PEYER'S PATCHES, BISPHENOL A INGESTION AND SELENIUM SUPPLEMENTATION IN MURINE MODELS.

João Araújo[1], Clarisse Fonseca[1], Marcos Paulo Carvalho Castro[1], Bianca Mickaela Santos Chaves[1], Ulisses de Sousa Rigon[2], Jordana Fonseca Reis[2], Sergio Guerra[1], Kelly Palombit[1], and Airton Conde Júnior[1]

[1]Universidade Federal do Piaui
[2]Universidade Federal de Piaui Departamento de Medicina

November 15, 2023

Abstract

Bisphenol A [2,2-bis(4hydroxyphenyl) propane, BPA], one of the endocrine disruptors, is of great concern due to its widespread use throughout the world. Several studies have shown that BPA has toxic effects when ingested, making contact with this substance a risk factor for the development of diseases in various organs, including the intestine and associated lymphoid tissue. Therefore, the objective of this work was to study the morphology of Peyer's patches in young rats supplemented with selenium and exposed to BPA. The study was approved by the UFPI Animal Use Ethics Committee, with protocol number 583/19. 28 male Wistar rats (*Rattus novergicus albinus*) were randomly divided into 4 groups: Control (CT), BPA, Se and BPA+Se. The pups were weaned on the 21st day and, from the 22nd postnatal day, the animals in the BPA group received daily doses of 5 mg/kg of BPA diluted in 0.3 ml of corn oil, administered orally. The Se group received 10 µg/kg of Se, the BPA+Se group received 5 mg/kg of BPA and 10 µg/kg of Se and the CT group did not receive any substance but was subjected to the oral gavage process. After 4 weeks of exposure, the puppies were anesthetized and euthanized for intestinal collection and subsequent histological, immunohistochemical and morphometric analysis. Our results showed that BPA caused damage to the epithelial layer of Peyer's patches, there was a structural disarray in the architecture of the follicular region of the BPA and BPA+Se groups, zones of inflammation with the presence of vacuoles in the tissue. A reduction in Ki-67 expression in Peyer's patches was also observed in the BPA group, as well as a significant reduction in the number of defense cells in the group. Animals exposed to BPA, but supplemented with Se, showed no damage to the epithelial layer and there was no reduction in Ki-67 expression. BPA, when ingested above the safe level, altered Peyer's patches tissue, and reduced cell proliferation. Additionally, BPA reduced the immune cell population in Peyer's patches. Se was able to reverse the observed damage, suggesting antioxidant potential.

TITLE: PEYER'S PATCHES, BISPHENOL A INGESTION AND SELENIUM SUPPLEMENTATION IN MURINE MODELS.

João Victor Silva Araujo[1], Clarisse Maria Barbosa Fonseca[1], Marcos Paulo Carvalho Castro[2], Bianca Mickaela Santos Chaves[2], Ulisses de Sousa Rigon[3], Jordana Fonseca Reis[3], Sergio Paulo Lima Guerra[4], Kelly Palombit[5], Airton Mendes Conde Junior[6]

[1]Doctoral Program in Technologies Applied to Animals of Regional Interest Federal University of Piauí, Brazil, Campus Ministro Petrônio Portella - Ininga.[2]Undergrad Student, Department of Nutrition, Federal University of Piauí, Brazil. [3]Undergrad Student, Department of Medicine, Federal University of Piauí, Brazil. [4]Master Degree, Department of Morphology, Federal University of Piauí, Brazil.[5]Doctoral Degree, Department of Morphology, Federal University of Piauí, Brazil. [6]Post-Doctoral Degree Department of Morphology, Federal University of Piauí, Brazil.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding statement: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest disclosure: The authors declare no conflict of interest.

Ethics approval statement: The research was approved by the Ethics Committee on the Use of Animals of the Federal University of Piauí with nº 583/19.

Acknowledgment: The authors wish to thank the Federal University of Piaui for making this research possible through the donation of animals

[1]Corresponding Author:

João Victor Silva Araújo1

Doctoral Program in Technologies Applied to Animals of Regional Interest Federal Federal University of Piauí, Campus Ministro Petrônio Portella - Ininga CEP: 64049-550 / Tel.: +55 86 3215-5868 / +55 86 99415-7112 Teresina – PI

Department of Morphology – Federal University of Piauí

E-mail: araujo.jvsbio@gmail.com

Research Highlights

1. BPA, when ingested above the safe level, altered PP tissue, and reduced cell proliferation.

2. In addition, BPA reduced the population of immune cells in PP.

3. Se has a possible protective effect against toxicity induced by BPA exposure.

ABSTRACT

Bisphenol A [2,2-bis(4hydroxyphenyl) propane, BPA], one of the endocrine disruptors, is of great concern due to its widespread use throughout the world. Several studies have shown that BPA has toxic effects when ingested, making contact with this substance a risk factor for the development of diseases in various organs, including the intestine and associated lymphoid tissue. Therefore, the objective of this work was to study the morphology of Peyer's patches in young rats supplemented with selenium and exposed to BPA. The study was approved by the UFPI Animal Use Ethics Committee, with protocol number 583/19. 28 male Wistar rats (*Rattus novergicus albinus*) were randomly divided into 4 groups: Control (CT), BPA, Se and BPA+Se. The pups were weaned on the 21st day and, from the 22nd postnatal day, the animals in the BPA group received daily doses of 5 mg/kg of BPA diluted in 0.3 ml of corn oil, administered orally. The Se group received 10 μg/kg of Se, the BPA+Se group received 5 mg/kg of BPA and 10 μg/kg of Se and the CT group did not receive any substance but was subjected to the oral gavage process. After 4 weeks of exposure, the puppies were anesthetized and euthanized for intestinal collection and subsequent histological, immunohistochemical and morphometric analysis. Our results showed that BPA caused damage to the epithelial layer of Peyer's patches, there was a structural disarray in the architecture of the follicular region of the BPA and BPA+Se groups, zones of inflammation with the presence of vacuoles in the tissue. A reduction in Ki-67 expression in Peyer's patches was also observed in the BPA group, as well as a significant reduction in the number of defense cells in the group. Animals exposed to BPA, but supplemented with Se, showed no damage to the epithelial layer and there was no reduction in Ki-67 expression. BPA, when ingested above the safe level, altered Peyer's patches tissue, and reduced cell proliferation. Additionally, BPA reduced the immune cell population in Peyer's patches. Se was able to reverse the observed damage, suggesting antioxidant potential.

