken, BPA uygulanan gruplarda mast hücre sayılarındaki artış istatistiksel olarak anlamlı bulundu ($p<0.05$). AB-SO 8GX kombine boyama sonuçlarında ise sindirim kanalının incelenen neredeyse her kesitinde AB (+), S (+) ve mix (+) mast hücrelerine rastlandı. Ancak S (+) ve mix (+) Mast hücrelerinin sayıları genel olarak bakıldığında AB (+) mast hücrelerine oranla oldukça düşüktü. Yine bu boyama metodu neticesinde BPA uygulanan gruplar da dahil olmak üzere yapılan semi kantitatif değerlendirmelerde bir fark tespit edilemedi. Sonuç olarak BPA'nın mast hücrelerinin heterojenitesine bir etkisi istatistiksel olarak tespit edilemese de dağılımlarına etkisi olduğu gözlendi. Bundan dolayı daha ileri çalışmaların yapılması gerektiği düşünülmektedir.

Anahtar Kelimeler: *Bisphenol A, Gastrointestinal kanal, Heterojenite, Mast hücresi.*

✉ *Corresponding author: halilcumhuryilmaz@yyu.edu.tr

INTRODUCTION

Bisphenol A is an organic compound classified in the group of phenols. According to the International Union of Pure and Applied Chemistry (UIPAC), its name is 4,4-dihydroxy 2,2-diphenyl propane (Cas no: 80-05-7) (Mikolajewska et al. 2015). First synthesized in 1891, BPA monomers are obtained as a result of a condensation reaction with two parts phenol and one-part acetone (O'Brien 2013). BPA is a chemical that can be used in polycarbonate plastics for food and beverage containers and epoxy resins in the lining of metal cans (Bodin et al. 2014). Bisphenol A is available in a wide range of products, including food storage containers, water bottles, the inner lining of food and beverage cans, electronic devices, thermal papers, medical devices, and materials used in dental treatment to which many people are exposed (Özaydın et al. 2018). In addition to all these, it is also used in the manufacture of many products such as baby bottles and pacifiers, glasses, lenses, compact discs, window panels, including children's toys (Mikolajewska et al. 2015).

This chemical, which is produced in very high quantities, can pass into food and beverages from the containers where it is used under normal conditions. High temperatures (boiling, heating) greatly increase the rate of exposure and penetration (Braniste et al. 2010). BPA can enter the body through ingestion, inhalation, or dermal contact (Mikolajewska et al. 2015). However, since it can leak into food and beverage containers, the main exposure is through the digestive system (Sztmanska et al. 2018). Research shows that when BPA enters the body, it is rapidly absorbed in the gastrointestinal tract and metabolized in the liver and intestines (Mikolajewska et al. 2015). This happens with the glucuronidation activity of the body (Sakamoto et al. 2002). But newborns and babies with low glucuronidation activity are at real risk (Mikolajewska et al. 2015).

The main way of exposure is diet. However, the endocrine effects on the intestinal barrier function in direct contact with oral BPA have not yet been elucidated (Braniste et al. 2010). Similarly, what is known about its effects on mast cells is very limited. Although there have been studies on mediator release from mast cell granules (O'Brien et al. 2014a, 2014b), no information has been found on the effect of BPA on the heterogeneity and distribution of these cells.

Mast Cells

Mast cells are one of the important effector cells of the immune system. Recent studies show that they have immunomodulatory roles in both health and disease (Wernersson and Pejler 2014). Mast cells are present in almost all vascularized tissues in mammalian and non-mammalian vertebrates (Ribatti 2018). In general, mast cells are concentrated in places where antigens such as the skin, respiratory and digestive systems can enter the body (Ertugrul et al. 2017). This is because mast cells are one of the first cells that help the body take precautions against the ingress of foreign substances (Welle 1997).

