# ANAIS

## IV INTERNATIONAL SYMPOSIUM OF ENTERIC NEURAL PLASTICITY

## III SYMPOSIUM OF GASTROENTEROLOGY

**DECEMBER 6-8, 2023**

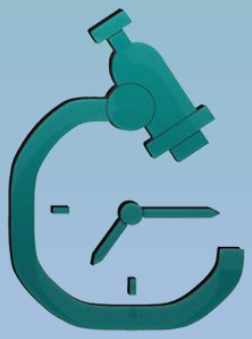
**LOCAL:** Auditório Professor Rodolpho Paulo Rocco e Auditório Professor Hélio Fraga
Av. Carlos Chagas Filho, 373 - Cidade Universitária, Rio de Janeiro - RJ, 21941-902

**Centro de Ciências da Saúde –**
**Universidade Federal do Rio de Janeiro (UFRJ)**

ISBN 978-65-83236-07-4

# Anais

# IV International Symposium of Enteric Neural Plasticity / III Symposium of Gastroenterology



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# · ANAIS ·

**ISBN 978-65-83236-07-4**

## Ficha catalográfica

I614

International Symposium Enteric Neural Pasticity (4. : 2023: Rio de Janeiro, RJ).
Anais do IV International Symposium Enteric Neural Pasticity [e] III Symposium of Gastroenterology, 06 a 08 de dezembro, Rio de Janeiro, RJ, Brasil / Juliana de Mattos Coelho Aguiar [et al]. – Rio de Janeiro: Ciência em Minutos, 2023. 89p.

ISBN: 978-65-83236-07-4

Demais membros da comissão organizadora e comitê científico: Ana Lucia Tavares Gomes, Diego Pinheiro Aguiar, Jacqueline Zanoni, Juliana Vanessa Colombo Martins Perles, Patricia Castelucci, Vivaldo Moura Neto.

1. Enteric Neural Pasticity . 2. Gastroenterology . 3. Symposium I. Aguiar, Juliana de Mattos Coelho. II. Gomes, Ana Lucia Tavares Gomes. III. Aguiar, Diego Pinheiro. IV. Zanoni, Jacqueline. V. Perles, Juliana Vanessa Colombo Martins. VI. Castelucci, Patricia. VII Moura Neto, Vivaldo. VIII Título

CDU 573

CDD 573.3

# SUMMARY

# PROGRAM


# FINAL PROGRAM

## WEDNESDAY, DECEMBER 06

**1:00pm - 3:00pm** - Registration

**3:00pm - 3:30pm** - Opening Ceremony

**3:30pm - 4:30pm - Plenary Conference**

**Chair:** Dra. Juliana M. Coelho-Aguiar (Professor at Federal University of Rio de Janeiro/Brazil)

**Dra. Shanti Srinivasan** (Professor at Emory University – Atlanta/USA)
Unraveling the Mysteries of Neurodegeneration in the Enteric Nervous System: Novel Mechanisms and Promising Therapeutic Strategies

**4:30pm - 6:00pm** - Welcome reception

## THURSDAY, DECEMBER 07

**9:00am – 10:00am - Plenary Conference**

**Chair: Dra. Patricia Castelucci** (Professor at São Paulo University, São Paulo/Brazil)

**Dr. Paulo Corrêa-de-Sá** (Professor at University of Porto – Portugal)
Tripartite myenteric synaptic plasticity – purines (ATP and adenosine) fine-tuning control metabolic, inflammatory and toxicological adaptations

**10:00am - 10:30am** - Coffee Break

**10:30am - 12:30pm - Thematic Panel – Enteric nervous system and inflammation 1**

**Chair: Dra. Jacqueline Nelisis Zanoni** (Professor at Maringá State University, Paraná/Brazil)

**Dra. Patricia Castelucci** (Professor at São Paulo University, São Paulo/Brazil)
Effects of intestinal inflammation on the enteric nervous system

**Dra. Juliana V. C. Martins Perles** (Professor at Maringá State University, Paraná/Brazil)
Does the Rheumatoid Arthritis Affect the Enteric Nervous System?

**Oral communications:**

- STUDY OF THE PRO-INFLAMMATORY ACTIVITY OF ENTERIC GLIA ON INTESTINAL EPITHELIAL CELLS. Mayara Melo, Liliane da Silva Ribeiro, Vivaldo Moura Neto, Profa. Juliana de Mattos Coelho Aguiar
- GUT MICROBIOTA DERIVED BUTYRATE PROTECT EXCITATORY AND INHIBITORY MYENTERIC NEURONS LOSS AFTER EXPERIMENTAL ULCERATIVE COLITIS. Marcos Antônio Ferreira Caetano, Roberta Figueiroa de Souza, Jheniffer Rayane de Lima Duarte, Victor dos Santos Silva, Profa. Patricia Castelucci


**12:30pm - 2:00pm** - Lunch

**2:00pm - 4:00pm - Thematic Panel -** Microbiome and environmental influences on the intestine and ENS

**Chair:** Dra. Juliana M. Coelho- Aguiar (Professor at Federal University of Rio de Janeiro/Brazil)

**Dr. Heitor Siffert P. Souza** (Professor at Faculdade de Medicina, Federal University of Rio de Janeiro/Brazil)
Pathogenic and therapeutic role of the Microbiome: focus on inflammatory bowel disease

**Dra. Renata de Britto Mari** (Professor at São Paulo State University - São Paulo/Brazil)
Enteric nervous system analyses: New biomarkers for environmental quality assessment.

**Oral communications:**

- ENVIRONMENTAL ISSUES: AZITHROMYCIN AFFECTS CROSSTALK BETWEEN MICROBIOTA, INTESTINAL BARRIER, AND ENTERIC NERVOUS SYSTEM IN FISH. Gabriela Pustiglione Marinsek, Isabelly Cristina Correia dos Santos de Oliveira, Marcos Antônio de Oliveira, Renata de Britto Mari
- NITRERGIC NEURONS AND VIPERGIC VARICOSITY ANALYSIS IN THE JEJUNUM OF RATS INDUCED TO COLORECTAL CARCINOGENESIS TREATED WITH MICROENCAPSULATED QUERCETIN AND PROBIOTIC. Maysa Pacheco Alvarez da Silva, Lucas Casagrande, Sabrina Silva Sestak, Carla Cristina de Oliveira Bernardo, Matheus Ferreira Zambonini, Marcos Yudi Nagaoka Godoy, João Victor Kuller, Lucas Sala Bellettini, Giovana Emanuele Derio De Lemos, Andressa Felipe Lima, Abygail Karlla Donadelli Damico, Lídia Rodrigues Cicero, Profa. Juliana Vanessa Colombo Martins Perles, Profa. Jacqueline Nelisis Zanoni

**4:00pm - 4:30pm** - Coffee Break

**4:30pm - 6:00pm** - Poster Session

## FRIDAY, DECEMBER 08

**9:00am - 10:15am** - **Thematic Panel – Enteric nervous system and inflammation 2**

**Chair: Dra. Ana Lucia Tavares-Gomes** (Professor at Federal Fluminense Universtity, Niterói/Brazil)

**Dra. Ana Carina Bon Frauches Oliveira** (Reasearcher at Francis Crick Institute – London/England)
Effect of the Gut Environment on the Homeostasis and Function of the Enteric Nervous System

**Dra. Beatriz Bastos de Moraes Thomasi** (Post-doc at Michigan State University/USA)
S100B influence on ENS excitability and function in health and post-inflammatory conditions

**10:15am - 10:45am** - Coffee Break


**10:45am - 12:30pm - Thematic Panel - Gastrointestinal tract in health and disease**

**Chair: Dra. Patricia Castelucci** (Professor at São Paulo University, São Paulo/Brazil)

**Dr. Moisés Tolentino** (Professor at University of Porto – Portugal)
Acute and Chronic Physical Exercise in Gastrointestinal Pathophysiological: "Risks and Benefits"

**Dra. Joana Hygino** (Post-doc at Instituto Biomédico, UNIRIO – Rio de Janeiro/Brazil)
The role of T cells expressing Toll-like receptors in the multiple sclerosis pathogenesis.

**Oral communication:**

EFFECTS OF HIGH PROTEIN DIET AND INDUCED-DSS ULCERATIVE COLITIS ON THE ILEUM OF MICE. Vinicius Balan Ramos Coronado, Camila Cristina Alves Machado, Giovanni Bruno Clivati Sodré, Alexandre Oba, Flávia Alessandra Guarnier, Eduardo José de Almeida Araújo

**12:30pm - 2:00pm** - Lunch

**2:00pm - 4:00pm - Thematic Panel - Gut-brain axis**

**Chair: Dra. Juliana V. C. Martins Perles** (Professor at Maringá State University, Paraná/Brazil)

**Dra. Ana Lúcia Tavares Gomes** (Professor at Federal Fuminense University, Niterói/Brazil)
Evidence of brain-gut axis in an animal model of Parkinson's disease: Involvement of enteric glia in intestinal inflammation and evaluation of enteric synaptic components

**Dr. Felipe Leite de Oliveira** (Professor at Federal University of Rio de Janeiro/Brazil)
Galectin-3: possible target to study gut-brain axis in experimental models of autism

**Oral communications:**

EFFECT OF SODIUM BUTYRATE TREATMENT ON GUT-BRAIN AXIS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE. Maria Carolina Garcia Ricciardi, Marianna Gonçalves de Carvalho, Isabela Nobrega Fialho Tavares, Profa. Ana Lucia Tavares Gomes

- PROJECT "SECOND BRAIN SPACE: FROM PRODUCTION TO THE POPULARIZATION OF KNOWLEDGE OF THE ENTERIC NERVOUS SYSTEM" - A GENERAL OVERVIEW OF THE PUBLIC SERVED FROM MAY 2022 TO SEPTEMBER 2023. João Victor Kuller, Marcos Yudi Nagaoka Godoy, Maysa Pacheco Alvarez da Silva, Andressa Felipe Lima, Lucas Sala Bellettini, Matheus Ferreira Zambonini, Lídia Rodrigues Cicero, Mariana Rodrigues Sanches, Giovana Emanuele Derio De Lemos, Sabrina Silva Sestak, Jacqueline Nelisis Zanoni, Juliana Vanessa Colombo Martins Perles

**4:00pm - 4:30pm** - Coffee Break

**4:30pm - 5:30pm** - Round table

**5:30pm - 6:00pm** – Awards

# ABSTRACTS –

# SELECTED FOR ORAL PRESENTATIONS


## STUDY OF THE PRO-INFLAMMATORY ACTIVITY OF ENTERIC GLIA ON INTESTINAL EPITHELIAL CELLS

ARAÚJO, M. M.[1]; RIBEIRO, L. S.[1]; DA SILVA; D.A. C.[1]; MOURA-NETO, Vivaldo; COELHO-AGUIAR, J. M.[1]

[1]Cellular Morphology Laboratory, ICB, UFRJ – Rio de Janeiro, Brazil

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**Introduction:** The enteric nervous system (ENS) is the intrinsic innervation of the gastrointestinal tract, and is composed of neurons and enteric glial cells. Enteric glia (EG) performs important functions, modulating intestinal motility, nutrient absorption, and maintenance of the intestinal epithelial barrier (IEB), reducing its permeability and acting on the immune response. Through the secretion of S-nitroso glutathione (GSNO), GE maintains the integrity of the IEB, by stimulating occludin and ZO-1 expression, helping the intestinal epithelium to maintain barrier properties during initial aggression. However, the role of EG in inflammation context will depend on its state, which can be “activated”, where it acts by increasing the trophic factors release, maintaining homeostasis, or “reactive”, where EG presents a deleterious response with increased pro-inflammatory functions, harmful to the integrity of the intestinal epithelium. **Objective:** To characterize the activity of GE in its active and reactive states, its pro-inflammatory activity, and its inhibition with palmitoylethanolamide (PEA). **Methodology:** Investigate in vitro how LPS 1ug/ml acts on enteric glial cells (rat EG cell lineage, CRL2690) in 24 hours, 3 days and 6 days, evaluating in each context the pro-inflammatory cytokines such as S100B, IL1B and TNFα, the levels of secreted trophic factors such as GDNF and GSNO, whether there will be an increase in GFAP, the activation of

the NFKB pathway, through ELISA, Western blotting and immunofluorescence techniques. Evaluate, in cultured Caco-2 or RKO intestinal epithelial cells, the responses to treatments with conditioned medium of EG, activated EG or reactive EG. How does the inhibition carried out by PEA acts. Next, we will investigate if the anti-inflammatory character of PEA reduces or prevents the inflammation caused by LPS, and when administered after LPS, whether it is capable of recovering the GE for control situation, leaving the pro-inflammatory state. **Results:** Our preliminary results point to an increase in the release of S100b by GE treated with LPS for 24h and even more for 72h. Intracellular labeling also increases. The immunostaining for cx43 does not appear to be altered, neither after treatment for 1 day nor for 3 days. Treatment of Caco2 with LPS causes disruption of the occlusion zones, which appears to be avoided when co-cultured with GE. RKO cells were treated with GE-LPS24h or GE-LPS72h conditioned medium. Analyzes are ongoing. **Conclusions:** We aim to characterize the activated and reactive phenotypes of GE according to its profile of S100b and inflammatory cytokines and activation of the NFKb pathway, and demonstrate the improvement of the inflammatory condition of intestinal epithelial cells, induced by reactive EG, with the use of PEA, which is an important finding for a possible treatment of ulcerative colitis. **Keywords:** SNE. Enteric Glia. Intestinal epithelial barrier. Acute colitis.


# GUT MICROBIOTA DERIVED BUTYRATE PROTECT EXCITATORY AND INHIBITORY MYENTERIC NEURONS LOSS AFTER EXPERIMENTAL ULCERATIVE COLITIS

CAETANO, Marcos Antônio Ferreira[1], SOUZA, Roberta Figueiroa[1], DUARTE, Jheniffer Rayane de Lima[1], SILVA, Victor dos Santos[1], CASTELUCCI, Patricia[1].

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[1]Department of Anatomy, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

**Introduction:** The enteric nervous system is affected by inflammatory bowel diseases (IBDs). Butyrate is a short-chain fatty acid, produced by gut microbiota from the fermentation of dietary fibers, which binds to G protein-coupled receptors, such as GPR43 receptor, and contributes to the maintenance of intestinal health. However, the effects of butyrate on IBDs and how it interacts in the different enteric neuronal classes remains unclear. **Objective:** This work aimed to study GPR43 receptor, and analyze the effect of Sodium Butyrate on mice myenteric neurons submitted to experimental ulcerative colitis (UC). **Methods:** For UC induction, 8 weeks-old C57BL/6 male mice received intrarectal injection of 100µL of 2,4,6-Trinitrobenzenesulfonic acid (TNBS group). SHAM group received the TNBS vehicle (ethanol 35%). After colitis induction, some animals were treated for 7 days with Sodium Butyrate (100mg/kg, Butyrate group) via gavage, while the SHAM and TNBS groups received an equivalent amount of saline (Approved by CEUA-ICB/USP-5482071122). The disease activity index (DAI) was daily analyzed. Animals were euthanized 7 days after TNBS injection. Large intestines were collected and processed for immunofluorescence double labelling for GPR43 receptor with Calretinin (Calr) and neuronal nitric oxide synthase (nNOS). The number of neurons/ganglia immunoreactive (-ir) for GPR43 receptor, Calr and nNOS were analyzed. Total, cytoplasmic and nuclear area of Calr-ir were measured. Morphological analyses were performed through histology. **Results:** The DAI results showed increased scores in the TNBS group, and decreased scores in the Butyrate group. Colocalization of GPR43 with Calr-ir and nNOS-ir neurons were observed. There was a reduction in the number of GPR43-ir, Calr-ir and nNOS-ir neurons by 20.3%, 38.5% and 41.1%, respectively, in TNBS group, compared to the Sham Group. In Butyrate group the number of these neurons increased by 20.9%, 35.4% and 38.3%, respectively, compared to TNBS group. These data are available on Table 1. A reduction by 18.01% and 28.5% in total and cytoplasmic area, respectively, was observed in Calr-ir neurons in the TNBS group. Butyrate group restored by 14.4% and 20.8% the total area and cytoplasmic area, respectively, returning values similar to the SHAM group. No difference in nuclear area

was observed. Histological analysis showed loss of mucosal integrity, mild submucosal edema, increased inflammatory infiltrate and cellular changes in myenteric neurons such as the presence of cytoplasmic vacuoles in TNBS group, compared to the SHAM group, while in the Butyrate group these findings were attenuated.