Keywords: Gastrointestinal tract, Immune system, Intestinal mucosa, Bisphenol A.

1. INTRODUCTION

The gastrointestinal tract is made up of several cell types such as epithelial cells, goblet cells, and immune cells that play a significant role in gut homeostasis. Since the gastrointestinal tract is exposed to a significant number of pathogens, structures such as Peyer's patches (PP´s) act in the identification of antigens and in the production of antibodies (Okumura; Takeda, 2017; Zhang; Wu, 2020; Reboldi; Cyster, 2016; Panneerselvam; Budh, 2020).

Chemical compounds such as endocrine disruptors are substances capable of interfering with the synthesis, secretion, and metabolism of hormones. In addition, these chemicals can modulate the body's immune response. Among them, Bisphenol A (BPA) stands out, known to bind to estrogen receptors and thus interfere with their actions (MALOY; POWRIE, 2011; VANDENBERG et al., 2012; Ratajczak-Wrona et al., 2020; MILANO et al., 2022).

Several routes of exposure to BPA have been described, vertical transmission (maternal-fetal), the respiratory system (inhalation) and the integumentary system (skin contact). However, the food route is the main form of contamination of this compound. (Almeida et al., 2018; López-Rodríguez et al., 2021; Faheem et al., 2021; Jang et al., 2022). The European Food Safety Authority has carried out a reassessment of the risks to public health related to the presence of BPA in foodstuffs. This organization established a reduction of the tolerable daily intake (TDI) of BPA from 4 μg/kg of body weight per day to 0.2 ng/kg of body weight per day (EFSA, 2023).

Selenium (Se) has become an element of great interest in view of its antioxidant effect. Critical functions of selenoenzymes include participation in the regulation of thyroid hormone synthesis, increased male fertility, and anti-inflammatory effects. Among the 25 selenoproteins identified in humans, GPxs and TrxRs are well-recognized antioxidant enzymes (Hariharan; Dharmaraj, 2020; Minich et al., 2022; Labunskyy; Hatfield; Gladyshev, 2014).

Previous studies have shown that using selenium alone ameliorated BPA toxicity in the liver, testicles, and lungs (Abedelhaffez et al. 2017; Amraoui et al., 2018; Khalaf et al., 2019; Ahmed Zaki et al., 2021; Bashir et al. 2022). Kaur et al. (2021) found that Se supplementation considerably restored the activities of antioxidant enzymes and reduced the expressions of stress-activated kinases, which further decreased apoptosis. Thus, Se supplementation has been shown to be effective against testicular damage caused by BPA.

In this sense, Se supplementation can reduce the risk of damage to various organs, such as the intestine, heart, lungs, and kidneys, as well as determine a protective effect against toxicity by substances such as BPA, which has already been demonstrated in some studies (Wetherill et al., 2007; Wang et al., 2015; Al-Amoudi, 2018). Thus, the objective of this study was to study the morphology of Peyer's plaques in young rats supplemented with selenium exposed to BPA.

2. MATERIALS AND METHODS

2.1 Ethical aspects

The research design is experimental. All the procedures described were approved by the Ethics Committee on the Use of Animals (CEUA), of the Federal University of Piauí, with protocol number 583/19. The procedures carried out in this study are in accordance with the ethical guidelines for animal experimentation recommended in the "European Communities Council Directive" of November 24, 1986 (86/609/EEC) on the recommendations for the care and use of laboratory animals.

2.2 Animals and Application of Bisphenol A

Twenty-eight young male Wistar rats (*Rattus novergicus albinus*) (n=7/group studied) were used, obtained from the Vivarium of the Center for Agrarian Sciences (CCA) of the Federal University of Piauí (UFPI). The animals were fed feed and water ad libitum.

The animals were kept with their sows until the 21st postnatal day in the Vivarium of the Department of Biophysics and Physiology of the Health Sciences Center (CCS) and randomly divided into four groups:

Control Group (TC); BPA Group, BPA+Se Group and Se Group.

From the 22nd postnatal day, the male pups in the BPA group (n=7) received daily doses of 5 mg/kg of BPA (a dose already used by the research group, Chem Service Inc., West Chester, PA) diluted in 0.3 ml of corn oil administered orally with gavage. ® The BPA+Se group (n=7) received daily doses of 5 mg/kg of BPA + 10 µg/kg of Se dissolved in water (used as sodium selenite - Na2SeO3 - Dynamic).® The Se group (n=7) received 10 µg/kg of Se dissolved in water (used in the form of sodium selenite - Na2SeO3 - Dynamic)® and the TC group (n=7) did not receive any substance, but underwent the oral gavage process (Al-Amoudi, 2018).

After 4 weeks of exposure and supplementation, the animals were taken to the Morphological Sciences Research Laboratory (LABCIM-UFPI), where the euthanasia protocol was performed in which they received a dose of analgesic (Tramadol 4mg/kg - intraperitoneally) and after 5 minutes they were anesthetized with an anesthetic combination of xylazine (10 mg/kg) and ketamine (80 mg/kg) administered by deep intramuscular route.

Then, the animals were euthanized using an anesthetic overdose, and then an incision was made in the abdominal region of the animal, exposing the intestinal loops to remove the material to be studied. The tissues were stored in 10% buffered formalin solution.

2.3 Histological Analysis

Samples of small intestine from the control (n = 7), BPA (n =7), BPA+Se (n = 7) and Se (n = 7) groups were immersed in 10% paraformaldehyde overnight. Then, the samples were treated with different concentrations of alcohol and xylene and were embedded in paraffin (Sigma). Samples from all groups were cut into 5 µm sections and stained with hematoxylin-eosin (H & E), as well as Masson's trichrome.

2.4 Immunohistochemistry

Immunostaining was performed with anti-Bcl-2 antibody (CONFIRM anti-bcl-2 (124) - Mouse Monoclonal Primary Antibady, Ventana, Roche, United States) and anti-Ki-67 (CONFIRM anti-Ki-67 (30-9) - Rabbit Monoclonal Primary Antibody, Ventana, Roche®®, United States) expressed in the cytoplasm and nucleus, respectively.