These tissue cells, which contain pronounced cytoplasmic granules, have an important role in allergic reactions. They are taking place in pathophysiological conditions such as congenital and acquired immunity, wound healing, fibrosis, tumors, and autoimmune diseases (Puxeddu et al. 2003). These cytoplasmic granules may have been previously synthesized and stored, or they may have been synthesized after stimulation (Ertugrul et al. 2017). The immunological activity of mast cells is explained by the release of these substances (Welle 1997). Together with these substances in the granules, mast cells are divided into two groups as connective tissue mast cell (CTMC) and mucosal mast cell (MMC) considering their location, origin, response to the detection solutions used, histochemical differences (Ertuğrul et al. 2018). Unlike rodents, their classification in humans is made according to their protease content. According to this classification, there are mast cells containing only tryptase (mast cell tryptase - MCT) and mast cells containing chymase, cathepsin G, and carboxypeptidase (mast cell tryptase, Chymase - MCTC) together with tryptase. Humans have a third type of mast cell, which carry only chymase (mast cell chymase-MCC) (Ergün 2016).

Differences in mast cells also manifest themselves in their detection and staining properties. Connective tissue mast cells can be stained after fixating with 10% neutral buffered formalin, while mucosal mast cells can only be stained if they are fixated using non-aldehyde fixatives such as Carnoy (ethanol + chloroform + acetic acid) (Tikoo et al. 2018). In light of all this information, Carnoy and BLA (Basic lead acetate) are recommended in any microscopic study to fixate mast cells (Strobel et al. 1981). The most commonly used dyes to distinguish mast cells under a microscope are Toluidine Blue (TB) and Alcian Blue/safranin o (AB-SO) dye combinations. Without separating the mast cell type, toluidine blue staining shows metachromasia by staining the cell granules from purple to red, and mast cells are easily distinguished (Ribatti 2018). In the combined staining used to determine heterogeneity, both types of mast cells can be stained with Alcian Blue, while safranin o stain gives positive results only in connective tissue mast cells (Uslu and Yörük 2013).

The gastrointestinal tract houses the largest population of mast cells in the body. Mast cells respond to external and internal stimuli thanks to the variety of receptors they have on their surfaces. With the increased passage of lumen antigens into the mucosa, the intestinal barrier is disrupted, which further facilitates mucosal mast cell activation, inflammatory responses, and mast cell-enteric nerve interaction (Albert-Bayo et al. 2019).

In this sense, it is not known how BPA affects the heterogeneity and distribution of mast cells after absorption in the intestines, where it is most commonly taken up in the body. This study aims to try to explain this issue.

MATERIAL AND METHODS

This study has been approved by the Local Ethics Committee of Van Yuzuncu Yil University (decision date: 07.03.2019 and numbered: 2019/2).

Experimental Design and Application of BPA

24 male Wistar Albino rats (these rats reached adult age in the same period) with an average weight of 200-250 g. were supplied as experimental animals from Van YYU Experimental Application and Research Center. These rats were divided into four different groups. Two of these four groups received BPA at doses of 25 mg/kg/day and 50 mg/kg/day (Schwetz and Harris 1993, Tolba and Mandour 2018). This BPA application was done by oral gavage by dissolving in corn oil (Menard et al. 2014; Aydemir et al. 2018; Özaydın et al. 2018; Tolba and Mandour 2018). No extra was applied to the control group. In the Sham group, corn oil oral gavage was applied for positive control. During the thirty-day exposure study (Tolba and Mandour 2018), their daily diet (pellet feed-ad libitum nutrition)

was continued as normal in all experimental groups (Aydemir et al. 2018).

The exposure study was terminated by using an isoflurane inhalation anesthetic in experimental animals (Veilleux-Lemieux et al. 2013). Tissue samples were then taken.

Microscopic Examination

Tissue samples taken from the organs forming the digestive tract (esophagus, ventriculus, duodenum, ileum, jejunum, cecum, colon) were fixated by immersion method using Carnoy (Strobel et al. 1981) and BLA (Basic Lead Acetate) (Becker et al. 1985) solutions. The tissues were then processed using routine histological technique and blocked with paraplast (Enerbeck 1966a). The sections prepared from these blocks were stained with 0.5% Toluidine Blue (TB) (Enerbeck 1966b) for identification and counting of mast cells and Alcian Blue-Safranin O 8GX (AB-SO 8 GX) combined dyes to determine their heterogeneity (Tung 1991; Bancroft and Cook 1994).