Table 1 - Neuronal density (neurons/ganglion) from myenteric neurons of mice submitted to experimental ulcerative colitis.

| IMMUNOREACTIVE NEURONS | GROUPS | | |
|---|---|---|---|
| | **SHAM** | **TNBS** | **Butyrate** |
| **GPR43 receptor** | 16.6±0.2[a] | 13.3±0.3[a] | 16.8±0.2[b] |
| **CaIr** | 9.5±0.3[a] | 5.9±0.4[a] | 9.1 ± 0.3[b] |
| **nNOS** | 8.1±0.2[a] | 4.8±0.1[a] | 7.7± 0.1[b] |

[a] SHAM group compared to TNBS group (*p<0.0001*); [b] Butyrate group compared to TNBS group (*p<0.0001*).

**Conclusions:** UC caused loss of GPR43-ir, CaIr-ir and nNOS neurons. The treatment with Sodium Butyrate was able to protect neuronal loss, attenuate DAI scores and morphological changes. Also, enteric neurons were immunoreactive for GPR43 receptor, suggesting that these neurons can respond to Butyrate binding and it may be a possible therapeutic tool for IBDs.

**Key words:** Inflammatory Bowel Disease; Enteric Nervous System; Short-chain fatty acids; Gut Microbiota; GPR43.


# ENVIRONMENTAL ISSUES: AZITHROMYCIN AFFECTS CROSSTALK BETWEEN MICROBIOTA, INTESTINAL BARRIER, AND ENTERIC NERVOUS SYSTEM IN FISH.

Gabriela Pustiglione Marinsek[1]; Isabelly Cristina Correia dos Santos de Oliveira[1]; Marcos Antônio de Oliveira[1]; Renata de Britto Mari[1].

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[1] São Paulo State University Júlio de Mesquita - Coastal Campus - Animal Morphophysiology Laboratory (LABMA)

**Introduction:** Environmental quality analyses are of paramount importance, particularly when it comes to the presence of emerging contaminants that are released daily into aquatic environments and lack current legislation. Among these compounds, antibiotics stand out due to their worldwide administration frequency, and their effects on aquatic organisms can be detrimental to both human health and the biota, as well as the spread of bacterial resistance genes. The gastrointestinal tract (GIT) is particularly susceptible to the impact of antibiotics, primarily due to its complex microbiological community, which has proven to be crucial in regulating important immune and neural pathways in the host.

**Objective:** Assessing changes in intestinal microbiota diversity in fish exposed to azithromycin and its relationship with intestinal barrier and density of non-adrenergic, non-cholinergic neurons.

**Methods:** After exposure to different concentrations of azithromycin (C- control; CA - 4ug/l and C2 - 16ug/l) for 15 days, *Poecilia reticulata* were euthanized and gut samples were collected. For neuronal density, the intestine was destined to NADPH-dp technique; for mucosal analysis, intestinal samples were subjected to a histological routine and stained with PAS/H. Goblet cells and intraepithelial lymphocytes (IELs) were quantified/100 enterocytes. Fecal DNA was extracted using the QAMP Fecal DNA kit for metagenomic analysis. Afterward, the 16S ribosomal DNA fragment was amplified and destined for NGS sequencing.

**Results:** The results are presented in Table 1. The presence of the antibiotic significantly increased the richness and diversity of the intestinal microbiota, especially at the lower concentration (4 µg/l). It is important to consider that the increase in richness/diversity of a microbial community is not always beneficial for the host due to the complexity of ecological interactions, which can facilitate the proliferation of pathogenic bacteria. Our results suggest that such a change may intensify inflammatory processes in the mucosa, leading to a considerable increase in the density of goblet cells, which serve as the primary physical barrier against harmful agents in the lumen. Furthermore, in the group exposed to the higher concentration, there was an extremely significant increase in NADPH-dp neurons, which can be explained by the neuroprotective role of nitric oxide

(NO). Interestingly, the lower concentration of azithromycin appeared to have a more detrimental impact on nitrergic neurons, possibly leading to cell death.

| | | Control | 4ug/l | 16ug/l |
|---|---|---|---|---|
| **Neuronal Density (mm²)** | **NADPH-dp** | 83.0 ± 14.0[a] | 42.5 ± 14.8[b] | 149 ± 16.5[c] |
| **Intestinal barrier** | **Goblet Cells/100 enterocytes** | 4.9 ± 1.6[a] | 8.8 ± 2.8[b] | 10.7 ± 3.0[b] |
| | **IELs/100 enterocytes** | 0.9 ± 0.3[a] | 1.6 ± 0.3 [b] | 1.2 ± 0.14 [b] |
| **Microbial Diversity** | **Chao Index** | 91.2 ± 2.3 [a] | 270.1 ± 21.4[b] | 163.9 ± 3.3[a,b] |
| | **Shannon Index** | 4.1 ± 0.02 [a] | 5.75 ± 0.05 b [b] | 4.94 ± 0.03[a,b] |
| | **Operational Taxonomic Units (OTUs)** | 88.03 ± 5.9 [a] | 240.4 ± 35.7[b] | 149.5 ± 18.5[a,b] |

**Conclusion:** The presence of the antibiotic azithromycin significantly increased the diversity and richness of the intestinal microbiota, which proved to be detrimental to the gastrointestinal tract of the organisms, intensifying inflammatory processes in the mucosa and altering the density of nitrergic neurons in both evaluated groups.

**Keywords:** antibiotic exposure; environmental monitoring; *Poecilia reticulata;* NADPH-dp neurons.


# EFFECTS OF HIGH PROTEIN DIET AND INDUCED-DSS ULCERATIVE COLITIS ON THE ILEUM OF MICE.

CORONADO[1], Vinicius Balan Ramos; MACHADO [1], Camila Cristina Alves; GUARNIER[2] , Flavia Alessandra; SODRÉ, Giovanni Bruno Clivati[1]; OBA[3] , Alexandre; ARAÚJO[1] , Eduardo José de Almeida.

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**Introduction:** Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that affects the large intestine, and part of the ileum. Its pathophysiology involves increased intestinal permeability and abnormal activation of the immune system. Excessive protein consumption can affect intestinal homeostasis, increase permeability and inflammation.
**Objective:** To investigate whether a high-protein diet interferes in the inflammatory process during the acute phase of UC.
**Methods:** C57BL/6 male mice were assigned into four experimental groups using sodium dextran sulfate (DSS) as UC inducer: CD (22% of protein – control diet), HP (44% of protein - hyperprotein diet), CD/DSS (22% protein - control diet and 3% DSS) and HP/DSS (44% protein - hyperprotein diet and 3% DSS). The body mass, stool consistency and presence of blood in the stools were checked daily to calculate the disease activity index (DAI). Ileal cross sections were performed to the quantification of carbonyl proteins, superoxide dismutase and reduced glutathione. Whole-mounted preparations were submitted to immunofluorescence technique to evidence the general neuronal population ($PGP9.5^{+}$) and specific subpopulations in the submucosal and myenteric plexi. GraphPad Prism was used for statistical analysis.
**Results:** Mice exposed to DSS and fed a high-protein diet presented a DAI even higher than mice fed a standard diet ($p<0.05$). The HP diet showed a negative influence on the conversion of superoxide radical into hydrogen peroxide during the acute phase of experimental UC. In the myenteric plexus, the inflammation caused an atrophy of nitrergic neurons (PGP9.5/nNOS+) (↓7.64%) ($p<0.05$) and a hypertrophy of estimated cholinergic neurons ($PGP9.5/nNOS^{-}$) (↑4.97%) ($p<0.05$) in animals that received the

(p<0.05). Related to the VIP-IR varicosities area, we found an increase of 35.55% in the CR group (vs C; p<0.05). There was a reduction of 19.64%, 26.14% and 23.49% in CQ, CP and CQP, respectively (vs CR; p<0.05). **Conclusions:** The CRC induction affected the nitrergic neurons and VIPergic varicosities, whilst quercetin and probiotic showed good results in the treatment of the carcinogenesis effects.

**Keywords:** Enteric nervous system; Small intestine; *Bifidobacterium animalis*; Myenteric plexus; Immunohistochemistry.


# EFFECTS OF HIGH PROTEIN DIET AND INDUCED-DSS ULCERATIVE COLITIS ON THE ILEUM OF MICE.

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standard diet. In animals that received the high-protein diet, inflammation caused an atrophy of nitrergic neurons even greater (↓↓22.3%) ($p$<0.05), but no cholinergic hypertrophy was observed. In animals that were not inflamed and that received a high-protein diet, there was an atrophy of the cholinergic neurons (↓9.80%) ($p$<0.05). In the submucosal plexus, both inflammation and a high-protein diet did not change the population density of PGP9.5 neurons without immunoreactivity for Calretinin (PGP9.5/Calr$^{-}$) or with weak (PGP9.5/Calr$^{+}$) or strong (PGP9.5/Calr$^{++}$) immunoreactivity (IR) for this protein. When observing the cell body area of submucosal neurons, it was found that inflammation did not cause any change in animals that received a standard diet. On the other hand, when fed a high-protein diet, inflammation caused atrophy in the two evaluated populations of submucous neurons: estimated cholinergic (PGP9.5/Calr$^{+}$) (↓12.2%) ($p$<0.05) and VIPergic (PGP9.5/Calr$^{++}$) (↓8.64%) ($p$<0.05). When the effect of diet alone on the area of these neurons was evaluated, this atrophy was observed only in neurons estimated to be cholinergic (PGP9.5/Calr$^{+}$) (↓5.22%) ($p$<0.05). Both inflammation and a high-protein diet did not change the population density of neurons in both plexus.

**Conclusions:** The high-protein diet (44% protein) worsens the clinical signs of UC in the acute phase of the disease, as well as exerting an influence on the size of neurons in the ileum of mice.

**Key words:** enteric nervous system; inflammatory bowel disease; diet; high protein; ulcerative colitis.


# EFFECT OF SODIUM BUTYRATE TREATMENT ON GUT-BRAIN AXIS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

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**Introduction:** Parkinson's disease (PD) is a neuroinflammatory disorder that affects the gut-brain axis. Our group has shown that the animal model of PD displays an enteric glial reactivity response in the colon, disrupting the intestinal epithelial barrier (IEB) and the presence of inflammatory markers. The enteric glial cells (EGCs) are recognized for their pivotal role in regulating intestinal homeostasis by controlling motility and epithelial barrier function and sensing microbial environment. Studies reveal that PD is linked to changes in the gut microbiome, resulting in an imbalance in microbiota metabolites that contribute to the progression of the disease.

**Objective:** We aim to investigate whether the oral sodium butyrate (NaB) treatment, a short-chain fatty acid, can rescue the alterations found in the gut-brain axis of the PD mice model induced by 6-hydroxydopamine administration in the striatum.

**Methodology:** Male C57/BL6 mice, 25-30g, were divided into three groups: Sham+Saline (control), submitted to stereotactic surgery and intrastriatal injection of saline with ascorbic acid; 6-OHDA+Saline and 6-OHDA+NaB, both submitted to stereotactic surgery to intrastriatal 6-hydroxydopamine administration. The 6-OHDA+NaB group received NaB (100mg/kg) after 48h of the stereotactic surgery via gavage for 5 days (CEUA-UFF: n° 6139030622). Motor abilities were assessed using the Pole test. Intestinal transit was measured based on the water content in the feces and the amount of fecal pellets observed in the colon. Western blotting and immunofluorescence evaluated the GFAP, occludin, and connexin-43 contents. It examined colon and spinal cord tissues.

**Results:** The 6-OHDA+Saline group showed an increase in the time to turn, orient downward, and fall compared to the control. However, the oral administration of NaB proved effective in rescuing motor dysfunction. The water content in feces in 6-OHDA+Saline animals was significantly lower, and the number of pellets found in the colon increased compared to the 6-OHDA+NaB and control groups. The Western blotting analysis of the colonic mucosal layer indicated that the GFAP and occludin protein content increased in the 6-OHDA+Saline and 6-OHDA+NaB groups compared to the control group. In the neuromuscular layer, the 6-OHDA+NaB and Saline groups maintained the increase in GFAP expression and the reduction of connexin-43 compared to the control group. The immunofluorescence of submucosal and myenteric ganglia revealed morphological alterations of the EGCs between the groups. In the 6-OHDA group, the EGCs displayed long branches and intense GFAP labeling, indicating reactive and stressed cells. In the 6-OHDA+NaB group, the staining pattern was heterogeneous, with specific points of intense staining for GFAP. However, the EGC morphology was similar to the control group. Both the 6-OHDA+Saline and 6-OHDA+NaB groups displayed an increase in GFAP expression, and only the 6-OHDA+NaB group increased the connexin-43 content in the spinal cord analysis compared to the control group.

**Conclusions:** The results point to EGCs as a critical element in the gut-brain axis in PD. The administration of NaB provided efficiency in promoting changes in EGCs and IEB in PD model animals, opening possibilities for future therapeutic strategies in the bottom-up scenario of the gut-brain connection.

**Key words:** Parkinson's disease; Enteric nervous system; Sodium butyrate; Gut-brais axis.

**Funding:** CAPES, CNPq, FAPERJ.


# PROJECT "SECOND BRAIN SPACE: FROM PRODUCTION TO THE POPULARIZATION OF KNOWLEDGE OF THE ENTERIC NERVOUS SYSTEM" - A GENERAL OVERVIEW OF THE PUBLIC SERVED FROM MAY 2022 TO SEPTEMBER 2023

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**Introduction:** Non-formal education is an important means of popularizing scientific research. At UEM, the main responsible for this education is the Interdisciplinary Dynamic Museum (MUDI/UEM), formed by several rooms, among them, the space linked to the Enteric Neural Plasticity Laboratory with the project entitled "Second brain space: from production to popularization of knowledge of the enteric nervous system". **Objective:** Evaluate the profile of the public served by the extension project from may 2022 to september 2023. **Methods:** Scheduling visits to the museum is done via the MUDI website, where the person responsible for scheduling indicates the education level and number of visitors, and can indicate between a general visit or a visit to specific rooms. During the visit, the public is divided into groups that pass through the different spaces of the museum, including the second brain space. The project aims teach about the enteric nervous system (ENS) and its relationship with other parts of the body, as well as its role in the development of diseases, the general functioning of drugs in the gastrointestinal tract and some pharmacologically active molecules studied by the enteric neural plasticity laboratory, which means, a short explanation about the dynamics of preclinical studies on the ENS. All these actions may also be done through traveling exhibitions held by the museum, in which the project participates in some of them. **Results:** In the period from May 2022 to September 2023, a total of 8282 people were served in the museum's physical space. Of these, 864 from special education, 450 from early childhood education, 789 from elementary school, 2853 from middle school and 3326 from high school. In addition to this public, more than 2000 other people were served by the project, through spontaneous visits and unscheduled visits. Another important point is the traveling exhibitions, which include more than 10000 people served by the project. Based on reports of project's monitors, most of the

served public did not know about them “second brain”, which indicates great potential and importance for the project and its scientific divulgation actions. **Conclusions:** Through the services provided by the second brain space project, children and adolescents of the most varied age groups can be sensitized with knowledge about the ENS, from physiology and pathophysiology to the dynamics of preclinical studies on the ENS. Furthermore, the majority of the public is reached through traveling exhibitions, which indicate a high potential for the expansion of the project.