The tissues were fixed, embedded in paraffin, and cut to approximately 4 µm thickness. They were then placed on positively charged TOMMO (VWR®) slides for IHC analysis. The slides containing the samples of interest were deparaffinized in a xylol bath, hydrated in ethyl alcohol, then subjected to automated reaction in the BENCHMARK IHC/ISH equipment (Ventana, Roche®, United States).

The ultraView DAB Detection Kit was used, the antibodies evaluated, the reagents, the contrast agents, and the contrast agents (Hematoxilin II and Bluing Reagent), as provided by the manufacturer.

2.5 Morphometric Analysis

The number of leukocytes was counted by examining all assembly preparations under a light optical microscope with a magnification of 1000 ×. All well-defined cells in each fragment were counted. For quantification, counts were made in five microscopic fields alternating for each animal in the region of the germinal center of the PP´s.

2.6 Image Analysis

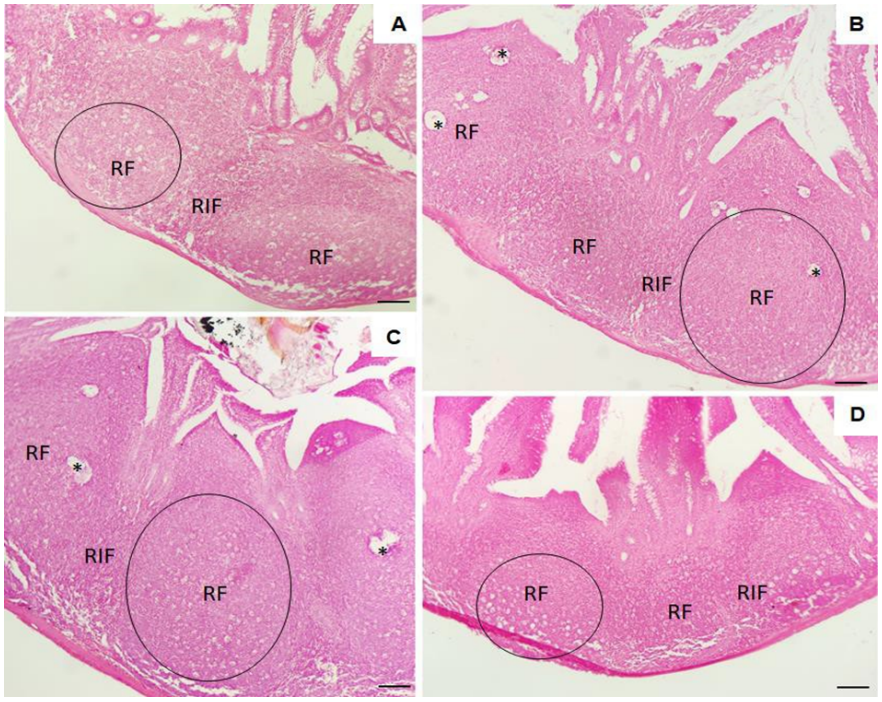
The photos of the tissues were obtained using a light microscope and the Xiaomi 11 mobile phone camera, Mi 11 Lite 5G version, (9216x6912). The boards were made using GIMP software version 2.10.34.

2.7 Statistical analysis

The data were statistically compared by analysis of variance (ANOVA), followed by Tukey's test, with a significance level of $p < 0.05$. The analysis was performed using GraphPad Software, San Diego, CA, USA.

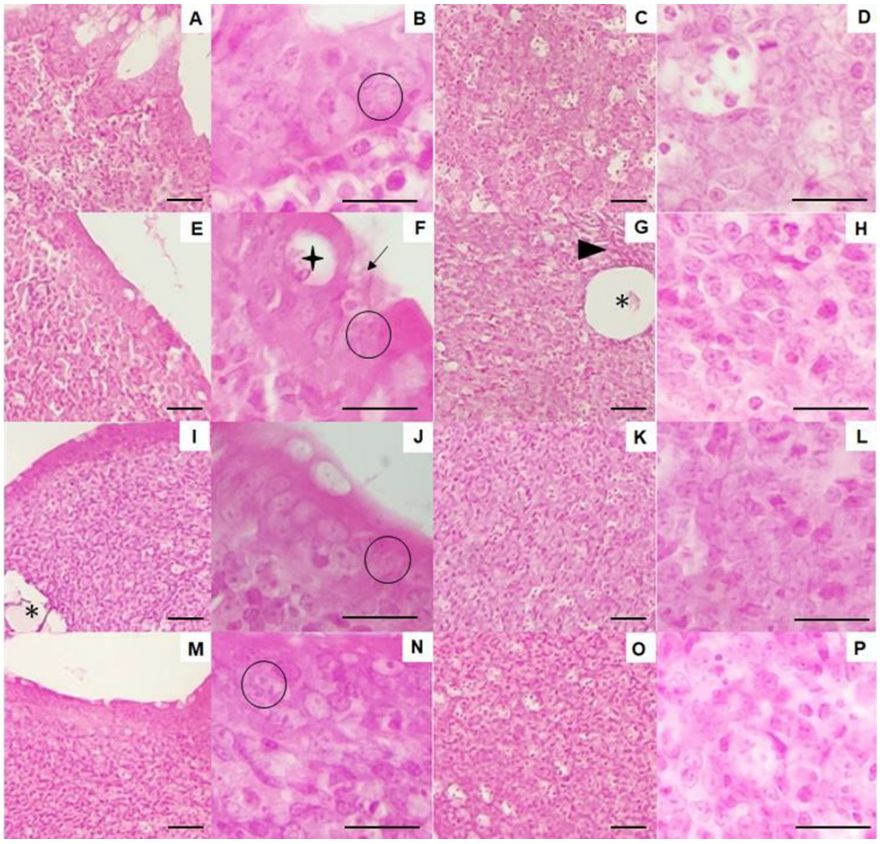
3. RESULTS

PP´s are in the mucous layer of the small intestine. Macroscopically, there was no qualitative morphological difference between the groups. Regarding the number of follicles per plaque, it was observed that it was variable in all groups, with the presence of two to three in each of them being more frequent (Figure 1).