In the sections prepared with Toluidine Blue, cell counts were performed on 24 randomly determined areas (since there was no previous study on the subject in the literature, the histological layers were ignored and the evaluation was made) without taking into account their histological layers. These counts were made using 100 square ocular micrometers (eyepiece graticule) at a lens magnification of 40. Arithmetic averages of the data obtained as a result of the enumeration of mast cells were taken. These data were then converted to the number of mast cells in a unit area of 1 mm^2 (Mulisch and Welsch 2015) (Romeiss mic. teq.).

Statistical Analysis

Descriptive statistics for continuous variables; expressed as average, standard deviation, minimum, and maximum values. In terms of the relevant feature; a 3-factor Factorial Analysis of Variance was performed to determine whether there was a difference between the levels of organ and group factors. Following the analysis of variance; In identifying different groups and organs Duncan's multiple comparison test was used. Since the interactions were found to be statistically important multiple comparisons were made at the level of subgroups. The statistical significance level was taken as 5% in the calculations and SPSS (ver: 20) statistical package program was used for the calculations.

RESULTS

Microscopic Findings

The first finding was that the Carnoy and BLA fixative solutions protected mast cells and granules quite well. Among these two fixative solutions, it was observed that the BLA fixative solution was more suitable than the Carnoy fixative solution (Figures 1A (BLA), 1B (Carnoy)). In histological staining, Toluidine Blue showed metachromasia in all organ sections taken and showed mast cells purple-violet color. In Alcian Blue-Safranin O staining, Alcian Blue positive (AB+), safranin positive (S+), and mix positive (Mix+) mast cells were easily distinguished, and the granules in the cells were mostly observed in homogenous appearance. (Figures 2A, 2B, 2C, 2D). In all organ sections, mast cells were mostly observed in the lamina propia and submucosa layers, while mast cell numbers were more or less sparse in the other layers. İn Toluidine Blue staining the shapes of the mast cells and granules were fully consistent with the general information about these cells. In the regions where the number of mast cells in the BPA groups increases, there is also a decreased appearance of granules (Figure 3B).

Statistical Analysis Results

When the statistical analysis results of mast cell count in toluidine blue staining were examined, more mast cells were detected in tissue samples detected with BLA fixation solution than Carnoy. When the number of mast cells was examined regardless of the fixative and experimental group, the highest number of mast cells was found in the cecum and the least in the ventriculus section. It is thought that this may be because there is more absorption in the intestinal parts than in the ventriculus. When the experimental groups are examined, the increase in the number of mast cells is noticeable in the groups where BPA is applied in doses of both 25 mg/kg/day and 50 mg/kg/day. When this increase is evaluated based on organs, it is seen that the number of mast cells in the jejunum, cecum, and colon sections increases (Table 1) (<0.05). According to the semiquantitative evaluations in tissue samples stained with AB-SO 8 GX Combined dye, no significant difference was found between the S+, AB+, or Mix+ features of mast cells. Again, it was seen that this situation was similar when evaluated separately within the experimental groups and fixatives (Table 2). Accordingly, no significant heterogeneity could be detected.