**Key words:** Enteric Nervous System; Scientific Divulgation; Science Museum; Non-formal Education

# ABSTRACTS –

# PRESENTED AS POSTERS


# WHEAT GLUTEN PROMOTES TISSUE INFLAMMATION WITH IMPACT ON MYENTERIC NEURONAL DENSITY OF THE JEJUNUM OF RATS

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**Introduction:** Non-celiac gluten sensitivity (NCGS) is an enteropathy caused by the presence of gluten containing food and presents clinical signs similar to Celiac Disease (CD). Gluten induces systemic disorders, primarily characterized by chronic inflammation of the intestinal mucosa due to an increase in intraepithelial lymphocytes (IELs), villous atrophy, crypt hyperplasia, and dysbiosis, leading to impairment of the barrier and intrinsic innervation.

**Objective:** To assess the number of intraepithelial lymphocytes (IELs) in the jejunal mucosa and the quantitative aspects of the myenteric neuronal population HuC/D in the jejunum of rats fed with diets containing different levels of wheat gluten.

**Methods:** The procedures were approved by the Ethics Committee on Animal Use (CEUA). Fifteen male Wistar *Rattus norvegicus* were used, allotted to three groups (n=5) fed with increasing levels of wheat gluten in their diet after weaning: SG group (0%), standard CG group (14%), and excess gluten EG group (42%). After 69 days, jejunal samples were collected, fixed in 4% paraformaldehyde (pH7.4) for histological and immunohistochemical analyses. Following routine histological processing, 5µm thick semi-serial histological sections were obtained and stained with Hematoxylin and Eosin to highlight IELs (intraepithelial lymphocytes). All the cells present in 30 randomly selected fields of the intestinal epithelium per animal were quantified, and the results were expressed as the number of cells per µm². For immunohistochemistry, jejunal samples were stored in a 0.08% sodium azide PBS solution at 4°C until the dissection of the myenteric plexus. Staining of the general

neuronal population (anti-HuC/D) was performed, and images were captured using a fluorescence microscope. All immunolabeled cell bodies were counted in 40 microscopic fields per animal in the intermediate region of the intestinal circumference. The data were analyzed through Analysis of Variance (One-way ANOVA), followed by Tukey's post test, with results presented as mean ± standard deviation. The significance level was set at 5% ($p<0.05$).
**Results:** There was a significant increase of 17.31% in the number of IELs in the epithelium in the EG groups compared with the CG group; however, the number of IELs decreased by 34.87% in the SG group compared with the control group (CG), whenwheat gluten was removed from the diet. There was also a significant reduction ($p<0.0195$) in the density of HuC/D neurons in the jejunum of rats fed 42% wheat gluten (EG) compared to the SG group and a non-significant difference compared with the CG group.
**Conclusions:** Food intake with 42% wheat gluten for 69 days increased the number of intraepithelial lymphocytes and reduced the density of the HuC/D myenteric neuronal population in the jejunum of rats. Excess gluten in the diet indicated a high inflammatory potential with an impact on myenteric neuronal density.

**Key words:** enteric nervous system, gliadin, myenteric plexus, small intestine.


# EFFECTS OF GOJI BERRY (*Lycium Barbarum*) ON MYENTERIC NEURONAL DENSITY IN THE PROXIMAL COLON OF OBESE WISTAR RATS

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**Introduction:** Obesity is characterized by the excessive accumulation of body fat resulting from a caloric intake that exceeds daily energy expenditure, leading to a state of moderate inflammation. In the large intestine, changes in motility and microbiota are associated with obesity. Research has been conducted with the goal of finding compounds useful in the prevention and/or treatment of obesity, such as *Lycium barbarum*, known as Goji Berry (GB), which is characterized as a functional food that has proven to be effective in obesity control.
**Objective:** Analyzing the effects of a high simple carbohydrate diet (HSCD), treatment, and prophylaxis with GB on biometric parameters, goblet cell index, and myenteric neuronal and glial density in the proximal colon.
**Methods:** Male Wistar rats, 21 days old, were used following an experimental design approved by an Animal Ethics Committee. All animals had access to diet and water at will. The CA group worked as a control group and received a standard rodent diet and sham oral gavage with water for 109 days. The groups OA, OPR and OGB were feed with HSCD for 109 days. OA group received sham oral gavage with water for 109 days. OPR and OGB groups received 250 mg/kg of GB extract via oral gavage. OPR received GB extract throughout the 109 days of HSCD feeding and OGB in the last 60 days. At 130 days of age, following euthanasia, fat deposits and the proximal colon (CP) were collected. CP samples were used for histological (HE and PAS histochemistry) and neuronal analysis of the intestinal intrinsic innervation, myenteric neurons (HuC/D+), and enteric glial cells (S100+).

**Results:** The HSCD led to a significant increase in final body weight, body mass index (BMI) and visceral fat percentage, with a reduction in the length of the CP. Treatment with GB (OGB group) had a positive effect, resulting in a reduction in final body weight and mesenteric fat weight. Prophylaxis with GB (OPR group) significantly reduced BMI and increased the number of goblet cells. Myenteric neurons were classified as small (<350 μm²), medium-sized (350 to 650 μm²) and large (>650 μm²). Feeding with HSCD did not have a significant effect on myenteric plexus glial and neuronal density but promoted a higher incidence of small neurons. Treatment with GB did not have a significant effect on the mentioned parameters, while prophylaxis increased the incidence of large neurons.
**Conclusions:** Treatment with GB extract prevented weight gain and an increase in mesenteric fat. On the other hand, prophylaxis resulted in a reduction of BMI, restoration of the goblet cell index, and an increase in the incidence of large neurons. The use of GB extract is a promising alternative for obesity control.

**Key words:** enteric nervous system, goblet cells, large intestine, obesity.


# MYENTERIC PLEXUS RESPONSES TO TRIBUTYLTIN EXPOSURE IN DUODENUM OF WISTAR RATS

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**Introduction:** The enteric nervous system (ENS) is composed of nerve cells and enteric ganglia, which regulate the functions of the gastrointestinal tract, independently of central inputs. In this way, they carry out *crosstalk* between the intestinal lumen and the brain, ensuring intestinal homeostasis. The nerve cells of the ENS have great plastic potential, responding in different ways to external agents. Considering the wide exposure of the food route through contaminated water and food, making

the ENS particularly vulnerable to the permeation of toxic substances. Among them, tributyltin (TBT) stands out, a toxic compound derived from tin used in chemical, industrial applications and in the formulation of antifouling paints on vessels and which stands out for its persistence in the environment and its ability to bioaccumulate in the trophic chain.

**Objective:** Investigate the responses of subchronic exposure to the anti-fouling agent Tributyltin (TBT) on the total and metabolically active neurons of the myenteric plexus of the duodenum in adult male Wistar rats.

**Methods:** 36 male Wistar rats were used and kept under controlled conditions. They were randomly divided into three groups receiving environmental concentrations: a control group (GC), an experimental group with 20ng/g of TBT (GE20), and an experimental group with 600ng/g of TBT (GE600). After 31 days, the animals were anesthetized, euthanized, and their duodenums were collected and destined for the neuronal techniques

of NADH-dp and Giemsa. Subsequently, the tissues were dissected to evaluate the neuronal density and morphometry. The results were statistically analyzed using One Way ANOVA with a significance level of 5%.

**Results:** The results indicate significant reductions in the density of general neurons, however, the density of metabolically active neurons (NADH-dp reactive) remained like the control group. Regarding cellular morphometry, reactive NADH-dp neurons showed a significant increase in the cellular and nuclear profile. The results corroborate possible episodes of cell death of general neurons, together with the reduction in the cellular profile and since the increase in the cellular and nuclear profile may indicate that the remaining neurons intensified their metabolism to meet the demands resulting from TBT.

| | | GROUPS | | |
|---|---|---|---|---|
| | | **GC** | **GE20** | **GE600** |
| **NADH-dp** | **Neuronal density** | 21.55 ± 2.92[a] | 23.87 ± 3.12[a] | 23.64 ± 1.82[a] |
| | **Cell profile (µm²)** | 401.75 ± 58.90[a] | 665.20 ±131.24[a] | 316.27 ± 71.36[b] |
| | **Nuclear profile (µm²)** | 158.70 ± 23.03[a] | 259.87 ± 54.71[a] | 91.06 ±31.39[a] |
| **Giemsa** | **Neuronal density** | 32.34 ± 0.43[a] | 21.04 ± 1.27[b] | 26.36 ± 1.77[c] |
| | **Cell profile (µm²)** | 307.14 ± 41.73[a] | 280.24 ± 35.93[b] | 222.93 ± 60.28[a] |
| | **Nuclear profile (µm²)** | 96.91 ± 9.52[a] | 99.62 ± 16.89[b] | 85.18 ±25.03[c] |

**Table 1**. The average density of neurons/ganglion and cellular and nuclear profile morphometry - Giemsa and NADH-diaphorase positive neurons from the GC, GE20, and GE600 groups. **Different letters indicate significant differences - One Way Anova p>0.05 and Holm-sidak post-hoc (p< 0,05).*

**Conclusion:** The responses of the myenteric plexus demonstrated it is plasticity when in contact with the TBT, elucidating mechanisms for maintaining homeostasis.

**Key words:** enteric nervous system, antifouling, neuronal density


# EVALUATION OF INTESTINAL HISTOPATHOLOGY IN LAMBARI *Astyanax lacustris* EXPOSED TO ENVIRONMENTAL CONCENTRATIONS OF FLUOXETINE

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**Introduction:** The fluoxetine hydrochloride (FLX) is the most prescribed antidepressant worldwide and in the past years, the population faced the COVID pandemic and other mental illness urged in post-pandemic scenery and the prescriptions of FLX raised. The effects of FLX in aquatic organisms and their digestive system are not well understood.
**Objective:** This work aimed to characterise the histopathology of intestinal tract of *Astyanax lacustris* exposed to environmental concentrations of FLX.
**Methods:** The experimental period was 10 days and was compounded by: control group (CT), 0.5 ng/L and 5.0 ng/L with 4 juvenile individuals in each group in duplicate. The parameters were kept standardised during all experiment. The CT and experimental groups tanks were partially renewed every 48 hours. Afterwards, the individuals were euthanized, the intestines collected and processed under routine histology. The slides were photographed. For morphometric analysis, measurements (µm) of villus height and submucosal thickness, measured by ImageJ software and the means and respective standard deviations were statistically analysed. Intraepithelial lymphocytes (IELs) and neutral goblet glandular stained with Periodic Acid-Schiff (PAS) were counted in relation to the number of enterocytes. All statistically verified using the GraphPad Prism software using the OneWay ANOVA-Tukey test, with a significance of $p<0.05$.
**Results:** In morphometry, the concentration of 0.5 ng/L showed lower villus compared to the control and 5.0 ng/L. At 5.0 ng/L, the submucosa was thicker than in the other groups. In both experimental groups, the IELs were higher compared to CT, indicating an inflammatory process. The PAS+ goblet cells were lower in experimental groups, being more exposed to pathogens and injuries/damages to others strata, such as in 5.0 ng/L, which the submucosa was thicker, crossing the intestinal barrier. The decreased PAS+ goblet cells also cause malfunction of absorption and lubrication of mucosa. See Table 1.

Table 1 - Morphometric data of intestinal tunics and quantitative data on IELs and goblet cells from the intestine of *Astyanax lacustris* exposed to environmental concentrations of fluoxetine with mean and standard deviation (SD).

| | | GROUPS | | |
|---|---|---|---|---|
| | | CONTROL | 0.5 ng/L | 5.0 ng/L |
| Morphometry (µm) | Villi height | $0.4336^{a} \pm 0.0989$ | $0.2805^{b} \pm 0.1097$ | $0.4537^{a} \pm 0.1127$ |
| | Submucosa | $0.0208^{a} \pm 0.0079$ | $0.0223^{a} \pm 0.0059$ | $0.0298^{b} \pm 0.0064$ |
| IELs/enterocytes | | $0.0790^{a} \pm 0.0131$ | $0.1290^{b} \pm 0.0022$ | $0.1175^{b} \pm 0.0029$ |
| Goblet cells/ enterocytes | | $0.1090^{a} \pm 0.0131$ | $0.0564^{b} \pm 0.0128$ | $0.0712^{b} \pm 0.0094$ |

** Values followed by different letters in the same line are statistically different.*

**Conclusions:** Lambari *A. lacustris* exposed to relevant environmental concentrations of FXL show changes in the intestinal barrier.

**Key words:** emerging contaminants; ecotoxicology; histopathology; antidepressants; teleostei.


# MORPHO-QUANTITATIVE CHANGES IN MYENTERIC PLEXUS OF A NON-OBESE TYPE 2 DIABETES RAT MODEL (GOTO-KAKIZAKI)

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**Introduction:** The Goto-Kakizaki rat (GK) is a non-obese model that spontaneously develops type 2 diabetes mellitus (T2DM) without needing diets or drugs. We reported that 120-day-old GK rats have glucose intolerance, insulin resistance, altered the morphology of villus (height and thickness) and crypt (depth) morphology, inflammation, and delayed intestinal transit with no difference in myenteric total population density.

**Objective:** To characterize the 120-day-old GK rat's myenteric plexus by evaluating the neurons' density cell body area and the enteric glia.

**Methods:** 120-day-old Wistar and GK rats were submitted to a glucose tolerance test (GTT) to confirm the diabetic state. The small intestinal segments, duodenum, jejunum, ileum, proximal, and distal colon were collected, washed, and fixed in paraformaldehyde for 4 hours. The myenteric plexus was evaluated by immunofluorescence in whole-mount preparation for the total neuronal population (HuC/D), the cholinergic (ChAT) and nitrergic (nNOS) subpopulations, and enteric glia (S100). The number of neurons and glia per ganglion were counted, and 100 cell bodies per segment and per staining were measured.

**Results:** The GTT confirmed the GK rats' glucose intolerance. The basal glycemia in the GK group was higher than in the control group and remained as such during the entire test. At the final period, 60 minutes, GK rats failed to return to their basal glycemia. There were no differences between GK and Wistar rats regarding the density of the total neuronal population and the nitrergic neurons. However, the GK group had decreased cholinergic neurons in the duodenum ($p < 0.001$), jejunum ($p < 0.01$), and ileum ($p < 0.01$). An increase of 39% in the enteric glia density was observed in the ileum of GK rats. The proximal colon of GK rats exhibited an increase in cholinergic neuron density compared to controls. The cell body area of the total population of neurons was higher in the jejunum (by 46%) and ileum (by 32%) of GK animals. No statistical differences were observed for neuronal density and cell body area in the large intestine. The analyses of the cell body area through histograms revealed a predominance of larger body area neurons in the small intestine of GK rats for the total population and in the ileum for the inhibitory neurons. In the large intestine, the GK group had a prevalence of smaller areas.

**Conclusions:** The increase in the neuron body areas and the enteric glial density could be promoted by hyperglycemia in GK rats. The increase in cholinergic neurons in the proximal colon of GK rats could be a compensatory mechanism in response to the decrease in excitatory neurons reported in the small intestine. The decrease in the density of the cholinergic myenteric plexus of 120-day-old GK rats might be involved in the delayed intestinal transit reported.

**Key Words:** GK rats; constipation, diabetic enteropathy, myenteric plexus, enteric glia.

Funding: FAPESP (2022/11249-9, 2018/09868-7), CNPq, CAPES, and Cruzeiro do Sul University.