Histological sections of Peyer's patches from male Wistar rats from the groups Control - CT (A), Bisphenol A - BPA (B), Bisphenol A + Selenium - BPA + Se (C) and Selenium - SE (D). RF: Follicular Region; RIF: Interfollicular Region; (*) inflammatory vacuoles. Scale bars: 40x increase.

In the CT group, it was observed that the histological structure of the epithelial layer of the PP´s is of the simple cylindrical type (Figure 2 A-B), with the presence of mucus-producing cells (goblet cells), enterocytes with brush borders indicating a certain absorptive function, and the presence of intraepithelial lymphocytes. It was also possible to observe the presence of lymphocytes in the follicular region with intact parenchyma, and with the absence of vacuoles in its structure.



Histopathological sections of Peyer's patches from male Wistar rats of the Control - CT (A, B, C and D), Bisphenol A - BPA (E, F, G and H), Bisphenol A + Selenium - BPA + Se (I, J, K, L) and Selenium - SE (M, N, O, P) groups. The CT group showed normal structure, with simple cylindrical epithelium, with the presence of intraepithelial lymphocytes (circle) and lymphocytes in the follicular region (C and D). The BPA group showed tissue alterations in the epithelial layer where apoptotic processes (arrow), presence of vacuoles (*) and immune cells (arrowhead) (G – H) were observed. The BPA + Se group did not present changes in the epithelial layer, but in the follicular region there was the presence of vacuoles (*) and high cellular activity. The Se group presented an architecture similar to the CT group. Scale bars: A – C, E – G, I -K, M and O – 100x magnification; B – D, F – H, J – L, N and P – 400x magnification.

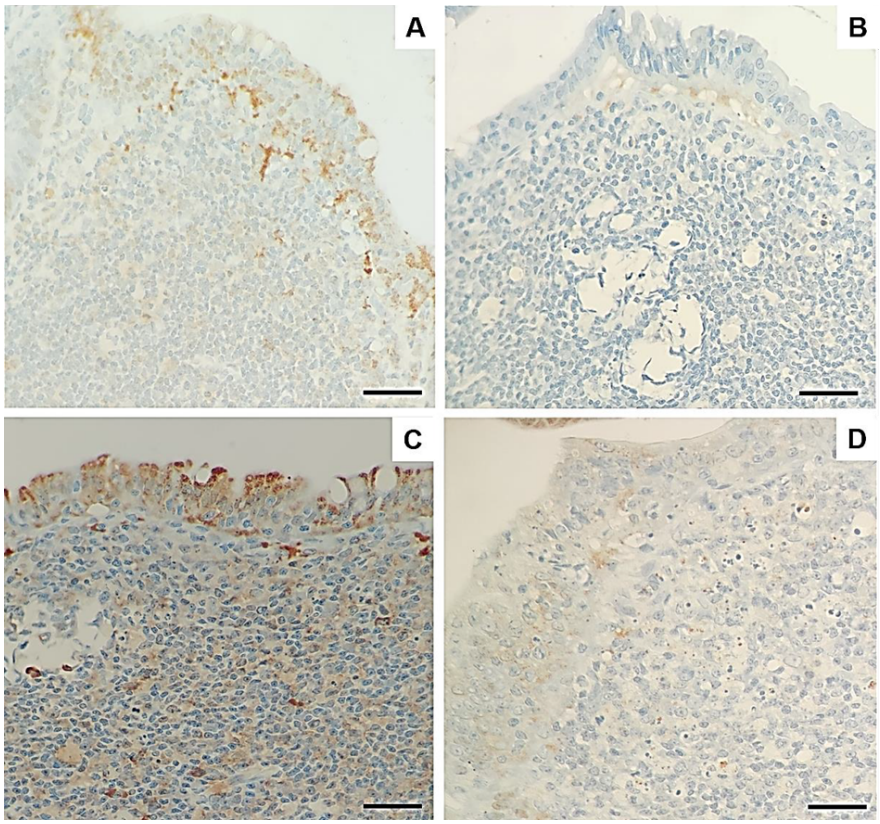
However, a disorganization of the epithelial layer of the BPA group was observed (Figure 2E), presenting a discontinuity of the same, and morphological changes in the goblet cells suggesting that this contaminant led to apoptotic processes in the epithelial layer, impairing repairs in the epithelial layer (Figure 2F). In the BPA+Se and Se groups (Figure 2 I-M), the epithelial layer was preserved when compared to the CT group (Figure 2 A-B).

It was found that there was a structural disarrangement in the architecture of the follicular region of the BPA and BPA+Se groups, where there are areas of inflammation, especially the basal region of the Peyer's patches with the presence of vacuoles in its structure (Figure 2G). It was noted that inflammation was less pronounced in the BPA+Se group when compared to the BPA group, which shows an antioxidant and anti-inflammatory activity of Se under tissue exposed to BPA.

The immunohistochemical evaluation of the cell markers revealed that there was a reduction in the cell proliferation process in the BPA group when compared to the other groups. In addition, there was an intense Ki-67 staining in the BPA+Se group, when compared to the other groups (Figure 3). However, there

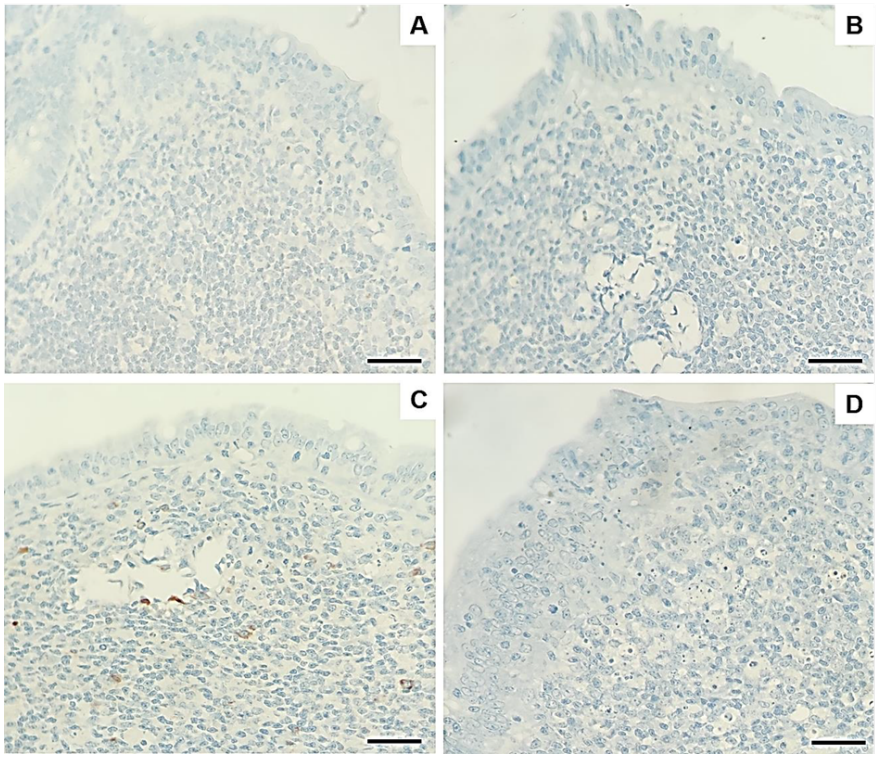
was no marking in the groups submitted to BCL-2 reaction (Figure 4).