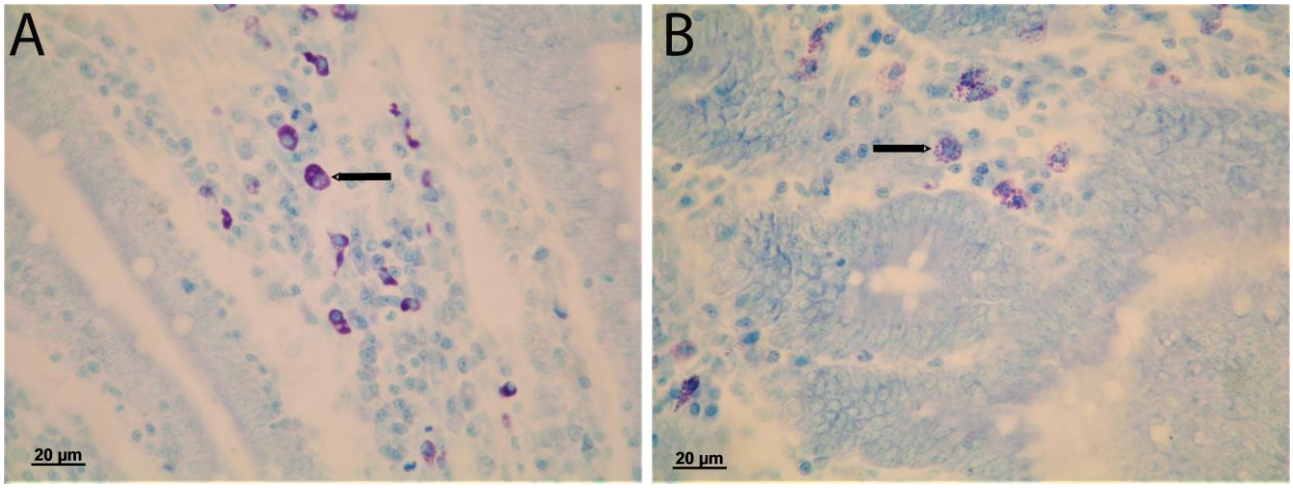

Figure 1. TB staining of mast cells in BLA (A) and Carnoy (B) solutions.