# STUDY OF ENTERIC NEURONS AND ENTERIC GLIAL CELLS IN CHRONIC EXPERIMENTAL ULCERATIVE COLITIS IN MICE DEFICIENT FOR THE P2X7 RECEPTOR (P2X7R -/-)

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**Introduction:** Inflammatory Bowel Disease (IBD) is a term used to describe prolonged inflammation of the gastrointestinal tract, including Chron's disease (CD) and Ulcerative colitis (UC). Ulcerative colitis affects enteric neurons and causes continuous mucosal inflammation, extending from the rectum to the proximal colon. The P2Z7 receptor (P2X7R) is activated by increased levels of extracellular ATP in intestinal inflammation and activates the NLRP3 inflammasome. The P2X7R participates in the regulation of the inflammatory response through the process of releasing pro-inflammatory cytokines, such as IL-1β, IL-18, and TNFα. **Objective:** This project aims to analyze enteric neurons and enteric glial cells in chronic experimental ulcerative colitis in mice deficient for the P2X7 receptor gene (P2X7R -/-, P2X7KO) and C57BL/6 Wild Type (WT).
**Methods:** Male mice were used. Colitis was induced by 3 cycles of 2% (2g/100ml) of Dextran Sodium Sulfate (DSS) dissolved in drinking water for 5 days (cycles 1 and 2 2% (2g/100ml) and 1.5% (1.5g/100ml) in cycle 3), followed by drinking water for the next 14 days (KO/DSS and WT/DSS groups). The status of the animals was monitored by general examination and body weight evolution. The KO/SHAM and WT/SHAM groups received water through the same period. The animals were euthanized after 57 days and the distal colon was removed. This study was approved by the Ethics Committee on Animal Use of the University of São Paulo, Brazil. Tissues were prepared by immunohistochemical methods with double labeling of the nitric oxide synthase neuronal (NOSn), acetylcholine transferase (ChAT), P2X7 Receptor (P2X7R),) and glial fibrillary acidic protein (GFAP). The number of NOSn-immunoreactive (ir) neurons (neuron/ganglion), ChAT-ir, P2X7R-ir, and glial cells positive for GFAP-ir were counted. Data were compared using ANOVA and Tukey's test, $p<0.05$ was statistically significant. (*WT/SHAM *vs* WT/DSS, **KO/SHAM *vs* KO/DSS). **Results:** Preliminary results show double labeling of neurons and glial cells with P2X7 receptor. The results are shown in Table 1. Histological studies revealed that the mucosa, lamina propria, and submucosal ganglia in the WT/SHAM and KO/SHAM groups were normal appearance. The submucosal of the WT/DSS and KO/DSS groups displayed increased thickness.

**Table 1.** Number of neurons/glia per ganglia expressing P2X7-receptor and for nNOS, ChAT, and GFAP positive for enteric glial cells in the distal colon myenteric plexus from the WT/SHAM, WT/DSS, KO/SHAM and KO/DSS groups.

| Groups | NOSn | ChAT | GFAP | P2X7R |
|---|---|---|---|---|
| **WT/SHAM** | 3.550±0.1610* | 8.280±0.1832* | 9.090±0.1759* | 8.880±2.447* |
| **WT/DSS** | 2.800±0.1385* | 5.740±0.1440* | 6.780±0.1474* | 9.120±2.280* |
| **KO/SHAM** | 3.950±0.1800 | 7.660±0.1860** | 8.310±0.1884** | 0 |
| **KO/DSS** | 3.450±0.1743 | 5.030±0.1672** | 5.890±0.1556** | 0 |

**Conclusions:** Our data conclude that myenteric neurons and glial cells of the distal colon were affected by ulcerative colitis and, that P2X7R Knockout mice were efficient in neuroprotection. Thus, these results demonstrate that the P2X7 receptor may be an important target in the therapeutic strategy.

**Keywords:** Purinergic receptor, Enteric Nervous System, Inflammatory Bowel Disease, P2X7R Knockout mice.


# EFFECTS OF MICROENCAPSULATED QUERCETIN AND *Bifidobacterium animalis* ON ENTERIC GLIAL CELLS IN THE ILEUM MUCOSA OF RATS WITH CHEMICALLY INDUCED COLORECTAL CARCINOGENESIS

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**Introduction:** Colorectal cancer is the third most common type of cancer in the world. For animal studies, this carcinogenesis is commonly chemically induced, leading to the formation of preneoplastic lesions like those found in humans. The systemic consequences generated by the disease, such as oxidative stress and systemic inflammation, can affect other intestinal regions as well as the Enteric Nervous System. **Objective:** This study aimed to evaluate enteric glial cells in the mucosa ($EGC_M$) of the ileum of rats with chemically induced colorectal carcinogenesis and the effects of supplementation with microencapsulated quercetin and *Bifidobacterium animalis*. **Methods**: Twenty-five 50-day-old male Wistar rats were randomly distributed into five groups (n=5): control (C); colorectal carcinogenesis (CR); colorectal carcinogenesis supplemented with microencapsulated quercetin (CQ); colorectal carcinogenesis supplemented with *Bifidobacterium animalis* (CP) and colorectal carcinogenesis supplemented with microencapsulated quercetin and *Bifidobacterium animalis* (CQP). For the induction of the carcinogenesis, DMH (40 mg/kg body weight, intraperitoneal route) was used twice a week for two weeks. The supplementation was made with microencapsulated quercetin (10 mg/kg/day) and the probiotic *Bifidobacterium animalis* subtype *lactis* ($5x10^7$ CFU/day). The experimental period was 16 weeks. After euthanasia, intestinal samples were collected, fixed and embedded in paraffin. Histological sections of 10 µm were made and submitted to immunohistochemistry to study $EGC_M$ immunoreactive (IR) GFAP and S100 in the ileal mucosa. The quantitative analysis of $EGC_M$ GFAP-IR was performed on 30 villi per animal and $EGC_M$ S100-IR performed by steriology considering thirty fields per animal. The significance level adopted was $p < 0.05$. All procedures performed followed ethical principles and were approved by the Ethics Committee on the Use of Animals, protocol nº 1126010419. **Results:** We observed an increase in the density of $EGC_M$-GFAP-IR and $EGC_M$-S100-IR in the ileal mucosa of animals with colorectal carcinogenesis. The supplementation of

microencapsulated quercetin and/or probiotic to animals with carcinogenesis significantly reduced the number of $EGC_M$-GFAP-IR: CQ (24%), CP (32%) and CQP (22%) (vs CR; p < 0 .0001). The density of $EGC_M$-S100-IR, however, did not show any change in the CQ and CP groups. On the other hand, joint supplements (CQP group) resulted in a reduction in the density of these CGEM-S100-IR (CQP vs CR; 16%; p = 0.048). **Conclusions:** The physiological changes promoted by DMH-induced colorectal carcinogenesis impacted the small intestine, leading to an increase in the number of $EGC_M$ and probably greater activity of these cells. Supplementations with microencapsulated quercetin and *Bifidobacterium animalis*, together or alone, showed positive effects, attenuating changes in $EGC_M$, probably due to their antioxidant and anti-inflammatory properties.

**Keywords:** Enteric Nervous System. Oxidative stress. Inflammation. Antioxidant. Probiotic.


# EFFECTS OF COLORECTAL CARCINOGENESIS INDUCED BY 1,2-DIMETHYLHYDRAZINE (DMH) ON THE ENTERIC NERVOUS SYSTEM OF RATS

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**Introduction:** Colorectal cancer is a neoplasm with a high worldwide incidence. In animal models, colorectal carcinogenesis can be induced by 1,2-dimethylhydrazine (DMH), leading to the formation of preneoplastic lesions, oxidative stress and inflammation. The systemic consequences of the disease can affect other intestinal regions, although little is known about the effects on the Enteric Nervous System (ENS). **Objective:** This study aimed to evaluate the enteric neuronal and glial population of the ileum of rats with chemically induced colorectal carcinogenesis. **Methods:** Ten 50-day-old male Wistar rats were randomly distributed into two groups (n=5): control (C) and colorectal carcinogenesis (CR). In carcinogenesis induction was used DMH (40 mg/kg body weight, intraperitoneal route) twice a week for two weeks. The experimental period was 16 weeks. After euthanasia, intestinal samples were collected, fixed and dissected to study the myenteric and submucosal plexus. For quantitative analysis of neurons and enteric glial cells (EGC) in both plexuses, thirty fields per animal were randomly captured using a 20X objective. Morphometric analysis was performed using 100 cell bodies per animal. The significance level adopted was $p < 0.05$. All procedures performed followed ethical principles and were approved by the Ethics Committee on the Use of Animals, protocol nº 1126010419. **Results:** We observed a reduction in the density and morphometry of neurons immunoreactive to HuC/D in the submucosal plexus of animals with colorectal carcinogenesis (CR vs C; $p< 0.01$) and no significant changes in the myenteric plexus. Meanwhile, the population of EGC immunoreactive to S100 in the myenteric plexus was significantly reduced (CR vs C; 15.5%; $p = 0.001$), with no change in the submucosal plexus. On the other hand, a significant increase in EGC morphometry was observed in the myenteric plexus (17%) and the submucosal plexus (7.5%) of animals with colorectal carcinogenesis (CR vs C; $p< 0.0001$). **Conclusions:** Carcinogenesis in the colon altered ENS cells in the small intestine, a probable effect of oxidative stress and systemic inflammation caused by the disease. More studies are needed to understand the effects of colorectal carcinogenesis on the small intestine.

**Keywords:** Oxidative stress. Inflammation. Small intestine. Enteric neurons. Enteric glial cells.


# ENTERIC NEUROTRANSMISSION IN THE JEJUNUM OF RATS WITH NON-SMALL CELL LUNG CANCER AND THE EFFECTS OF ADMINISTERING MELATONIN

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**Introduction:** Respiratory tract cancers have a high global incidence becoming a major public health issue. The most common subtype of lung cancer is non-small cells (NSCLC), is treated traditionally with chemotherapy and surgery, however, still presents a bad prognosis. In this sense, advances toward better therapeutics are necessary. Melatonin administration is well-documented as a potential resource for NSCLC since it is oncostatic and presents effects modulating inflammation and oxidative state. However, studies regarding systemic effects of NSCLC and melatonin administration are scarce. **Objective:** evaluate enteric neurotransmission alterations caused by NSCLC Development and by 10 mg/kg melatonin administration. **Methods:** All procedures were performed with approval from the ethics committee in research with animals (UEM, #8812040521). Twenty male Wistar rats were randomly distributed into four groups: control (C), control administered with melatonin 10 mg/kg (CM), NSCLC (A), and NSCLC administered with melatonin 10 mg/kg (AM) for 14 days. Tumor induction was performed by inoculating 1,0 x $10^7$ A549 cells on the right flank. Melatonin was given by gavage, daily. After euthanasia, the tumors were removed, and samples of the jejunum were collected, fixated, and dissected for study. To evaluate body parameters, animals and tumors were weighted for calculation of cachexia index (CI), with a threshold of >10%. To study enteric neurotransmission, we performed immunohistochemistry for neuronal subpopulations of immunoreactive to the vasoactive intestinal peptide (VIP-IR) on submucosal plexus and neuronal nitric oxide synthase (nNOS-IR) on myenteric plexus. Quantitative analyses were made in 30 fields captured on intermediate regions, per animal, with the 20X lens, for both populations. Morphometric analyses used 100 neurons per animal, captured with the 20X lens, for both markers. Data was analyzed by block design with Fisher's post-test and the significance level adopted was $p < 0.05$. **Results:** 3 animals in group A presented CI > 10% and the group's average was 8.68%. In group AM, only 1 animal presented CI > 10% and the group's average was 7.79%. The VIP-IR neurons increased by 36.31% in density in NCSLC (A compared to C; $p<0.0001$) and melatonin decreased the population by 16.90%

(AM compared to A, $p<0.0001$). Melatonin also increased the density in healthy animals 36.69%, (CM compared to C, $p<0.0001$). The disease caused an 8.02% increase in cell area (C compared to A, $p=0.0002$) and melatonin prevented this (9.3%, A compared to AM, $p < 0.0001$). Myenteric nNOS-IR neurons presented a 29,48% reduction (AM compared to A, $p < 0.0001$). In morphometry, there was a reduction of 6.57% (A compared to C, $p = 0.001$), an increase of 14.04% (AM compared to A, $p < 0.0001$), and an increase of 5.36% (CM with C, $p = 0.009$). **Conclusions:** The development NSCLC alters enteric neurotransmission, increasing density and size of VIP-IR and nNOS-IR neurons. Melatonin administration can prevent part of these changes. The administration of melatonin can prevent part of the changes.

**Keywords:** Lung Cancer; Neurons; Neurotransmission; Plasticity; Melatonin.


# ANTI-TNF ALPHA TREATMENT IS EFFICIENT IN THE RECOVERY OF MYENTERIC NEURONS EXPRESSING TUMOR NECROSIS FACTOR-ALPHA 2 RECEPTOR (TNFR2) FOLLOWING EXPERIMENTAL ULCERATIVE COLITIS

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**Introduction:** Ulcerative colitis and Crohn's disease have pathophysiological processes such as necrosis of enteric neurons. Also, the main inflammatory mediator is tumor necrosis factor alpha (TNFα). **Objective:** This project aims to study the effects of anti-TNFα, (Adalimumab, ADA) on the myenteric plexus expressing tumor necrosis factor-alpha 2 receptor (TNFR2) in experimental ulcerative colitis.
**Methods:** Male C57BL/6 mice were used. Mice received 3% dextran sodium sulfate (DSS group) dissolved in drinking water for 7 days. The Sham group received water for the same period. The DSS+ADA group received anti-TNFα (Adalimumab, 50mg/Kg) on the second day of DSS intake. The ADA group received water for the entire period and anti-TNFα (Adalimumab, 50mg/Kg) on the second day. The scoring of the disease activity index (DAI) was analyzed in all groups. The number of neurons/glia per ganglion, and the profile area neuronal of the neuronal nitric oxide synthase (nNOS), choline acetyltransferase (ChAT), PGP9.5 (pan-neuronal), TNFR2 receptor, and Glial Fibrillary Acid Protein (GFAP, pan enteric glial cell) are immunoreactive (ir) were evaluated. The data were compared statistically by two-way ANOVA and Tukey test, $p<0.05$. **Results:** a) The Disease Activity Index (DAI) of the DSS group were higher compared to the sham group and, there was a decrease in the DSS+ADA group compared to the DSS group; b) There were double labeling of myenteric neurons with TNFR2-receptor; c) there was a decrease in the number of neurons/ganglia in the DSS group and recovery in the DSS+ADA group; d) there was an increase in the TNFR2-receptor-ir/ganglia and GFAP-positive glial cells/ganglia in the DDS group and decrease in the DSS+ADA group and, e) there was a reduction in the nNOS- and ChAT-ir neuronal profile in the DSS group and recover in the DSS+ADA group. The submucosal of the DSS group displayed increased thickness.

**Table 1**. Number of cells per ganglia and neurons/glia per ganglia expressing TNFR2-receptor and cell profile area (µm2) from the Sham, ADA, DSS and DSS+ADA groups.

| **Cell per ganglia** | **Sham** | **ADA** | **DSS** | **DSS+ADA** |
|---|---|---|---|---|
| TNFR2-receptor | 26.7± 0.1 | 22.0 ±1.2# | 30.6 ±0.3* | 19.1±0.3** |
| nNOS | 7.9±0.05 | 8.1±0.2 | 5.6±0.2* | 7.2±0.1** |
| ChAT | 9.8±0.2 | 7.7±0.1 | 6.7±0.08* | 8.2±0.2** |
| PGP9.5 | 19.2±0.1 | 17.9±0.3 | 15.06±0.2* | 17.6±0.2** |
| GFAP | 17.07±0.1 | 19.04±0.07 | 19.5±0.1* | 17.9±0.2** |
| **cell profile area (µm$^2$)** | **Sham** | **ADA** | **DSS** | **DSS+ADA** |
| NOSn | 522.8±45.5 | 459.8±3.9 | 218.4±4.3* | 440.9±22.8** |
| ChAT | 222.9±9.6 | 239.7±6.8 | 187.8±0.1* | 201.3±16.1** |
| PGP9.5 | 189.4±10.3 | 193.6±18.6 | 146.3±14.5 | 150.4±17.0 |

*DSS group compared to Sham group;** DSS+ADA group compared to DSS group; #ADA group compared to Sham group); (*, #, ** p<0.05).

**Conclusions:** It is concluded that myenteric neurons are immunoreactive to the TNFR2 receptor and Adalimumab treatment was efficient in recovery of the myenteric neurons and enteric glial cells. Thus, these results demonstrate that anti-TNFα may be an important target in the therapeutic strategy.