Figure 3. Photomicrograph of Peyer's patches of Wistar rats of CT, BPA, BPA+Se and Se groups labeled with Ki-67.



Histological sections of Peyer's patches from male Wistar rats of the groups Control - CT (A), Bisphenol A - BPA (B), Bisphenol A + Selenium - BPA + Se (C) and Selenium - SE (D) submitted to immunohistochemistry technique for antigen expression. It was observed that cell proliferation was reduced in the BPA group when compared to the other groups. Ki-67 staining was intense, especially in the epithelial layer of Peyer's patches. Scale bars: 200x magnification.

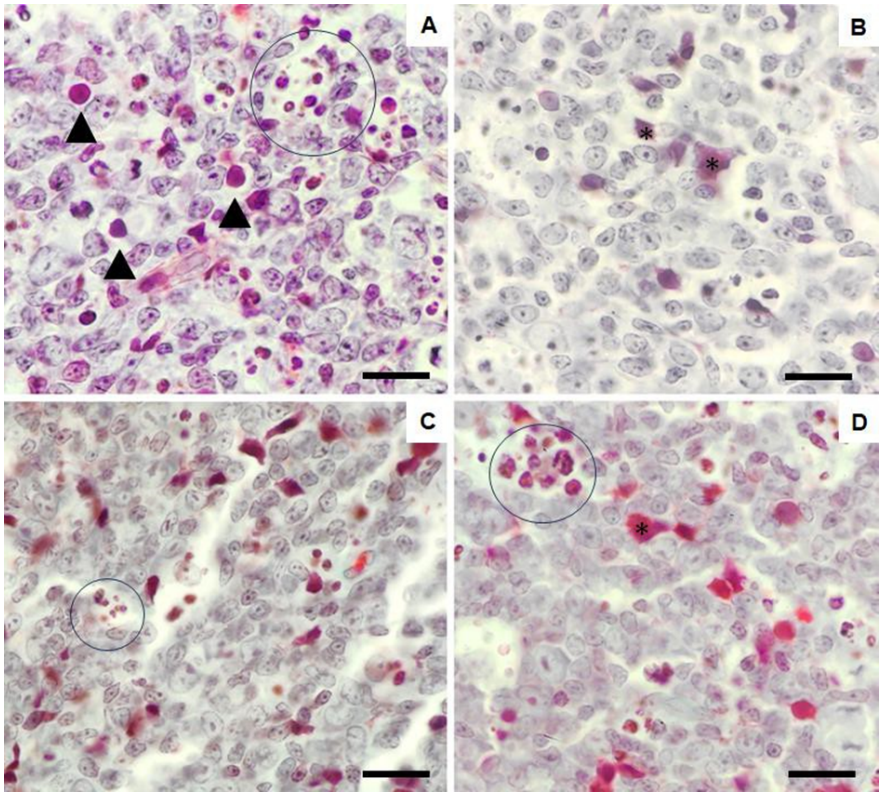
Figure 4. Photomicrograph of Peyer's patches from Wistar rats of CT, BPA, BPA+Se and Se groups labeled with BCL-2.



Histological sections of Peyer's patches from male Wistar rats from the groups Control - CT (A), Bisphenol A - BPA (B), Bisphenol A + Selenium - BPA + Se (C) and Selenium - SE (D). No BCL-2 markings were observed in the Peyer patches of the groups studied, except in the BPA + Se group. Scale bars: 200x magnification.

Masson's special trichrome staining technique revealed a differentiation between the immune cells that make up Peyer's plaques and the connective tissue belonging to it (Figure 5).

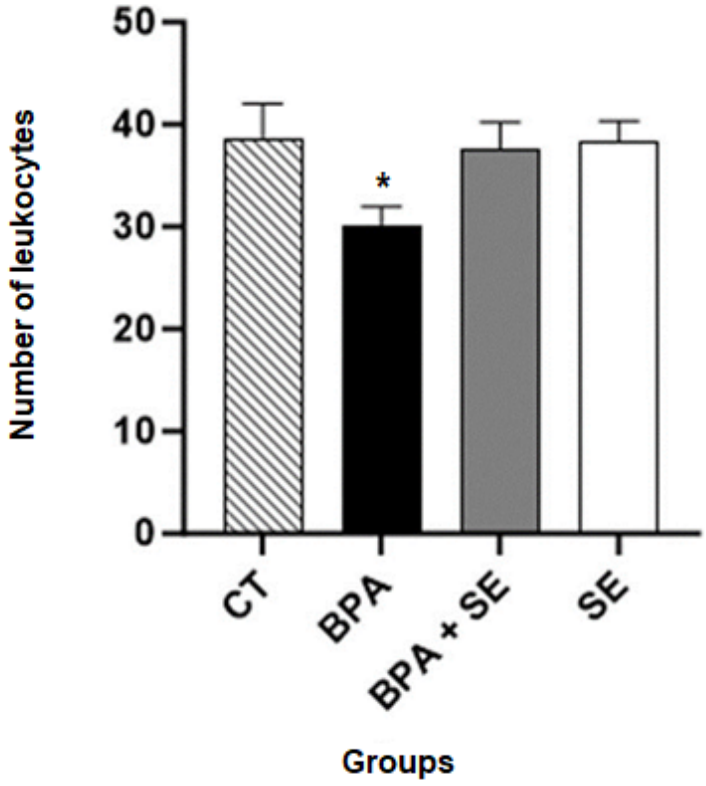
Figure 5. Photomicrograph of Peyer's patches of Wistar rats of CT, BPA, BPA+Se and Se groups stained by Masson's Trichrome method.