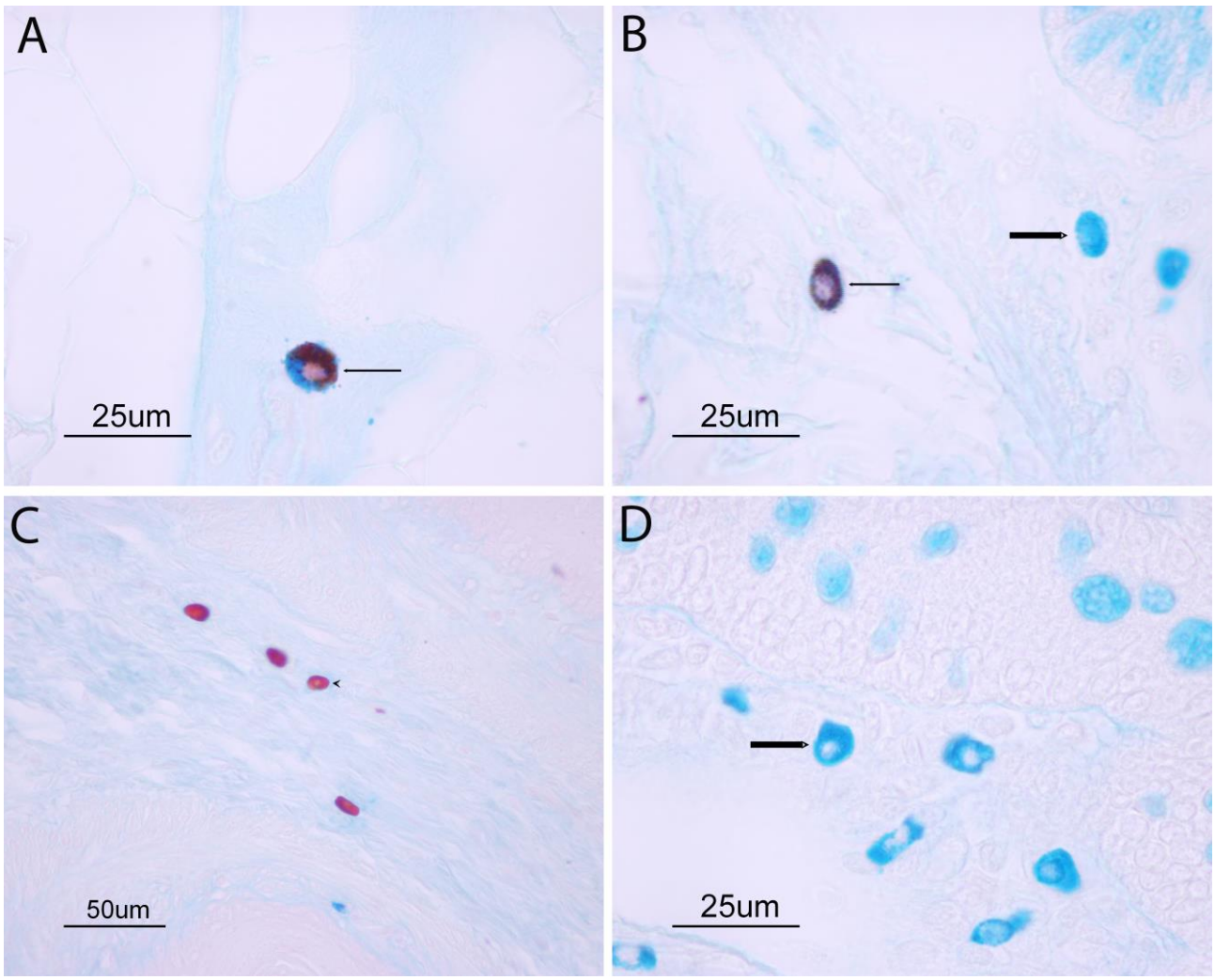

Figure 2. Mast cells in AB-SO staining. Mix+ (A-B), S+ (C), AB+ (B-D).

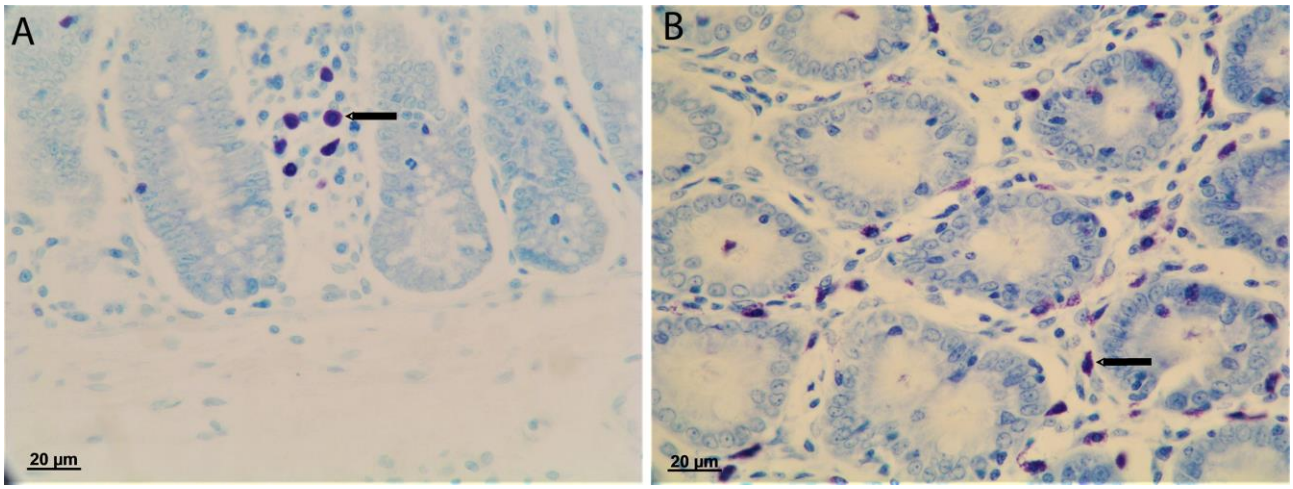

Figure 3. Mast cells in TB staining. The appearance of mast cells in Sham (A) and 50 mg/kg/day (B) groups in the cecum.

Table 1. Descriptive statistics and comparison results of mast cells.