**Keywords:** Tnf-a, Enteric Neuron, Enteric Glial Cells, Ulcerative Colitis, Adalimumab.


# ENVIRONMENTAL CONCENTRATIONS OF FLUOXETINE MODULATES THE DEVELOPMENT OF ENTERIC NERVOUS SYSTEM AND SEROTONERGIC NEURONS IN EARLY EMBRYOS OF ZEBRAFISH *Danio rerio*

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**Introduction:** The fluoxetine hydrochloride (FLX) is a class of antidepressants that acts as selective serotonin (5-HT) reuptake inhibitor (SSRI). In the previous years, the population faced the COVID pandemic and more prescriptions of psychotropic drugs have been reported. However, the effects of environmental concentrations of FLX on the Enteric Nervous System (ENS) of aquatic organisms still need to be better analyzed and defined.

**Objective:** The aim of this study was to evaluate the effect of environmental concentrations of FLX on enteric neurogenesis (general and serotonergic) during development of zebrafish (*Danio rerio)*.

**Methods:** The control group (CT) only with E3 medium, and the experimental groups: 50 $ng.L^{-1}$ (C1); 500 $ng.L^{-1}$ (C2); 5,000 $ng.L^{-1}$ (C3); and 50,000 $ng.L^{-1}$ (C4) of FLX diluted in E3 medium. The experiment was carried out from 3-hour post fertilization (3hpf) to 4 days post fertilization (4 dpf) and the solutions were changed daily and the animals kept on standardized conditions with 10 individuals in each group. A transgenic/mutant TgBAC(neurod:EGFP)nl1/Casper line was used for the experiment. At 4 dpf, the embryos were euthanized and proceeded to immunofluorescence routine. We used

whole-mounted embryos to analyze on confocal microscope. The counting was estimated based on the cells visible on microscopy field: number of enteric neurons (EN), number of 5-HT in general, and serotonergic neurons (SN) and SN/EN in percentage. The data were statistically evaluated using Prism GraphPad 8.0.1 software with OneWay-Anova-Tukey test, $p<0.05$.

**Results:** The EN population presented lower estimated neurons in C3 and C4 compared to C1, C2 and CT. However, the estimated SN subpopulation was higher only in C2 compared to all groups including CT. Nevertheless, the proportion of SN in EN was higher in concentrations above C2; so, the EN plasticity from neural crest were modulated to develop more SN to keep the homeostasis of neuronal 5-HT.

**Table 1.** The estimated number of Enteric Neurons (EN) and Serotonergic Neurons (SN) in the gut of zebrafish at 4 dpf exposed to environmental concentrations of FLX and SN/EN in percentage. Different letters in the same column mean statistically differences (OneWay-Anova-Tukey test, $p<0.05$). Mean values and their respective standard deviations (±SD).

| | Enteric Neurons (EN) | Serotonergic Neurons (SN) | Ratio SN/EN (%) |
|---|---|---|---|
| **CT** | $99.25 \pm 8.18^{a}$ | $8.25 \pm 0.50^{a}$ | $9.08 \pm 1.85^{a}$ |
| **C1** | $83.25 \pm 13.96^{a}$ | $9.00 \pm 1.15^{a}$ | $11.49 \pm 1.02^{a}$ |
| **C2** | $98.25 \pm 12.12^{a}$ | $12.50 \pm 2.08^{b}$ | $19.46 \pm 2.42^{b}$ |
| **C3** | $44.75 \pm 4.79^{b}$ | $10.00 \pm 1.15^{a}$ | $23.85 \pm 2.97^{b}$ |
| **C4** | $47.20 \pm 3.42^{b}$ | $10.00 \pm 1.41^{a}$ | $19.31 \pm 0.48^{b}$ |

**Conclusions:** The concentrations of 500 $ng.L^{-1}$ and higher of FLX modulated significantly the development of enteric and serotonergic neurons in the early embryos of zebrafish gut.

**Key words**: fluoxetine, enteric neurons, ecotoxicology, pathology, contaminant


# ILEUM RESPONSES TO TRIBUTYLTIN: CHANGES IN METABOLICALLY ACTIVE NEURONS AND PRX2 DENSITY.

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**Introduction:** The gastrointestinal tract (GIT) is a significant biomarker due to its adaptability. Indeed, the presence of xenobiotic compounds in the lumen can alter its morphophysiology, particularly the plasticity of the enteric nervous system (ENS). Oxidative stress is a predominant factor in inducing intraluminal alterations, with the generation of ROS capable of intensifying inflammatory processes, resulting in an imbalance in organismal homeostasis. Among the key proteins involved in ROS control, peroxiredoxin 2 (PRx2) stands out due to its ability to decompose organic and inorganic peroxides, recruit immune system cells, and neuronal protection.

**Objective:** To assess whether the presence of the persistent environmental contaminant tributyltin (TBT) can influence the density and morphology of metabolically active neurons (NADH-dp) in the ileum and whether these changes are related to alterations in the redox system.

**Methods:** 36 male Wistar rats were divided into three groups: control group (GC) and two experimental groups with environmental concentrations of TBT: 20ng/g (GE20) and 600ng/g (GE600). After 30 days, the animals were euthanized, and the ileum segment was destined for the neuronal techniques of NADH-dp (density and morphometry) and for the western blot technique to quantify the PRx2 (ab191535). Subsequently, the tissues were evaluated for neuronal density and morphometry. The results were statistically analyzed using One-Way ANOVA with a significance level of 5%.

**Results:** The results regarding neuronal density, neuron morphology, and intestinal PRx2 density are presented in Table 1. Interestingly, more significant alterations were observed in the group exposed to the lower concentration (20ng/kg), which proved to be more toxic in terms of reducing neuronal density. Although reduced in density, the nuclear profile of neurons increased, possibly due to heightened neuronal metabolism to compensate for losses caused by cell death, possibly induced by reactive species. PRx-2 is a protein widely involved in neuroprotection, explaining its increased presence in organisms of this same group. However, it is important to note that, while the initial increase in PRx-2 is advantageous for maintaining intestinal functions by decomposing

peroxides generated by the presence of the contaminant, chronic exposures may lead to enzyme hyperoxidation, inhibiting its catalytic activity.

**Table 1**. The average density of neurons/ganglion and cellular and nuclear profile morphometry of NADH-diaphorase positive neurons and densitometry from Peroxirredoxin-2 (PRx-2) from the GC, GE20, and GE600 groups. **Different letters at the same line indicate significant differences by - One Way Anova test*

| | | GROUPS | | |
|---|---|---|---|---|
| | | **GC** | **GE20** | **GE600** |
| **NADH-dp** | **Density** | 22.5 ± 5.9[a] | 16.14 ± 0.55[a,b] | 27.05 ± 10.01[a] |
| | **Cell profile ($\mu m^2$)** | 393.4 ± 51.03 | 447.2 ±100.6 | 348.9 ± 47.6 |
| | **Nuclear profile ($\mu m^2$)** | 142.03 ± 14.8[a] | 176.1 ± 35.1[a,b] | 102.0 ± 19,02[a] |
| **PRx2** | **Densitometry** | 0.38 ± 0,14[a] | 1.26 ± 0.59[b] | 0.65 ± 0.21[a] |

**Conclusion:** Our findings indicate that TBT exhibits heightened toxicity at lower concentrations, primarily due to specific toxicity mechanisms. This results in a decrease in the density of metabolically active neurons and an upsurge in the metabolism of the remaining neurons. Additionally, the presence of reactive species induced by the contaminant increases the density of PRx2, potentially posing a long-term threat to intestinal homeostasis.

**Keywords:** Enteric nervous system; NADH-dp; peroxiredoxin-2; oxidative stress; western blot


# TRIBUTILTIN: TOXICITY UNDER THE MYENTERIC PLEXUS OF THE JEJUNUM IN WISTAR RATS

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**Introduction:** Due to the frequent exposure of the gastrointestinal tract (GIT) to contaminants through the ingestion of contaminated water and food, it is essential for this system to encompass complex structures capable of ensuring intestinal homeostasis. Among these, the enteric nervous system (ENS) stands out, comprised of a network of neurons organized into two plexuses: the myenteric and submucosal plexuses. These neurons exhibit remarkable plasticity, responding diversely to external agents, in this way, considering the vital importance of this system in maintaining homeostasis, it becomes clear that it has significant potential as a biomarker for investigating toxicological effects. A notable substance capable of infiltrating the GIT is Tributyltin (TBT), an organotin compound extensively utilized in industrial and chemical applications, especially in antifouling paint formulations. Due to it is considerable toxicity, TBT has a propensity to accumulate in aquatic organisms and biomagnify up the food chain, ultimately affecting human health.

**Objective:** Investigate the responses of subchronic exposure to the anti-fouling agent Tributyltin (TBT) on the metabolically active neurons (NADH-dp) and the morphophysiology of the myenteric plexus of the jejunum in Wistar rats.

**Methods:** 36 male Wistar rats were used and kept under controlled conditions and divided into three groups receiving different environmental concentrations: a control group (GC), group with 20ng/g of TBT (GE20), and a group with 600ng/g of TBT (GE600). After the period of 30 days, the animals were euthanized, and their jejunum were collected for the

NADH-dp neuronal technique. Subsequently, the collected tissues were dissected to assess neuronal density, density of isolated neurons and plexus morphology. The results obtained were analyzed using One Way ANOVA with a significance level set at 5%.

**Results:** Exposure to TBT resulted in changes in the morphology of the plexus, significantly reducing the neuronal density of metabolically active neurons (NADH-dp reactive). In addition to the reduction, there were changes in the arrangement of neurons, and an increase in isolated neurons, outside the ganglia (table 1). The results indicate possible episodes of cell death, and imbalances in other subpopulations since the reduction in neuronal density and the increase in isolated neurons suggests a reorganization to ensure the maintenance of intestinal homeostasis.

| | | GROUPS | | |
|---|---|---|---|---|
| | | **GC** | **GE20** | **GE600** |
| **NADH-dp** | Isolation neurons density | $0.43\pm0.10^{a}$ | $2.45\pm0.48^{b}$ | $2.11\pm0.48^{b}$ |
| | Population density | $29.41 \pm 2.07^{a}$ | $21.66 \pm 1.87^{b}$ | $23.64 \pm 3.15^{b}$ |

**Table 1 -** The average density of isolation neurons and neurons/ganglion - NADH-dp from the GC, GE20, and GE600 groups. **Different letters indicate significant differences in the same line- One Way Anova p>0.05 and Holm-sidak post-hoc (p< 0,05).*

**Conclusion:** Exposure to TBT resulted in disorganization of the myenteric plexus, through a reduction in neuronal density and an increase in isolated neurons, demonstrating the toxicity of environmental concentrations of TBT.

**Key words:** neuronal migration, NADH-dp, metabolic active, cell death, plexus organization.


# ANALYSIS OF VIP IMMUNOREACTIVE NEURONS IN THE SUBMUCOSAL PLEXUS OF ARTHRITIC RATS ADMINISTERED WITH MICROENCAPSULATED QUERCETIN

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**Introduction:** Rheumatoid arthritis (RA) is a systemic inflammatory disease that affects the joints and also organs such as the intestine. Conventional treatment of RA consists of non-steroidal anti-inflammatory drugs, like ibuprofen, but these are known to cause harmful side effects. Quercetin is a natural compound with antioxidant properties and the potential to treat RA. Submucosal VIPergic neurons are related to the control of water and electrolyte circulation, mucus release, blood flow and proliferation of epithelial cells in the intestinal mucosa. **Objective:** To evaluate the density and morphometry of VIP immunoreactive (IR) cells in the submucosal plexus of arthritic rats administered with microencapsulated quercetin. **Methods**: Thirty-three 56-day-old male Holtzman rats were randomly assigned to five groups: control (C), quercetin-treated control (CQ), arthritic (AIA), ibuprofen-treated arthritic (AI), and quercetin-treated arthritic (AQ). RA was induced by intradermal injection of complete Freund's adjuvant containing 0.1 mL of 5% *Mycobacterium tuberculosis* suspension in the plantar region of the left hind paw. The CQ and AQ received quercetin-loaded microcapsules by gavage at a dose of 10 mg/kg, while the AI group received ibuprofen at a dose of 17.5 mg/kg also by gavage for sixty days. After euthanasia, intestinal samples were collected, fixed and dissected to study the submucosal plexus. For quantitative analysis of neurons VIP-IR, thirty-five fields per animal were randomly captured using a 20X objective. Morphometric analysis was performed using 100 cell bodies per animal. The significance level adopted was $p < 0.05$. All procedures performed were in accordance with ethical principles and approved by the Ethics Committee on the Use of Animals, protocol nº 4462180216. **Results:** The neuronal bodies of the VIP-IR neurons of the submucosal plexus in the AIA group were 6% higher than those in group C ($p < 0.05$), while those in group AQ were 17% smaller in comparison to AIA ($p < 0.05$). VIP-IR submucosal neurons showed reduced density in the AIA group (vs C; $p < 0.05$), and in AQ there was a 20% reduction when compared to AIA (p 0.05). **Conclusions:** The increase in cell body morphometry of VIP-IR neurons in the submucosal plexus of arthritic rats possibly occurred due to compensation for the

decrease in this neuronal phenotype. Quercetin was unable to prevent this change, causing an even greater reduction.

**Keywords:** Enteric Nervous System. Oxidative stress. Inflammation. Antioxidant.


# IMPACT OF MELATONIN ADMINISTRATION ON GFAP⁺ GLIAL INTERMEDIATE FILAMENTS IN THE JEJUNUM OF RATS WITH NON-SMALL CELL LUNG CANCER

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**Introduction:** Cancer is a disease characterized by disordered cell growth that leads to tissue damage. Oxidative stress and inflammation are two key mechanisms in the development of the disease and affect the enteric nervous system. Substances with antioxidant and immunomodulatory properties, such as melatonin, have been researched in modulating the pathophysiology of cancer. **Objective:** The goal of this study was to evaluate the effects of melatonin administration on GFAP⁺ glial intermediate filaments in the myenteric plexus of the jejunum of rats with non-small cell lung cancer. **Methods:** This study was approved by the Animal Research Ethical Committee of the State University of Maringá (UEM) under approval number 8812040521.Twenty-four male Wistar rats were used and randomly divided into 4 different groups containing 6 animals each: control (C), control administered with melatonin 10 mg/kg orally (CM), tumor-bearing rats lineage A549 (A), tumor-bearing rats lineage A549 administered with melatonin 10 mg/kg orally (AM). The animals in the A and AM group were inoculated subcutaneously with a $1x10^7$ suspension of A549 tumor cells in 0.3 mL of PBS in the right rear flank, while the control groups inoculated with PBS undergoing the same stress protocol. After the 14-day experimental period, the jejunum was collected to perform the immunohistochemical technique. Thirty-two images were captured per animal and the occupancy area by the GFAP⁺ glial intermediate filaments were measured using ImageJ® software version 1.43° (NIH). The statistical analysis was performed using one-way ANOVA analysis of variance was performed, followed by the Fisher's Test. The $p < 0.05$ were considered significant. **Results:** The animals with cancer (A group) increased 18% ($p = 0.03$) for the occupancy area by the GFAP⁺ glial intermediate filaments compared to C group. Comparing the administration of melatonin in the cancer group (AM) with A group, decrease 34% in AM ($P = 0.000447$) of the occupancy area by the GFAP⁺ gliofilaments. No significative changes were observed comparing C and CM groups. **Conclusions**: It is concluded that cancer increased GFAP+ glial intermediate filaments. Studies in the literature indicate increased expression of GFAP in several chronic diseases, including cancer. The administration of melatonin promoted the reduced of intermediate filaments. The reduction of these filaments may have been a beneficial effect of melatonin to bring these values closer to control animals. Melatonin had no effects on intermediate filaments in healthy animals.