Histological sections of Peyer's patches from male Wistar rats of the groups Control - CT (A), Bisphenol A - BPA (B), Bisphenol A + Selenium - BPA + Se (C) and Selenium - SE (D) stained by Masson's trichrome method. The presence of lymphocytes (arrowhead), leukocyte groups (circle) and macrophages (*) was observed in all groups studied. Scale bars: 1000x magnification.

At higher magnification (1000x), leukocytes of varying sizes and staining intensities were observed. After measurement, it was observed that there was a significant reduction ($p < 0.05$) in the number of defense cells in the PP's in the BPA group (30.08±1.87) when compared to the other groups (TC: 38.72±3.30; BPA+Se: 37.56±2.61; If: 38.36±1.98) (Figure 6). However, a greater presence of macrophages was observed in the BPA and BPA+Se groups. No significant differences in the number of immune cells were observed in the other groups.

Figure 6. Number of leukocytes in Peyer's patches.



Number of cells in Peyer's patches in the germinal center region of the groups Control - TC, Bisphenol A - BPA, Bisphenol A + Selenium - BPA+Se and Selenium - SE, ($p<0.05$) (* versus groups CT; BPA+Se and Se).

4. DISCUSSION

This study showed that BPA treatment promoted an alteration in the epithelial layer of PP´s, inducing possible apoptotic processes and morphological changes in goblet cells. These results were also observed in the work of Feng et al. (2019), where they found that in addition to leading to apoptotic processes, BPA also reduced the number of goblet cells, inhibited the expression of tight junction proteins, and altered the diversity and structural composition of the microbiota, eventually reducing intestinal permeability in mice.

These results do not corroborate the findings of Özaydin et al. (2018), where no changes were observed in their experimental groups. The differences in results are due to the time of exposure to the substance and the dose administered in the groups studied.

Furthermore, publications in Romania reveal that the toxicity caused by BPA has been able to cause tissue changes in other organs. The effects of BPA depend on several molecular and epigenetic mechanisms that determine whether the endocrine or reproductive system is affected, whereby pre-existing benign lesions can become cancerous (Dumitrascu, et al., 2020).

Ambreen et al. (2019) performed an in vivo evaluation in BPA-intoxicated rats and demonstrated similarities with the present study, in which histological analysis revealed changes in the intestinal tissue of BPA-treated rat groups compared to the control. In addition, the group treated with low doses of BPA had a lesion in the small intestine. On the other hand, the group that received high doses had a significant fissure in the

intestine, with hyperplasia of the cells in the lamina propria along with shrinkage and rupture of the villi. Therefore, the severity of the trauma depends on both the time of exposure and the amount of dose received.

The protective capacity of Se has also been documented against BPA-induced oxidative stress. Rafiee et al. (2021) found that, overall, Se reduced mitochondrial oxidative stress and effectively improved mouse sperm survival and motility, suggesting that Se may improve mitochondrial damage caused by BPA and impaired mouse sperm quality by preventing oxidative stress.

Khalaf et al. (2018) concluded that co-administration of Se and nano-Se (NSe) attenuated BPA-induced reproductive toxicity via improved antioxidant activity, genetic alterations, and restoration of testicular tissue almost like their control group. These results indicated that both forms of Se and NSe can be used as reproductive protective agents against the detrimental effect induced by BPA. Our study corroborates these studies, where it is possible to perceive a possible protective effect of the Se against the injury induced by exposure to BPA. In addition, we noticed a reduction in inflammation caused by the administration of BPA in the follicular region of the Peyer's patches.

Our results indicate that there was a reduction in the process of cell proliferation in the epithelial layer of Peyer's patches in the BPA group. Previous studies showed that BPA was able to inhibit cell proliferation in human colonic epithelial and goblet cells (QU et al., 2018; ZHAO et al., 2019). These findings indicate BPA's potential to disrupt intestinal barrier function. However, our study did not correlate the effects of BPA and the induction of apoptosis due to the absence of markers in PP´s. Qu et al. (2018) demonstrated that this substance promoted cell apoptosis, weakening the functions of the intestinal epithelial barrier, altering intestinal permeability.

Our results also corroborate the work of Wang et al. (2021) where they concluded that BPA exerts deleterious effects on the epithelial layer, in which it activates an innate immune response that immediately disrupts the balance between cell damage and repair. Consequently, the intestinal epithelial barrier and permeability are disrupted, leading to intestinal damage.

The data from this study showed that Se has a protective effect against epithelial barrier dysfunction caused by BPA. This study shows that Ki-67 expression levels were increased in animals given BPA. The protective effect of Se under conditions of oxidative stress has already been reported by Dou et al. (2023), where they demonstrated that the protective effect of SeNPs on intestinal epithelial barrier injury is closely associated with the mitochondria-lysosome crosstalk signaling pathway mediated by TBC1D15/Rab7.

Canter et al. (2021) pointed out that selenium had a potential role in the integrity of the intestinal barrier and structural changes in glandular goblet and mucin-producing cells in the mucosa and submucosa of the colon. Liu et al. (2020) showed that Se attenuated oxidative stress-induced intestinal mucosal disruption, which was associated with elevated mucosal antioxidant capacity and improved intestinal barrier functions. Xu et al. (2018) also reported that selenium particles promoted the growth and proliferation of porcine intestinal epithelial cells, human colonic epithelial cells, and macrophages derived from human acute monocytic leukemia cells.

These results corroborate the findings of Afzal et al. (2022) where in their study a significant reduction in hemoglobin (Hb), lymphocytes, globular volume (PCV), red blood cells (RBC) and monocytes was observed in a dose-dependent manner compared to their control group. They suggest that due to the toxic accumulation of BPA, there was a decrease in pH, plasma volume in the blood, as well as a low oxygen supply to the red blood cells. Tran et al. (2020) found that BPA had potential negative effects on the T cell response as it decreased telomerase activity via an ER/GPR30-ERK signaling pathway.