Fixative	Organ	GROUP															
		1-Control				2-Sham				3-BPA-25mg/kg				4-BPA-50mg/kg			
		Av.	Std. dev.	Min.	Max.	Av.	Std. Dev.	Min.	Max.	Av.	Std. Dev.	Min.	Max.	Av.	Std. Dev.	Min.	Max.
1-Carnoy	Esophagus	b 50.72	14.35	33.28	70.56	Cd# 38.72	6.26	32.64	49.28	a 55.72	21.08	35.92	84.00	c 44.27	9.59	35.20	57.92
	Ventriculus	c 30.37	11.25	19.20	52.00	d 30.05	9.71	17.92	44.64	b 37.60	9.08	29.28	53.28	c 35.52	11.28	21.92	49.92
	Duodenum	ab 66.93	18.46	44.64	95.20	a 77.71	13.38	61.28	92.64	a# 74.51	18.22	60.00	108.64	b 66.83	15.40	45.92	92.00
	Ileum	b# 56.83	9.58	48.64	73.92	ab# 58.51	22.77	41.28	96.64	a# 61.81	14.92	36.64	75.20	b 74.69	10.96	59.20	89.28
	Jejunum	Bab 62.0	19.60	48.64	101.92	B# 50.13	14.40	36.64	71.20	B# 67.95	15.91	49.28	90.56	Aa# 93.28	12.58	78.56	109.28
	Cecum	Ba# 78.40	15.25	64.00	105.28	Bab# 69.60	24.55	40.64	97.92	Ba# 64.27	15.11	51.20	90.56	Aa# 100.79	16.32	71.20	115.20
	Colon	Bc# 15.49	2.53	12.64	18.56	Ba# 19.25	12.41	5.28	41.28	Ab# 32.48	9.60	23.20	48.64	Ac# 38.03	13.35	18.56	57.28
2-BLA	Esophagus	Bd 38.13	5.75	30.56	47.20	ABc 47.04	3.80	41.92	53.28	Ad 53.95	10.97	43.20	69.28	ABd 43.07	11.75	28.00	57.92
	Ventriculus	d 33.04	4.97	25.28	37.92	c 43.15	17.49	25.92	69.92	d 42.03	9.79	34.56	60.00	d 34.72	12.55	25.92	59.20
	Duodenum	bc 94.37	28.66	55.20	126.56	b 85.49	31.57	28.64	121.92	b 106.16	20.97	84.64	135.20	c 83.07	17.77	68.64	105.92
	Ileum	b 106.08	15.33	80.00	121.28	b 90.03	19.42	73.92	127.20	b 105.47	14.53	81.92	127.20	bc 95.63	21.49	76.00	121.92
	Jejunum	Bc 81.39	19.58	67.20	118.40	B 82.85	5.02	77.28	92.00	Ab 116.93	11.66	100.00	131.20	Ab 110.37	12.57	96.64	128.64
	Cecum	Ba 134.03	26.42	84.64	163.20	Ba 138.75	12.57	121.28	151.20	Aa 185.31	37.43	153.92	249.92	Aa 173.25	16.86	150.56	194.56
	Colon	Bc 60.37	13.73	39.20	76.00	B 58.05	20.86	29.28	92.00	A 79.25	14.06	60.64	97.92	Abc 95.15	11.24	80.00	113.92

A. B. C → The difference between groups that receive different capital letters within the same fixative and organ (on the same line) is statistically significant ($p<0.05$) (Comparison of groups.).

a. b. c ↓ The difference between the organs receiving different lowercase letters within the same group and fixative (in the same column) is statistically significant ($p<0.05$) (Comparison of organs.).

#: The difference from the second fixative is significant ($p<0.05$).

Table 2. Results of semi-quantitative evaluation of AB-SO staining of mast cells.

		CONTROL		SHAM		BPA-25mg/kg		BPA-50mg/kg	
		Carnoy	BLA	Carnoy	BLA	Carnoy	BLA	Carnoy	BLA
Esophagus	AB	+++	+	+++	+	+++	+	+++	+
	SO	++	++	++	+	+	+++	+	+++
	Mix	++	+++	+	+++	++	++	++	++
Ventriculus	AB	+++	+	+++	++	+++	+	+++	++
	SO	++	+	+	+	+	+	+	+
	Mix	++	+++	++	+++	++	+++	++	+++
Duodenum	AB	+++	+++	+++	+++	+++	+++	+++	+++
	SO	-	-	±	-	±	-	-	±
	Mix	±	±	-	±	±	±	±	±
Ileum	AB	+++	+++	+++	+++	+++	+++	+++	+++
	SO	-	±	-	-	-	-	-	-
	Mix	-	±	±	-	±	-	-	-
Jejunum	AB	+++	+++	+++	+++	+++	+++	+++	+++
	SO	-	±	±	-	±	-	±	-
	Mix	-	±	-	-	±	-	±	-
Cecum	AB	+++	+++	+++	+++	+++	+++	+++	+++
	SO	±	±	+	-	±	±	±	+
	Mix	+	+	++	+	++	++	+	++
Colon	AB	+++	+++	+++	+++	+++	+++	+++	+++
	SO	-	-	-	±	±	±	±	-
	Mix	-	±	±	±	±	+	±	±