**Key words:** Enteric glial cells, Inflammation, Oxidative stress, Anti-inflammatory.


# EFFECTS OF QUERCETIN-LOADED MICROCAPSULES AND *Bifidobacterium animalis* IN THE VIP IMUNOREACTIVE VARICOSITIES OF JEJUNUM MUCOSA IN EXPERIMENTAL MODEL OF COLORECTAL CARCINOGENESIS

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**Introduction:** Colorectal cancer is the third most common type of cancer in the world. For animal studies, colorectal carcinogenesis is commonly chemically induced by 1,2-dimethylhydrazine (DMH), leading to the formation of preneoplastic lesions. The disease's consequences, such as oxidative stress and inflammation, can affect the enteric nervous system. **Objective:** This study aimed to evaluate the varicosities immunoreactive to VIP (VIP-IR) in the jejunum mucosa of rats with chemically induced colorectal carcinogenesis and the effects of supplementation with quercetin-loaded microcapsules and *Bifidobacterium animalis*. **Methods:** Thirty 50-day-old male Wistar rats were randomly distributed into five groups (n=6): control (C); colorectal carcinogenesis (CR); colorectal carcinogenesis supplemented with quercetin-loaded microcapsules (CQ); colorectal carcinogenesis supplemented with *Bifidobacterium animalis* (CP) and colorectal carcinogenesis supplemented with quercetin-loaded microcapsules and *Bifidobacterium animalis* (CQP). The carcinogenesis induction was performed by the administration of DMH (40 mg/kg) for two weeks, twice a week. The quercetin-loaded microcapsules were administrated in a dose of 10 mg/kg/day and the *Bifidobacterium animalis* ($5x10^7$ CFU/day). The experimental period was 16 weeks. After euthanasia, intestinal samples were collected, fixed and embedded in OCT. Histological sections of 10 µm were made and submitted to immunohistochemistry to label VIP-IR varicosities in the jejunal mucosa. The area of 300 varicosities per animal were measured (40X objective) and the quantitative analysis were made by stereology in 30 fields randomly captured in the lamina propria region of the crypt (40X objective). The significance level adopted was $p < 0.05$. All procedures performed were approved by the Ethics Committee on the Use of Animals, protocol nº 1126010419. **Results:** Our results demonstrated an increase in the morphometry of VIP-IR varicosities in the jejunum mucosa of animals with carcinogenesis (CR vs C; $p < 0.01$) and no significant difference in these varicosities quantification. In animals supplemented with quercetin-loaded

microcapsules, was observed an increase in the morphometry of VIP-IR varicosities (CQ vs CR; $p < 0.01$) and no relevant change in quantification compared with the CR group. The administration of *B. animalis* promoted an increase of 19% in the quantification of these varicosities in the jejunal mucosa (CP vs CR; $p=0.01$) and no change in the morphometry results. The combined treatment did not promote changes when compared with the CR group. **Conclusions:** Colorectal carcinogenesis promoted physiological changes in the small intestine, leading to an increase in the size of VIP-IR varicosities. After the trial with quercetin-loaded microcapsules, *B. animalis*, and their combination, was observed that the quercetin attenuated the growth in the VIP-IR varicosities of jejunum mucosa and the probiotic promoted an increase in the quantification of these varicosities, what was not observed in the CR group. The administration of both (quercetin and probiotic) did not promote changes in the disease effect. In an overview, new studies are needed to conclusions more specifics about the use of probiotic and/or quercetin.

**Keywords:** Inflammation. Enteric Nervous System. Probiotic. Antioxidant.


# EFFECTS OF TRIBUTYLTIN (TBT) EXPOSURE ON GASTRIC BARRIER PLASTICITY

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**Introduction:** Tributyltin (TBT) is a chemical product with biocidal properties used in different industrial sectors. Due to its high toxicity, its use as an anti-fouling agent in boat paints has been banned by the International Maritime Organization. However, TBT is very persistent in the aquatic environment and, consequently, its environmental level, toxicity, bioaccumulation, and human exposure are of current concern. Food is the main route of entry for contaminants, making the gastric barrier one of the first regions exposed.

**Objective:** To evaluate the effects of sub-chronic exposure to TBT on different members of the gastric barrier.

**Methods:** Male Wistar rats were separated into three groups (5 animals/group): GC received only the vehicle (corn oil), GE20, and GE600, which received, environmentally relevant concentrations of 20ng/g and 600ng/g of TBT, respectively. After 30 days of exposure, the stomachs were collected and sent for histological processing to reveal the metabolically active neuronal population using the NADH-dr technique. The histological slides were stained with HE for stratigraphic evaluation of the mucosal layer. The membrane preparations of the glandular and aglandular regions had 40 ganglia photographed to obtain the neuronal/ganglion density. The results were submitted to the One-way ANOVA test.

**Results:** The results show non-monotonic responses to TBT, which is common for molecules characterized as endocrine disruptors. Significant differences were observed in the thickness of the mucosal layer (Table 1), with GE20 animals

showing a 54% reduction compared to the other groups, due to changes in the cell renewal process, altering the availability of the mucosa, causing a reduction in the contact and absorption surface. In GE600, there were significant changes in the myenteric plexus, with a higher neuronal/ganglion density in the glandular and aglandular regions compared to the other groups (Table 1). This is a consequence of the search for greater protection of the myenteric neurons within the ganglia and/or greater metabolic activity of neuronal subpopulations, which express neuroprotective molecules since TBT causes acidification of the intracellular pH and activation of apoptotic pathways. As TBT becomes more bioavailable at alkaline pH, the changes in GE600 may also have occurred via systemic contamination, through the absorption of TBT in the intestinal segments.

Table 1 - Mucosal layer thickness (μm) and neuron/ganglion density in the glandular and aglandular regions of Wistar rats in the Control Group (CG), Experimental Group exposed to 20ng/g of TBT (EG20), and Experimental Group Exposed to 600ng/g (EG600)

| | Mucosa | Density of neurons/ganglia Glandular region | Density of neurons/ganglia – Aglandular region |
|---|---|---|---|
| GC | 1087,62±139,73[a] | 19,33±0,83[a] | 16,73±2,26[a] |
| GE20 | 502,30±234,81[b] | 16,18±2,67[a] | 18,28±3,24[a] |
| GE600 | 1115,04±118,07[a] | 26,38±7,60[b] | 22,98±3,74[b] |

** Different letters in the same column show a significant difference (p<0.001).*

**Conclusions:** It is concluded that synchronous exposure to TBT at environmentally relevant concentrations affects the plasticity of different gastric barrier components.

**Key words:** NADH-d+, neuronal metabolism, gastrointestinal tract, anti-fouling.


# MORPHOMETRIC ANALYSIS OF IMMUNOREACTIVE VARICOSITIES TO CGRP IN THE ENTERIC NERVOUS SYSTEM OF ARTHRITIC RATS TREATED WITH MICROENCAPSULATED QUERCETIN

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**Introduction:** Rheumatoid arthritis (RA) is an autoimmune, inflammatory and multisystemic disease that affects not only joints but also the digestive system. Non-steroidal anti-inflammatory drugs, such as ibuprofen, are indicated for treatment, but their side effects have led to the search for other alternatives, such as quercetin, which is a flavonoid with antioxidant and anti-inflammatory properties, among others. In the enteric nervous system (ENS), there is the expression of the neurotransmitter calcitonin gene-related peptide (CGRP), which has a regulatory function in the ENS and is important in both physiological and pathological states, as it is involved in motility, secretion, absorption functions. and microcirculation. **Objective:** To evaluate the morphometry of CGRP immunoreactive (IR) varicosities in the myenteric and submucosal plexuses of the jejunum of arthritic rats treated with microencapsulated quercetin. **Methods:** Thirty 56-day-old Holtzman rats were divided into five groups: control (C), control treated with microencapsulated quercetin (CQ), arthritic (AIA), arthritic treated with ibuprofen (AI) and arthritic treated with microencapsulated quercetin (AQ). RA was induced by intradermal injection of complete Freund's adjuvant, containing 0.1 mL of 5% *Mycobacterium tuberculosis* suspension in the plantar region of the left hind paw. The CQ and AQ groups received microcapsules loaded with quercetin by gavage at 10 mg/kg, while the AI group received ibuprofen at a dose of 17.5 mg/kg also by gavage for sixty days. After euthanasia, intestinal samples were collected, fixed and dissected to study the myenteric plexus and submucosal plexus. Morphometric analysis was performed with a 40X objective, measuring 400 varicosities per animal with an area expressed in $\mu m^2$. All procedures were conducted in accordance with ethical principles, CEUA nº 4462180216. The significance level adopted was 5%. **Results:** CGRP-IR varicosities in the myenteric plexus were larger in the CQ group (11%) and in AIA (17%) (vs C; $p< 0.05$). In the AI and AQ groups, there was a reduction of 24% and 14%, respectively (vs AIA; $p< 0.05$). In the submucosal plexus, the area of CGRP-IR varicosities was reduced by 12% in CQ and 27% in AIA (vs C; $p< 0.05$). The size of CGRP-IR varicosities was increased in the AQ group and decreased in the AI group (vs AIA; $p< 0.05$). **Conclusions:**

Antagonistic results were observed regarding the size of CGRP-IR varicosities in the myenteric and submucosal plexuses. There was an increase in size in the AIA group and a reduction in the CQ group. On the other hand, the AI and AQ groups showed similar results in both plexuses. In the myenteric plexus, the reduction in varicosity size was probably a consequence of an anti-inflammatory effect, both for ibuprofen and quercetin.

**Keywords:** Antioxidant. Inflammation. Jejunum. Oxidative stress.


# EFFECT OF MICROENCAPSULATED QUERCETIN ON MYENTERIC NITRERGIC INNERVATION IN THE JEJUNUM OF ARTHRITIC RATS

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**Introduction:** Rheumatoid arthritis (RA) is an autoimmune disease that affects the body systemically, including the gastrointestinal tract. It is often treated with non-steroidal anti-inflammatory drugs, such as ibuprofen, which can lead to harmful side effects. Therefore, therapeutic alternatives have gained prominence in the treatment of RA. One example is quercetin, a natural flavonoid with antioxidant and anti-inflammatory properties. In the enteric nervous system, nitric oxide (NO) is a neurotransmitter produced by the enzyme neuronal nitric oxide synthase (nNOS), and changes in NO levels occur due to systemic pathologies like RA. **Objective:** To evaluate the density and morphometry of nNOS immunoreactive (IR) cells in the myenteric plexus of the jejunum of arthritic rats treated with microencapsulated quercetin. **Methods:** Thirty 56-day-old male Holtzman rats were randomly distributed into five groups: control (C), control treated with quercetin (CQ), arthritic (AIA), arthritic treated with ibuprofen (AI), and arthritic treated with quercetin (AQ). RA was induced by intradermal injection of complete Freund's adjuvant, containing 0.1 mL of 5% *Mycobacterium tuberculosis* suspension in the plantar region of the left hind paw. The CQ and AQ groups received microcapsules loaded with quercetin by gavage at 10 mg/kg, while the AI group received ibuprofen at a dose of 17.5 mg/kg also by gavage for sixty days. After euthanasia, intestinal samples from the proximal jejunum were collected, fixed, and dissected for the study of the myenteric plexus. For the quantitative analysis of nNOS-IR neurons, thirty-five fields per animal were randomly captured using a 40X objective. Morphometric analysis was performed using a hundred cell bodies per animal. The adopted significance level was 5%. All procedures were performed by ethical principles and were approved by the Animal Use Ethics Committee, protocol nº 4462180216. **Results:** The area of nNOS-IR neurons was reduced by 24% in the AIA group (vs C; $p < 0.05$). In the AQ group, the area was 6% larger (vs AIA; $p < 0.05$). There was a 20% increase in neuronal density in the CQ group (vs C; $p < 0.05$) and a 21% decrease in the AQ group (vs AIA; $p < 0.05$). **Conclusions:** A greater

phenotypic expression was observed in neurons expressing nNOS in the CQ group, indicating a higher density. Regarding the size of nNOS neurons, rheumatoid arthritis led to a reduction and consequently lower expression of the enzyme, and the treatment positively altered this outcome.

**Keywords:** Antioxidant. Enteric Nervous System. Inflammation.


# CATALASE ACTIVITY ON THE OXIDATIVE STATE IN THE JEJUNUM OF RATS INDUCED TO COLORECTAL CARCINOGENESIS TREATED WITH MICROENCAPSULATED QUERCETIN AND PROBIOTIC.

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**Introduction:** Colorectal cancer is the third most occurring type of cancer in the world. The effects of this disease affect not only the large intestine, but can also affect other parts in the digestive system, like the jejunum, causing alterations in the oxidative state of the enteric nervous system, leading to other systemic consequences.**Objective:**The aim of this study was measure the activity of the antioxidant catalase enzyme in the jejunum of rats induced to colorectal carcinogenesis treated with microencapsulated quercetin and the probiotic *Bifidobacterium animalis*. **Methods:** Forty 50-days-old male rats were randomly divided into five groups: Control (C), Colorectal carcinogenesis (CR), Colorectal carcinogenesis treated with microencapsulated quercetin (CQ), Colorectal carcinogenesis treated with *Bifidobacterium animalis* (CP) and Colorectal carcinogenesis treated with microencapsulated quercetin and *Bifidobacterium animalis* (CQP). For the induction of the carcinogenesis, DMH (40 mg/kg body weight, intraperitoneal route) was used twice a week for two weeks. The supplementation was made with microencapsulated quercetin (10 mg/kg/day) and the probiotic *Bifidobacterium animalis* subtype *lactis* ($5x10^7$ CFU/day). The experimental period was 16 weeks. After euthanasia, intestinal samples were collected and frozen. Homogenates (50 mg/mL) were prepared in 10 mM monobasic potassium phosphate buffer pH 7.4/0.9% NaCl. Each sample was subjected to homogenization by Ultra-Turrax in an ice bath. The supernatants were obtained by centrifugation at 11300 x g for 15 min at 4ºC and used to compose the catalase activity analysis assay. The significance level adopted was $p < 0.05$. All procedures performed followed ethical principles and were approved by the Ethics Committee on the Use of Animals, protocol nº 1126010419. **Results:** There was no difference in catalase enzyme activity in CR (vs C; $p > 0.05$). In CQ there was a 793% increase in enzymatic activity (vs CR; $p < 0.05$), in CP and CQP there was no difference (vs CR; $p > 0.05$). **Conclusions:** Catalase is an antioxidant enzyme that breaks down hydrogen peroxide, a highly harmful reactive oxygen species, into water and oxygen. The administration of microencapsulated quercetin demonstrated a significant increase in the activity of the enzyme catalase, an endogenous antioxidant, observed in the group treated with quercetin. This indicates that quercetin can stimulate the body's antioxidant systems, helping to neutralize the harmful effects of oxidative stress playing a crucial role in protecting against oxidative stress, contributing to the maintenance of redox balance.

**Key words:** Antioxidant, Oxidative Stress, Flavonoid, *Bifidobacterium animalis*.


# EVALUATION OF THE TOTAL ANTIOXIDANT CAPACITY IN OXIDATIVE STRESS ANALYSIS OF THE JEJUNUM OF RATS SUBMITTED TO SOCIAL ISOLATION

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**Introduction:** The Oxidative Stress happens when an imbalance between the amount of oxidative compounds and antioxidants in the organism. The damages caused by the disbalance of the oxidative and antioxidant substances are related to the etiological process of a lot of diseases. Social isolation was used as the principle method of prevention against the COVID-19, however this type of stress is a factor that influences the generation of radicals and oxygen reactive species.**Objective:** Evaluate the impact of the comportamental model of social isolation in the total antioxidant capacity to measure the generation of oxidative damages in the long intestine of rats submitted to this stress. **Methods:** Male rats with 21-days-old were divided in 2 groups (n=6), Control Group (C) and the Social Isolation Group (Is). The experimental period was 15 days. The rats were divided into boxes of 3 in the control group, and in individual boxes for the social isolated group. They were manipulated just for the cleaning routines. After the euthanasia, the intestinal samples were collected and frozen The samples were submitted to the total antioxidant capacity analysis. Homogenates (15 mg/mL) were prepared in 10 mM monobasic potassium phosphate buffer pH 7.4/0.9% NaCl. Each sample was subjected to homogenization by Ultra-Turrax in an ice bath. The supernatants were obtained by centrifugation at 11000 x g for 15 min at 4ºC and used to measure the total antioxidant capacity. All procedures performed followed ethical principles and were approved by the Ethics Committee on the Use of Animals, protocol nº 9639231121**. Results:** There was no significant difference when comparing the Control Group and the Social Isolated Group. ($p > 0.05$). **Conclusions:** The social isolation does not affect the total antioxidant activity in the jejunum of rats submitted to this type of stress. More studies are needed to evaluate the impact of this experimental model in the oxidative state of animals in the puberal age.