Our findings do not corroborate those of Ye et al. (2023) who found an increase in immune cells infiltrated in the colon and increased expression of GHSR (hormone receptor secretagogue) and pro-inflammatory cytokines and chemokines, such as Il6e Ccl2, in the colon mucosa. However, our results are similar to those of this study, since exposure to BPA increased the number of macrophages in the groups exposed to BPA. The authors suggested that GHSR signaling from the nutrient-sensitive ghrelin receptor is involved in modula-

ting the effect of BPA on macrophages and when GHSR expression increases, it activates innate immunity systemically.

Özaydeun et al. (2018) noticed that CD8+ and CD4+ lymphocytes in ileal PP´Ss, exposed to BPA, were mainly located in the interfollicular region (IFR). Notwithstanding, there was a significant increase in CD8+ lymphocyte count and a decrease in CD4+ lymphocytes compared to the control and vehicle groups. It is worth noting that in physiological situations there is a predominance of CD4+ in PP´s. Our study does not corroborate these findings, suggesting that this change in the number of leukocytes may be related to the time of exposure and BPA dosage, significantly altering the immune response performed by PP´s.

We observed that in the animals supplemented with Se, the defense cells remained in a similar amount to the control group. The literature shows that selenium can act as an antioxidant, protecting against oxidative stress, aiding in cell survival and growth, thus playing a chemopreventive role (SELENIUS, et al., 2010; RAZAGHI, et al., 2021).

Köse; Naziroğlu (2014) showed in their work that Se supplementation also protected neutrophils from endogenous oxidative stress. Huang; Rose; Hoffmann (2012) suggest that selenium supplementation, for the most part, is immunostimulating, which is measured by a wide range of parameters, including T cell proliferation, NK cell activity, innate immune cell functions, and many others depending on the dose.

Our study corroborates these findings, as intense cell proliferation was observed in PP´s and we suggest that Se is necessary for optimal immune function, in addition to minimizing oxidative damage caused by BPA. However, selenium at the administered dose did not show a significant difference in the number of defense cells when compared to the control group, suggesting the need for a more refined evaluation of how selenium affects different types of immune responses.

5. CONCLUSIONS

According to the results, it was observed that the ingestion of Bisphenol A above the levels considered safe resulted in changes in the tissues of Peyer's patches, causing a reduction in cell proliferation. In addition, BPA decreased the population of immune cells in these structures. Crucially, selenium has been shown to have a possible protective effect against BPA-induced toxicity. The results suggest that Se plays a crucial role in the immune functions of Peyer's patches. The findings of this study not only contribute to the current understanding of the immune function of these structures, but also provide a solid foundation for future research. It is believed that this work can broaden our understanding of the impacts of BPA and serve as a starting point for further investigation.

6. REFERENCES

ABEDELHAFFEZ, A. S. et al. Lung injury induced by Bisphenol A: A food contaminant, is ameliorated by selenium supplementation. Pathophysiology, v. 24, n. 2, p. 81–89, jun. 2017. AFZAL, G. et al. Bisphenol A Induces Histopathological, Hematobiochemical Alterations, Oxidative Stress, and Genotoxicity in Common Carp (Cyprinus carpio L.). Oxidative Medicine and Cellular Longevity, v. 2022, p. 1–14, 28 jan. 2022. AL-AMOUDI, M. W. Antioxidant activity of selenium on bisphenol-induced apoptosis and testicular toxicity of rats. African Journal of Pharmacy and Pharmacology, v. 12, n. 21, p. 278–289, 8 jul. 2018. ALMEIDA S. et al. Bisphenol A: Food Exposure and Impact on Human Health. Comprehensive Reviews in Food Science and Food Safety, v. 17, n. 6, p. 1503– 1517, 5 set. 2018. AMBREEN, S. et al. In Vivo Evaluation of Histopathological Alterations and Trace Metals Estimation of the Small Intestine in Bisphenol A-Intoxicated Rats. Canadian Journal of Gastroenterology and Hepatology, v. 2019, p. 1–7, 9 dez. 2019. AMRAOUI, W. et al. Modulatory Role of Selenium and Vitamin E, Natural Antioxidants, against Bisphenol A-Induced Oxidative Stress in Wistar Albinos Rats. Toxicological Research, v. 34, n. 3, p. 231–239, 15 jul. 2018. CANTER, J. A. et al. Selenium and the 15kDa Selenoprotein Impact Colorectal disrupting cellular repair and injury homeostasis in vivo and in vitro. Biomedicine & Pharmacotherapy, v. 137, p. 111270, maio 2021. DOU, X. et al. Biogenic selenium nanoparticles alleviate intestinal epithelial barrier injury by regulating mitochondria-lysosome crosstalk. Food & Function, v. 14, n. 10, p. 4891–4904, 22 maio 2023. DUMITRASCU, et al.