(-) Not available (±) Very rare (+) Rare (++) Few (+++) Too many.

DISCUSSION AND CONCLUSION

This study, it was aimed to investigate the effects of BPA taken by digestion on the heterogeneity and distribution of mast cells in the organs that make up the gastrointestinal tract. There is only a limited amount of literature on the effects of BPA on immune system cells and especially on mast cells (O'Brien et al. 2014a, 2014b).

Results in direct proportion to the literature information were obtained from the fixative solutions used in the study. In the literature, it is emphasized that better results are obtained with BLA and IFAA detections in the respiratory tract of geese and ducks (Uslu and Yörük 2013). Similarly, in a study conducted on dog skin, a greater number of mast cells were fixated in the fixative of BLA (Becker et al. 1985). In a study conducted in the digestive tract of turkeys, it is stated that basophilic granules are observed more clearly in the tissues fixated by BLA (Uslu and Yörük 2008). The research findings are in parallel with all these studies in both types of mast cells.

The most preferred dye for the demonstration of mast cells in histological preparations is TB staining, which shows metachromasia. But for the determination of heterogeneity, AB-SO combination dye is preferred. Metachromasia was easily distinguished in this study, as in TB staining of geese and duck (Uslu and Yörük 2013), turkey (Uslu and Yörük 2008), quail and chicken (Kurtdede and Yörük 1995), cattle, sheep and goat (Ertuğrul and Kurtdede 2017). As a result of AB-SO staining, AB+, S+, and mix+ mast cells were detected along the rat digestive tract. Tung Wang (1991) has observed that as a result of AB-SO 8GX staining in the small intestines of chickens, mast cells in the villi contain only blue-colored dyed granules, while those in the submucosa contain both blue and red granules. In their study of the digestive tract, Yörük and Uslu (2008) report that connective tissue mast cells are stained red and mucosal mast cells are painted blue, and S+ stained mast cells are very rare. Again, in the study conducted by Yörük and Karaca (2004), similar results were obtained in terms of the AB-SO staining properties of mast cells and the regions where they are located. The findings obtained as a result of AB-SO staining in all these studies are directly proportional to this study.

In this study of rats' digestive tract, mast cells were seen in the esophagus, stomach, and colon parts of the digestive tract, mostly in the submucosa layer. In other parts of the digestive tract, the density is in the areas where the villi and crypts are. Statistically, mast cells were detected at least in the stomach (avg: 35,81) and at most in the cecum (avg: 118, 04) parts. In the groups that received BPA, the number of mast cells increased considerably compared to the non-applied groups. It is possible to see that this increase is especially in the jejunum, cecum, and colon parts (Table 1).

In the studies of Strobel (1981) in human jejunum, Ertuğrul (2017) in the urinary systems of cattle, sheep, and goats, and Uslu (2013) in the goose respiratory tract, the density of mast cells detected in lamina propria in the regions where villus and crypts are located is seen as in this research. The increase in the number of mast cells was similarly detected in a study conducted on the canine intestinal mucosa (Eren et al. 2000). Likewise, Sokol (2013) says that mast cell counts are always higher in inflammatory bowel disease (IBD) situations. Mast cells increase in inflammatory skin diseases such as atopic dermatitis and psoriasis, as in IBD, but also in diseases such as celiac, and irritable bowel syndrome (Frossi et al. 2018).