**Key words:** Enteric nervous system; Oxidative Stress; Social Isolation; Antioxidant; TRAP.


# NITRITE CONCENTRATION AND 3-NT EXPRESSION IN NITRERGIC NEURONS IN THE JEJUNUM OF RATS INDUCED TO COLORECTAL CARCINOGENESIS TREATED WITH MICROENCAPSULATED QUERCETIN AND PROBIOTIC

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**Introduction:** Colorectal cancer (CRC) is the third most common type of cancer. In this disease, we observe oxidative stress and dysbiosis, affecting the Enteric Nervous System (ENS), located on the gut wall. Quercetin is a flavonoid with antioxidant effect, what makes it a promising treatment for CRC. The pectin and casein microcapsules increase the quercetin bioavailability, for better use of the substance. *Bifidobacterium* strains have been studied in the treatment of CRC because of its functions such as the maintenance of the microbiota homeostasis. **Objective:** Analyze the amount and percentage of nNOS-IR (immunoreactive to nitric oxide synthase) and 3-NT-IR (immunoreactive to 3-nitrotyrosine) nitrergic neurons in the myenteric plexus, to understand how many nitrergic neurons were affected by 3-NT, and quantify total nitrite in the jejunum of rats induced to colorectal carcinogenesis and the treatment with microencapsulated quercetin and *Bifidobacterium animalis* subtype lactis probiotic and the association of both. **Methods:** All procedures were previously approved by the Animal Use Ethics Committee of the State University of Maringa under the number 1126010419. Male rats were divided in 5 groups (n=5): control (C), colorectal carcinogenesis (CR), colorectal carcinogenesis administrated with *Bifidobacterium animalis* probiotic $5x10^7$CFU (CP), colorectal carcinogenesis administrated with microencapsulated quercetin 10 mg/Kg (CQ) and colorectal carcinogenesis administrated with microencapsulated quercetin and *Bifidobacterium animalis* probiotic (CQP). CRC was induced by injections of 1,2-dimethylhydrazine (DMH). The treatment lasted 112 days. We collected the jejunum of the rats for for total nitrite quantification by Griess reagent test and for immunohistochemistry to visualize nNOS-IR and 3-NT-IR neurons, which were quantified. **Results:** We found no difference in the proportion of 3-NT-IR and nNOS-IR in CR (22.29%) compared to C (18.37%). In CQ (15.73%) there was a reduction in 3-NT expression in nNOS-IR neurons (vs CR; $p<0.05$). In the case of 3-

NT-IR neuron density, we found an increase of 37.14% in the CQP group (vs CR; $p<0.05$). There was no significant difference in the nitrite concentration analysis. **Conclusions:** CRC did not affect the 3-NT and nitrite expression in the jejunum. Quercetin and probiotic, on the other hand, showed some response. More studies are needed for better comprehension of the effects of carcinogenesis, quercetin and probiotic.

**Keywords:** Enteric nervous system; *Bifidobacterium animalis*; Oxidative stress; Immunohistochemistry.


# EFFECTS OF TRIBUTYLTIN TOXICITY ON THE INTESTINAL BARRIER OF THE DUODENUM-JEJUNUM ON WISTAR RATS

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**Introduction:** The gastrointestinal tract (GIT) has characteristics that allow it to be used as a biomarker of contamination since the food pathways route stands out as a potential route of entry for contaminants. In this way, the GIT acts as a highly selective barrier that blocks stress factors and allows water, nutrients, and electrolytes to be absorbed into the intestinal lumen, together with the components of the intestinal epithelium, such as enterocytes and goblet cells. Among environmental contaminants, Tributyltin (TBT) stands out for its high toxicity. TBT, an organotin compound and polymeric stabilizer, is widely used in industries such as chemicals and textiles as an antifouling agent and stands out for its highly toxic action as a biocide. This contaminant has a high bioaccumulative capacity, persisting in the trophic chain and interfering with the health of the different organisms present in it.

**Objective:** Investigate the effects on the intestinal barrier of the duodenum-jejunum after chronic exposure to tributyltin (TBT).

**Methods:** 36 male Wistar rats were separated into three experimental groups administered different concentrations of TBT by gavage, established according to environmental concentrations: control (CG), 20ng/g (GE20) and 600ng/g (GE600). After 30 days, the animals were anesthetized and euthanized, and their duodenumjejunum were collected. Subsequently, the tissues were prepared for

Hematoxylin and Eosin, Periodic Acid-Schiff, and Alcian Blue staining techniques to evaluate the intestinal layers and neutral and acid goblet cells. The results were statistically analyzed using One Way ANOVA.

**Results:** Exposure to TBT resulted in changes in the thickness of the intestinal barrier and the mucosecretory cells and IELs (Table 1). GE20 of duodenum resulted in a reduction in all the intestinal barrier, associated with limiting the contact surface with the contaminant in the intestinal lumen. Unlike, in the jejunum, there was only a significant reduction in the villi of GE20, in relation to the control and GE600, interfering in villus interaction, unbalancing the cell renewal process. Also, in the jejunum, there was an increase in the submucosa in the experimental groups, probably associated with the recruitment of immune system cells. Both segments simultaneously showed an increase in PAS+ goblet cells, which produce mucins with an immunological composition, providing the body with a greater defense against the stressor.

| | | | GROUPS | | |
|---|---|---|---|---|---|
| | | | **GC** | **GE20** | **GE600** |
| **Intestinal barrier (µm)** | **Villus** | **Duodenum** | 959.50±168.27$^{a}$ | 513.25±167.15$^{b}$ | 818.00±148.67$^{a}$ |
| | | **Jejunum** | 608.50±114.52$^{a}$ | 466.50±117.87$^{b}$ | 601.53±67.27$^{a}$ |
| | **Submucosa** | **Duodenum** | 421.50±127.32$^{a}$ | 277.00±91.86$^{b}$ | 426.00±73.34$^{a}$ |
| | | **Jejunum** | 201.75±12.94$^{a}$ | 228.50±60.70 $^{a}$ | 167.92±21.24$^{b}$ |
| **Globet cells and IELs/ enterocytes** | **PAS+** | **Duodenum** | 60.17± 1.6$^{a}$ | 33.37±8.01$^{a}$ | 13.77 ±4.77$^{a}$ |
| | | **Jejunum** | 16.65±0.79$^{a}$ | 24.26±1.85$^{b}$ | 15.91 ± 0.90$^{a}$ |
| | **AB+** | **Duodenum** | 86.80 ± 11.23$^{b}$ | 34.52± 5.73$^{a}$ | 13.07±3.46$^{a}$ |
| | | **Jejunum** | 9.59 ± 0.51$^{a}$ | 18.18 ± 0.34$^{b}$ | 17.0±0.43$^{ab}$ |
| | **IELs** | **Duodenum** | 65.25 ± 7.1$^{a}$ | 38.65 ± 2.51$^{a}$ | 27.87 ±2.58$^{b}$ |
| | | **Jejunum** | 3.33 ±0.45$^{a}$ | 7.47 ±0.77$^{b}$ | 8.29±0.,69$^{b}$ |

**Table 1**. The average thickness of intestinal barrier (µm): villus and submucosa, and the ratio of PAS+ goblet cells/enterocytes, AB+/enterocytes, and IELs/enterocytes of GC, GE20 and GE600. **Different letters indicate significant difference in the same line - One Way Anova p>0.05 and Holm-sidak post-hoc (p< 0,05).*

**Conclusions:** The results elucidate different mechanisms the intestinal barrier employs to maintain it is functions and ensure organism homeostasis. **Key words:** Neurogastroenterology; toxicology; Antifouling; Globet cells; Morphophysiology.


# ANALYSIS OF VARICOSITIES OF VIP-IMMUNOREACTIVE NEURONS IN THE COLON OF MICE INDUCED WITH C26 COLORECTAL CARCINOMA

ZAMBONINI[1], Matheus Ferreira SANCHES[1], Mariana Rodrigues; SILVA[1], Maysa Pacheco Alvarez; KULLER[1], João Victor; GODOY[1], Marcos Yudi Nagaoka; LEMOS[1], Giovana Emanuele Derio; SECORUN[1], Anna Lara; ZANONI[1] Jacqueline Nelisis; PERLES[1], Juliana Vanessa Colombo Martins

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**Introduction:** Colorectal cancer (CRC) is one of the cancers that most affect the world population and has a high mortality rate. As a result of this disease, cachexia syndrome is seen, observed in some cases of cancer, which differs from anorexia because it is a syndrome that results in a constant and involuntary loss of muscle, adipose and skeletal tissue. In experimental models, the C26 lineage, originating from colon cancer, was developed and is focused on the result of cachexia due to accelerated muscle atrophy and increased catabolism of proteins associated with hypoanabolism, resulting in a net reduction of total muscle mass. In the myenteric and submucosal plexuses, there are neurons that synthesize and release the active vasointestinal peptide (VIP), which acts in neuroprotection and has anti-inflammatory properties. **Objective:** To morphometrically evaluate VIP-immunoreactive varicosities in the myenteric plexus of mice induced with colorectal cancer (C26) comparing control groups and groups with different study periods. **Methods:** With prior approval from the Ethics Committee on the Use of Animals (CEUA) of UEL (nº 18592201887) the groups were randomly divided into 4 groups (N=5): Control 7 days (C7), control 14 days (C14), pre-cachectic 7 days (PCQ7) and cachectic 14 days (CQ14). Groups PCQ7 and CQ14 underwent subcutaneous inoculation in the dorsal region with 1x106 colon carcinoma cells (C26), and C7 and C14 were inoculated with a sterile saline solution in order to undergo the same stress protocol. After a period of 7 and 14 days, the animals were killed and, in sequence, colon samples were separated to undergo the immunohistochemistry process, to highlight the labeled neurons in the myenteric plexus that are immunoreactive to the VIP marker. We measured the size of 400 varicosities in each animal, followed by the possible block design and Fisher's post-test. **Results:** In the measured size of the C14 and CQ14 groups, there was no significant difference in their total area when comparing both groups. However, a significant increase was obtained comparing the C7 and CQ7 groups of approximately 3,40%. **Conclusions:** After analyzing the data found, the experimental model of the C26 cancer line showed significance only in a period of 7 days.

**Key words:** immunohistochemistry, cachexia, vasointestinal peptide (VIP)


# THE ROLE OF ACTIVE AND REACTIVE ENTERIC GLIAL CELLS ON THE COLORECTAL TUMOR

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**Introduction:** Under normal physiological conditions, enteric glial cells (EGC) are essential for numerous functions, such as maintaining the intestinal epithelial barrier (IEB). However, in pathological conditions, it becomes reactive and assumes a pro-inflammatory phenotype, overexpressing markers such as S100B and nitric oxide (NO). It has already been described that, in the context of colorectal cancer, EGC acts promoting the proliferation of tumor stem cells, stimulating tumorigenesis. **Objective:** Our work seeks to understand how EGC becomes reactive in the face of inflammatory and tumor situations, and how this reactive state affects colorectal cancer. **Methods:** We performed culture experiments on the HCT116 colorectal tumor line with or without conditioned medium from EGC cells challenged with LPS or previously challenged with HCT116's own conditioned medium. We performed a cell viability assay by MTT, and immunocytochemistry assay of tumor cells to evaluate the ZO-1 protein using Caco2 cells as control model. An ELISA assay was performed measuring the S100B released by EGC under a reactive state with LPS. We made immunocytochemistry assays using the markers S100 and GFAP evaluating their expression on control and reactive conditions of enteric glia during 24h. A nitric oxide (NO) assay was made with collected conditioned mediums of EGC with 5 and 10 µg/ml of LPS during 24h. **Results:** In the HCT116 24h MTT, there was a similar cell viability in all conditions (see Table 1). In the immunocytochemistry assay, HCT116 had a more widespread staining of ZO1 compared to Caco2. In the 48h MTT, the 20 µg/ml LPS treatment significantly reduced cell viability, indicating greater toxicity. The concentration of 2.5 µg/ml had cell viability in the range of 70%, suggesting use in future experiments. In the ELISA assay, EGC conditioned mediums with LPS secreted more S100B compared to the control. S100 increased as LPS levels were higher in 24h and 48h immunocytochemistry assays. GFAP had higher staining in 48h with higher LPS concentration. NO levels at 10 ug/ml in 24 hours were higher than the control; 5 ug/ml had a small increase, but was close to the control.
Table 1 - List of experimental conditions and markers for experiments using EGC and HCT116 cells.

| Conditioned mediums | 24h MTT | 48h MTT | ZO1 Staining | S100β (ELISA) | S100β (Immunocytochemistry) | 48h - GFAP | NO Dosage (24h) |
|---|---|---|---|---|---|---|---|
| EGC Control | Similar to 1µg/ml | Similar to 1µg/ml | NP | Similar to 1µg/ml | Similar to 1µg/ml | Similar to 1µg/ml | Similar to 1µg/ml |
| EGC + 1 µg/ml LPS | Similar to control | Similar to control | NP | Twice as much S100β was secreted compared to the control | Increase with LPS | NP | NP |
| EGC + 2.5 µg/ml LPS | Cell viability around 70% | Cell viability around 70% | NP | NP | Increase with LPS | NP | NP |
| EGC + 5 µg/ml LPS | NSRD | NP | NP | NP | Increase with LPS | Increase in LPS levels compared to control | Similar levels to control |
| EGC + 10 µg/ml LPS | NSRD | NP | NP | NP | Increase with LPS | Increase in NO levels compared to the control | Levels were higher than the control |
| EGC + 20 µg/ml LPS | Significant reduction in cell viability rate | NP | NP | NP | NP | NP | NP |
| Caco2 - Control | NSRD | NSRD | Well-defined staining | NP | NP | NP | NP |
| HCT116 + CM (EGC +HCT116) | NP | NP | Diffuse staining | NP | NP | NP | NP |
| HCT116 + MC (EGC +LPS -1µg/ml LPS) | NSRD | NSRD | Diffuse staining | NP | NP | NP | NP |

Legends:
NP: Non-participant
NSRD: No statistically relevant difference

**Conclusion:** Our preliminary results indicate that under conditions where EGC-secreted factors were present, there was similar cell proliferation compared to control and ZO1 staining is more diffuse on HCT116 compared to Caco2 cells. We also concluded that 20 µg/ml of LPS is a toxic concentration for the cells due to its death level. Furthermore, we conclude that there is greater release of S100B in enteric glia with LPS. GFAP and S100 increased in higher LPS levels on immunocytochemistry, corroborating with the previous data available in the literature. Also, NO release tends to be bigger in higher LPS concentrations. This work will allow us to understand the role of EGC in its normal or reactive state in the colorectal tumor context.

**Key words: Cancer, enteric nervous system, enteric glial cells activity.**


# EVALUATION OF THE JEJUNAL WALL OF AGING RATS SUPPLEMENTED WITH ASCORBIC ACID

LEMOS[1], Giovana Emanuele Derio; BELLETTINI[1], Lucas Sala; LIMA[1], Andressa Felipe; GODOY[1] , Marcos Yudi Nagaoka; SANCHES[1], Mariana Rodrigues; CICERO[1], Lídia Rodrigues; SILVA ALVAREZ[1], Maysa Pacheco; KULLER[1], João Victor; PERLES[1], Juliana Vanessa Colombo Martins; ZANONI[1], Jacqueline Nelisis.