Carcinogenic effects of bisphenol A in breast and ovarian cancers (Review). Oncology Letters, v. 20, p. 282, 2020. EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2023. Scientific opinion on the re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal 2023;21(4):6857, doi: 10.2903/j.efsa.2023.6857. FAHEEM, N. M.; EL ASKARY, A.; GHARIB, A. F. Lycopene attenuates bisphenol A-induced lung injury in adult albino rats: a histological and biochemical study. Environmental Science and Pollution Research International, v. 28, n. 35, p. 49139–49152, 1 set. 2021. FENG, L. et al. Bisphenol A increases intestinal permeability through disrupting intestinal barrier function in mice. Environmental Pollution, v. 254, p. 112960, nov. 2019. HARIHARAN, S.; DHARMARAJ, S. Selenium and selenoproteins: it's role in regulation of inflammation. Inflammopharmacology, p. 1–29, 6 mar. 2020. HUANG, Z.; ROSE, A. H.; HOFFMANN, P. R. The Role of Selenium in Inflammation and Immunity: From Molecular Mechanisms to Therapeutic Opportunities. Antioxidants &amp Redox Signaling, v. 16, n. 7, p. 705–743, 1 abr. 2012. immunophenotype. Scientific Reports, v. 10, n. 1, 20 fev. 2020. JANG, M. et al. A sensitive environmental forensic method that determines bisphenol S and A exposure within receipt-handling through fingerprint analysis. Journal of Hazardous Materials, v. 424, n. Pt B, p. 127410, 15 fev. 2022. KAUR, S. et al. Selenium attenuates bisphenol A incurred damage and apoptosis in mice testes by regulating mitogen-activated protein kinase signalling. Andrologia,v. 53, n. 3, p. e13975, 1 abr. 2021. KHALAF, A. et al. Protective effects of selenium and nano-selenium on bisphenol-induced reproductive toxicity in male rats. Human & Experimental Toxicology, v. 38, n. 4, p. 398–408, 9 dez. 2018. Knudsen, Xenoestrogen action in prostate cancer: pleiotropic effects dependent on androgen receptor status, Canc. Res. 65 (1) (2005) 54–65. KÖSE, S. A.; NAZIROĞLU, M. Selenium Reduces Oxidative Stress and Calcium Entry Through TRPV1 Channels in the Neutrophils of Patients with Polycystic Ovary Syndrome. Biological Trace Element Research, v. 158, n. 2, p. 136–142, 18 mar. 2014. LABUNSKYY, V. M.; HATFIELD, D. L.; GLADYSHEV, V. N. Selenoproteins: Molecular Pathways and Physiological Roles. Physiological Reviews, v. 94, n. 3, p. 739–777, 1 jul. 2014. LIU, L. et al. Selenium-Enriched Yeast Alleviates Oxidative Stress-Induced Intestinal Mucosa Disruption in Weaned Pigs. Oxidative Medicine and Cellular Longevity, v. 2020, p. 1–11, 19 mar. 2020. LÓPEZ-RODRÍGUEZ, D. et al. Multi- and Transgenerational Outcomes of an Exposure to a Mixture of Endocrine-Disrupting Chemicals (EDCs) on Puberty and Maternal Behavior in the Female Rat. Environmental Health Perspectives, v. 129, n. 8, p. 087003, ago. 2021. MALOY, K. J.; POWRIE, F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature, v. 474, n. 7351, p. 298–306, 1 jun. 2011. MILANO, W. et al. Obesity and Its Multiple Clinical Implications between Inflammatory States and Gut Microbiotic Alterations. Diseases, v. 11, n. 1, p. 7, 29 dez. 2022. MINICH, W. B. Selenium Metabolism and Biosynthesis of Selenoproteins in the Human Body. Biochemistry (Moscow), v. 87, n. S1, p. S168–S177, jan. 2022. Molecular Sciences, v. 22, n. 19, p. 10651, 30 set. 2021. OKUMURA, R.; TAKEDA, K. Roles of intestinal epithelial cells in the maintenance of gut homeostasis. Experimental & Molecular Medicine, v. 49, n. 5, p. e338–e338, may 2017. ÖZAYDIN, T. et al. The effects of bisphenol A on some plasma cytokine levels and distribution of CD8+ and CD4+ T lymphocytes in spleen, ileal Peyer's patch and bronchus associated lymphoid tissue in rats. Acta Histochemica, v. 120, n. 8, p. 728–733, 1 nov. 2018. PANNEERSELVAM, D.; VAQAR, S. Peyer Patches. Disponível em: <https://www.ncbi.nlm.nih.gov/books/NBK557457/&gt>. Acesso em: 18 out. 2023. QU, W. et al. Bisphenol A suppresses proliferation and induces apoptosis in colonic epithelial cells through mitochondrial and MAPK/AKT pathways. Life Sciences, v. 208, p. 167–174, set. 2018. RAFIEE, Z. et al. Protective effects of selenium on Bisphenol A-induced oxidative stress in mouse testicular mitochondria and sperm motility. JBRA Assisted Reproduction, 2021. RATAJCZAK-WRONA, W. et al. Effect of bisphenol A on human neutrophils RAZAGHI, A. et al. Selenium stimulates the antitumour immunity: Insights to future research. European Journal of Cancer, v. 155, p. 256–267, 1 set. 2021. REBOLDI, A.; CYSTER, J. G. Peyer's patches: organizing B-cell responses at the intestinal frontier. Immunological Reviews, v. 271, n. 1, p. 230–245, 18 abr. 2016. SELENIUS, M. et al. Selenium and the Selenoprotein Thioredoxin Reductase in the Prevention, Treatment and Diagnostics of Cancer. Antioxidants & Redox Signaling, v 12, n. 7, p. 867–880, abr. 2010. TRAN, H. T. T.; HERZ, C.; LAMY, E. Long-term exposure to "low-dose" bisphenol A decreases mitochondrial DNA copy number, and accelerates telomere shortening in human CD8 + T cells. Scientific Reports, v. 10, n. 1, 25 set. 2020. VANDENBERG, L. N. et al. Hormones and Endocrine-Disrupting

Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses. Endocrine Reviews, v. 33, n. 3, p. 378–455, jun. 2012. WANG, K.; ZHAO, Z.; JI, W. Bisphenol A induces apoptosis, oxidative stress and inflammatory response in colon and liver of mice in a mitochondria-dependent manner. Biomedicine & Pharmacotherapy, v. 117, p. 109182, 1 set. 2019. WANG, N. et al. Influence of bisphenol a on thyroid volume and structure independent of iodine in school children. PLoS ONE, v. 10, n. 10, p. 1–12, 2015. XU, C. et al. Biogenic Synthesis of Novel Functionalized Selenium Nanoparticles by Lactobacillus casei ATCC 393 and Its Protective Effects on Intestinal Barrier Dysfunction Caused by Enterotoxigenic Escherichia coli K88. Frontiers in Microbiology, v. 9, 18 jun. 2018. Y.B. Wetherill, N.L. Fisher, A. Staubach, M. Danielsen, R.W. de Vere White, K.E. Knudsen, Xenoestrogen action in prostate cancer: pleiotropic effects dependent on androgen receptor status, Canc. Res. 65 (1) (2005) 54–65. YE, X. et al. Nutrient-Sensing Ghrelin Receptor in Macrophages Modulates Bisphenol A-Induced Intestinal Inflammation in Mice. Genes, v. 14, n. 7, p. 1455, 16 jul. 2023. ZHANG, M.; WU, C. The relationship between intestinal goblet cells and the immune response. Bioscience Reports, v. 40, n. 10, out. 2020. ZHAO, Z. et al. Bisphenol A inhibits mucin 2 secretion in intestinal goblet cells through mitochondrial dysfunction and oxidative stress. Biomedicine & Pharmacotherapy, v. 111, p. 901–908, 1 mar. 2019.