In a study on the activation of mast cells (Akin 2017), two conditions are identified in which this activation can cause pathological clinical symptoms. The first are situations in which mast cells are produced qualitatively and quantitatively abnormally, and the second is when mast cell activation is disproportionate to the need to protect the body from perceived danger. This can occur when there is an imminent threat from infections, physical triggers, poisons, or allergens. An extreme example of abnormal mast cell activation is anaphylaxis. In this study, as a result of BPA application, it was observed that there was an increase in the number of mast cells in the digestive tract, especially in the cecum section, and that there was a decrease in cell granules, especially in the villi areas of the digestive tract. In light of this information, it is thought that BPA activates mast cells quite a lot. The increase and abnormal activation of mast cell counts in the digestive tract may also affect released mediators, visceral sensitivity, and intestinal barrier function, as noted in another study (Ramsay et al. 2010).

BPA, a monomer of polycarbonate plastics and epoxide resins, has been shown to function as an endocrine active compound and increase the inflammatory response (O'Brien et al. 2014a). According to the statistical analysis results obtained in this study, the increase in the number of mast cells in the experimental groups where BPA was applied and the decreased appearance in the granules of mast cells that stood out at the microscopic level also support this information.

In the literature, O'Brien et al. have two studies investigating the release of BPA from mast cell lines through mediators (2014a, 2014b). In the first of these studies, the relationship between BPA exposure in the perinatal period and asthma pathogenesis in the adult period was examined. To determine this relationship, asthma-related inflammatory mediators were evaluated through mediators of bone marrow-derived mast cells (BMMC). In this study, it was determined that the release of cysteinyl leukotrienes (CysLT), PGD2, TNF-a, and IL-13 from BMMCs increased in adulthood as a result of perinatal BPA exposure, and a link was established between perinatal BPA exposure and irregularity of mast cells in adulthood (O'Brien et al. 2014b). The second study investigated whether short-term BPA exposure in mice (amounts thought to be exposed to humans) would increase the release of histamine and CysLTs from BMMCs. In this study, acute BPA exposure increased the release of histamine and CysLTs from mast cells in vitro. (O'Brien et al. 2014a) The findings obtained from these two studies that BPA can stimulate mast cells and increase mediator release coincide with the findings of our study. (Figures 3A,3B)

As can be seen from the results of statistical analysis (Table 1), the increase in mast cell numbers occurring in the digestive tract of experimental animals where BPA was administered through oral gavage was detected in the jejunum, cecum, and colon. In their study, Sakamoto et al. (2002) showed that orally administered BPA by dissolving in olive oil was glucuronized, by UDP-glucuronyltransferase UGT2B1 in the liver in rats, and excreted into the bile. They found that most of the Bisphenol A glucuronide (BPA-GA), which passes through bile into the small intestine, is deconjugated in the cecum. After BPA administration (after 15 min), they detected BPA-GA in the small intestine, but free BPA in the contents

of the cecum and colon. They have stated that BPA is reabsorbed in the colon because the free BPA detected in the colon is less than in the cecum. This information suggests that the increase in the number of mast cells detected in the cecum and colon may be related to the digestive parts where free BPA is present.

BPA is a chemical that is widely used around the world and can have negative effects on the lives of almost all living things. It was concluded that further studies were needed to increase knowledge about the effects of this chemical on immune system cells, disease, tissue, and organ. In this study, the heterogeneity and distribution of mast cells in BPA-treated experimental animals were tried to be revealed. Since there is not enough literature information in terms of the effects of BPA on immune system cells, especially mast cells, it is thought that this study will contribute to the literature on this subject.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ACKNOWLEDGMENT

This research was supported by Van Yuzuncu Yil University Scientific Research Projects Coordination Unit (Project ID: TDK-2019-8358).

This research article was summarized from the first author's PhD thesis.

This study has been approved by the Local Ethics Committee of Van Yuzuncu Yil University (decision date: 07.03.2019 and numbered: 2019/2).

AUTHOR CONTRIBUTIONS

Idea / Concept: HCY, MY
Supervision / Consultancy: HCY, MY
Data Collection and / or Processing: HCY, MY
Analysis and / or Interpretation: HCY, MY
Writing the Article: HCY, MY
Critical Review: HCY, MY

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