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**Introduction:** Aging is a dynamic and progressive phenomenon. It is a process in which many physiological changes occur, such as those generated by free radicals which increase over time, promoting degenerative aging diseases and cell death. **Objective:** The aim of this study was to analyse the effect of ascorbic acid supplementation (1 g/L/day) on the jejunal mucosa of aging Wistar rats (*Rattus norvegicus*). **Methods:** 25 animals were divided into groups with 5 animals each: J90 (young rats 90-days-old), E345 (aged rats 345-days-old), E428 (aged rats 428-days-old), EA345 (aged rats treated with ascorbic acid from the 90th to the 345th day of age) and EA428 (aged rats treated with ascorbic acid from the 90th to the 428th day of age). Ascorbic acid was administered orally by drinking water. The jejunum was collected and submitted to histology routine to obtain 2 µm semi-serial sections which were then stained with haematoxylin and eosin (HE). The metaphase index and 30 measurements of total wall height, mucosal height, villi height and crypt depth and tunica muscularis height were analysed. The analyses were subjected to statistical treatment with a significance level of 5%. **Results:** The metaphase index was similar between all groups. The crypts of animals in the E345 and E428 groups were larger than those in the J90 group. The mucosal height of the E345 group was 11.5% higher ($p < 0.05$) than that of the J90 group. The E428 group had a 13.10% ($p < 0.05$) reduction in mucosal height compared to the E345 group. Animals in the EA428 group showed a 20.9% reduction in mucosal height (vs. E428; $p < 0.05$). The height of the villi in the young aging group (J90) was lower (16% and 4.58% $p < 0.05$) than in the E345 and E428 groups. Animals in the EA345 and EA428 groups had lower villus heights (6.92% and 13.06% $p < 0.05$) than the E345 and E428 groups, respectively. The crypts of the animals in the E345 and E428 groups were deeper than those in the J90. Animals in the EA345 and EA428 groups had a 10.44% and 9.53% ($p < 0.05$) reduction in crypt depth when compared to the E345 and E428 groups. The crypt depths of the E90 and EA345 groups were similar. The total wall and tunica muscularis of the animals in the E345 and E428 groups increased compared to the young animals; the

aging condition itself promoted a reduction in the intestinal wall and tunica muscularis. Ascorbic acid supplementation reduced the height of the tunica muscularis and total wall in the EA345 and EA428 groups. **Conclusions:** In conclusion, the aging process promoted an increase in the total wall, tunica mucosa, and muscle, and supplementation with ascorbic acid reverted these parameters to values close to that of a young animal suggesting a protective role of the compound, in addition, the metaphase index was similar between all groups.

**Key words:** senescence, intestinal mucosa, antioxidant.


# STUDY OF THE SHORT-CHAIN FATTY ACID RECEPTOR GPR41 IN THE ENTERIC NEURONS OF MICE SUBMITTED TO EXPERIMENTAL ULCERATIVE COLITIS

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**Introduction:** Inflammatory bowel diseases (IBD) are characterized by inflammation in the gastrointestinal tract. Ulcerative colitis is a serious health problem that can lead to death. Butyrate, a short-chain fatty acid (SCFA), produced by the intestinal microbiota through the fermentation of dietary fiber, SCFA can bind to G-coupled protein receptor, such as GPR41, and trigger intracellular pathways that leads to improvement of the intestinal barrier and intestinal health functions. **Objective:** The aim of this study was to analyze the GPR41 receptor in neurons immunoreactive (-ir) to PGP9.5 (pan-neuronal marker) and analyze the effect of sodium butyrate on neurons of the myenteric plexus of mice submitted to experimental ulcerative colitis. **Methods:** 100µL of 2,4,6-Trinitrobenzenesulfonic acid was injected intrarectally into 8 weeks-old male C57BL/6 mice (TNBS group). The Sham group received ethanol (vehicle). The BUT group was treated with Sodium Butyrate via gavage after injection with TNBS. The animals were euthanized after 7 days. The disease activity index (DAI) was analyzed. A double labeling reaction of the GPR41 and PGP9.5 receptor was performed with analysis of colocalization, number of neurons/ganglion and area of neurons. (Approved by CEUA-ICB/USP nº 6507140420). **Results:** The DAI (mice weight, stool consistency and fecal bleeding) demonstrated a decrease in weight and increase in fecal bleeding and soft stools in the TNBS group, with these data recovering in the BUT group. The Sham group showed no changes. There was the presence of the GPR41-ir receptor in PGP9.5-ir enteric neurons. The count of PGP9.5-ir neurons revealed a 9% reduction in the TNBS group (13±0.2) compared to the Sham group (14±0.2; $p<0.001$). The BUT group (15±0.2; $p<0.001$) showed a recovery of 14% compared to the TNBS group. The areas of cell body neurons (235±4 $\mu m^2$) and the cytoplasmic area (127±3 $\mu m^2$) of PGP9.5-ir neurons were reduced by 13% in the TNBS group compared to the Sham group but did not differ from the BUT group. **Conclusions:** The experimental ulcerative colitis induction protocol using TNBS was successful with characteristic clinical, macroscopic and microscopic manifestations of the disease. A reduction in the number of PGP9.5-ir neurons affected by experimental ulcerative colitis was observed. Treatment with sodium butyrate

attenuated the clinical effects and the reduction of PGP9.5-ir neurons in animals in the BUT group.

**Key words:** Inflammatory Bowel Disease; Enteric Nervous System; Sodium Butyrate: Short-chain fatty acids; GPR41.


# ANALYSIS OF COLON AND REACTIVITY ENTERIC GLIA IN ANIMAL MODELS OF ANXIETY DISORDERS

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**Introduction**: Anxiety is among the most common psychiatric disorders. Studies indicate a relationship between mood disorders, such as anxiety, and changes in gut-brain communication. The gut-brain axis can be described as a bidirectional communication network that links the gastrointestinal tract (GIT) and central nervous systems. The GIT functions are controlled by the enteric nervous system (ENS), also called the second brain, given its importance in intestinal and general health maintenance. The ENS is composed of enteric neurons and glial cells, and the enteric glia is a key element that modulates the intestinal epithelial barrier, participates the neurotransmission and motility, talks actively with immune cells, and other roles. A recent study highlighted that a high-fat diet alters glial expression along the gut-brain axis, evoking anxiogenic behaviors. Thus, there is an interest in the literature to understand the role of enteric glia in the context of mood disorders.

**Objective:** This study aims to evaluate possible alterations in the gastrointestinal tract and the enteric glia found in the large intestine of animal models of anxiety disorder.

**Methods:** Male Wistar rats previously selected for high (CHF - Carioca High-conditioned Freezing) (n=6) and low (CLF - Carioca Low-conditioned Freezing) (n=7) levels of freezing in response to contextual cues were used in this study and compared with control animals (n=5). The present study was approved by the CEUA-UFF under protocol number 1707150622. Possible alterations in the large intestine and in the gastrointestinal transit were observed by measuring colon length and counting the number of pellets. Western blot was performed to determine the protein content of glial fibrillary acidic protein (GFAP) in the neuromuscular layer since GFAP is a glial protein commonly used to evaluate the response of the glial network.

**Results:** We found a decrease in gastrointestinal motility in CHF animals ($P=0.0794$) and a longer colon in CHF animals compared to CLF animals ($P<0,05$). Further, we found an increase in GFAP in CHF animals compared to the CLF animals ($P<0,05$), suggesting a major reactivity enteric glia in the colon of Carioca High-conditioned Freezing.

**Conclusions:** The animals with exacerbated anxiety-like behavior demonstrated a decrease in gastrointestinal transit and an enhancement in the reactivity of enteric glial cells in the neuromuscular layer. These findings point to glia as a mediating agent of the modulation induced by anxiety-like behavior in the gastrointestinal tract function, opening perspectives for future therapeutic interventions.

**Key words:** anxiety, enteric glia, enteric nervous system, gut-brain axis.


# EXCESS PROTEIN INTAKE INDUCES COLONIC NEURONAL ALTERATIONS IN A DEXTRAN SULFATE SODIUM-INDUCED ULCERATIVE COLITIS MODEL AND AGGRAVATES THE CLINICAL SIGNS OF DISEASE

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**Introduction:** Today, there is a greater understanding about the role of the Enteric Nervous System (ENS) in generating the main signs and symptoms of Ulcerative colitis (UC). However, little is known about how the interaction of the ENS with dietary nutrients can modulate the clinical course of UC. **Objective:** To evaluate the effects on the colon of C57BL/6 mice of consuming a high-protein diet during the active phase of UC. **Methods:** Male mice were distributed into groups: SD (fed standard diet); HP (fed high-protein diet); SD/DSS (fed standard diet and induced to UC); and HP/DSS (fed high-protein diet and induced UC). The standard diet contained 22% protein while high-protein diet 44%. Mice had free access autoclaved water containing 3% DSS for seven days to induced UC. Gastrointestinal transit and disease activity index (DAI) were assessed. Proximal and distal colon was assayed by immunofluorescence to evaluate the general (PGP9.5$^+$), nitrergic (nNOS$^+$) and estimated cholinergic neurons (PGP9.5$^+$/nNOS$^-$) in the myenteric plexus (MP), as well as the general (PGP9.5$^+$) and calretinin (CALR$^+$) neurons in the submucosal plexus (SMP). Strong and weak CALR fluorescence were distinguished for identification of cholinergic and VIPergic neurons, respectively. **Results:** The DAI score of HP/DSS group was significantly higher during the acute phase of UC ($p<0.01$). The number of faecal pellets expelled per daily significantly reduced in SD/DSS and HP/DSS ($p<0.05$), and in seventh day the decrease in the HP/DSS was approximately 82%, 72% and 52% compared respectively with HP, SD and SD/DSS. A reduction of around 38% in the distal colon area was observed in the SD/DSS and HP/DSS when compared to the control groups ($p<0.01$). Shortening of the proximal colon occurred only in SD/DSS ($p=0.02$; ≈29%). The colonic weight and gastrointestinal transit were not altered ($p>0.05$). It was observed in MP of distal colon an increase in number of general and estimated cholinergic neurons in the HP/DSS ($p=0.03$; ≈117%) and SD/DSS ($p=0.02$; ≈154%), respectively. In SMP, we found in DSS groups a reduction in number of VIPergic neurons of proximal colon of DSS groups ($p<0.05$; ≈57%), and an increase of cholinergic neurons of HP group in the distal colon ($p=0.01$;

≈82%). In the proximal colon, general myenteric neurons from the HP/DSS group had a smaller cell body when compared to SD/DSS ($p<0.01$), while nitrergic myenteric neurons were significantly larger in of all experimental groups when compared to SD ($p<0.01$). Besides, all submucosal neurons presented atrophied when compared to SD ($p<0.01$). In the distal segment, specifically in MP, the cell body area of general neurons was larger in the SD/DSS and HP/DSS when compared to SD ($p<0.01$), and same occurs with the estimated cholinergic neurons of SD/DSS in relation to SD ($p=0.04$) and HP/DSS ($p<0.01$). Atrophy of nitrergic neurons of SD/DSS and HP/DSS was also observed ($p<0.01$). In the SMP, general, cholinergic and VIPergic neurons presented a reduction in cell body in SD/DSS ($p<0.01$). **Conclusions:** The results found highlight that nutrients present in the intestinal lumen can modulate the activity of enteric neurons, especially during the acute UC.

**Key words:** Inflammatory bowel disease. Enteric Nervous System. Diet. Protein.

# AWARDS

# Postgraduate students with abstracts selected for oral communication

## Honorable mention to:

- **Gabriela Pustiglione Marinsek**
  ENVIRONMENTAL ISSUES: AZITHROMYCIN AFFECTS CROSSTALK BETWEEN MICROBIOTA, INTESTINAL BARRIER, AND ENTERIC NERVOUS SYSTEM IN FISH. Gabriela Pustiglione Marinsek, Isabelly Cristina Correia dos Santos de Oliveira, Marcos Antônio de Oliveira, Renata de Britto Mari

- **João Victor Kuller**
  PROJECT "SECOND BRAIN SPACE: FROM PRODUCTION TO THE POPULARIZATION OF KNOWLEDGE OF THE ENTERIC NERVOUS SYSTEM" - A GENERAL OVERVIEW OF THE PUBLIC SERVED FROM MAY 2022 TO SEPTEMBER 2023. João Victor Kuller, Marcos Yudi Nagaoka Godoy, Maysa Pacheco Alvarez da Silva, Andressa Felipe Lima, Lucas Sala Bellettini, Matheus Ferreira Zambonini, Lídia Rodrigues Cicero, Mariana Rodrigues Sanches, Giovana Emanuele Derio De Lemos, Sabrina Silva Sestak, Jacqueline Nelisis Zanoni, Juliana Vanessa Colombo Martins Perles

- **Marcos Antônio Ferreira Caetano**
  GUT MICROBIOTA DERIVED BUTYRATE PROTECT EXCITATORY AND INHIBITORY MYENTERIC NEURONS LOSS AFTER EXPERIMENTAL ULCERATIVE COLITIS. Marcos Antônio Ferreira Caetano, Roberta Figueiroa de Souza, Jheniffer Rayane de Lima Duarte, Victor dos Santos Silva, Patricia Castelucci

## Honorable mention to:

- **Maria Carolina Garcia Ricciardi**
  EFFECT OF SODIUM BUTYRATE TREATMENT ON GUT-BRAIN AXIS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE. Maria Carolina Garcia Ricciardi, Marianna Gonçalves de Carvalho, Isabela Nobrega Fialho Tavares, Profa. Ana Lucia Tavares Gomes

- **Mayara Melo**
  STUDY OF THE PRO-INFLAMMATORY ACTIVITY OF ENTERIC GLIA ON INTESTINAL EPITHELIAL CELLS. Mayara Melo, Liliane da Silva Ribeiro, Vivaldo Moura Neto, Juliana de Mattos Coelho Aguiar

- **Maysa Pacheco Alvarez da Silva**
  NITRERGIC NEURONS AND VIPERGIC VARICOSITY ANALYSIS IN THE JEJUNUM OF RATS INDUCED TO COLORECTAL CARCINOGENESIS TREATED WITH MICROENCAPSULATED QUERCETIN AND PROBIOTIC. Maysa Pacheco Alvarez da Silva, Lucas Casagrande, Sabrina Silva Sestak, Carla Cristina de Oliveira Bernardo, Matheus Ferreira Zambonini, Marcos Yudi Nagaoka Godoy, João Victor Kuller, Lucas Sala Bellettini, Giovana Emanuele Derio De Lemos, Andressa Felipe Lima, Abygail Karlla Donadelli Damico, Lídia Rodrigues Cicero, Juliana Vanessa Colombo Martins Perles, Jacqueline Nelisis Zanoni

- **Vinicius Balan Ramos Coronado**
  EFFECTS OF HIGH PROTEIN DIET AND INDUCED-DSS ULCERATIVE COLITIS ON THE ILEUM OF MICE. Vinicius Balan Ramos Coronado, Camila Cristina Alves Machado, Giovanni Bruno Clivati Sodré, Alexandre Oba, Flávia Alessandra Guarnier, Eduardo José de Almeida Araújo

# Abstracts Presented as Posters

## Undergraduate students' category

### Honorable mention to:

- **Felipe Teixeira Santana**
  EVALUATION OF INTESTINAL HISTOPATHOLOGY IN LAMBARI Astyanax lacustris EXPOSED TO ENVIRONMENTAL CONCENTRATIONS OF FLUOXETINE. Felipe Teixeira Santana, Kainã Rocha Cabrera Fagundes, Renata de Britto Mari

- **Gabriele Domingos Jardim**
  THE ROLE OF ACTIVE AND REACTIVE ENTERIC GLIAL CELLS ON THE COLORECTAL TUMOR. Gabriele Domingos Jardim, Gabrielle Sobrinho de Souza, Juliana de Mattos Coelho Aguiar

- **Isabela Nobrega Fialho**
  ANALYSIS OF COLON AND REACTIVITY ENTERIC GLIA IN ANIMAL MODELS OF ANXIETY DISORDERS. Isabela Nobrega Fialho Tavares, Yasmin Nazareth, Maria Carolina Garcia Ricciardi, Marianna Gonçalves de Carvalho, Silvia Soares Maisonnette, Jesus Landeira Fernandez, Paula Campello Costa Lopes, Ana Lucia Tavares Gomes

- **Isabelly Cristina Correia dos Santos de Oliveira**
  MYENTERIC PLEXUS RESPONSES TO TRIBUTYLTIN EXPOSURE IN DUODENUM OF WISTAR RATS. Isabelly Cristina Correia dos Santos de Oliveira, Gabriela Pustiglione Marinsek, Lucas Correia,Regina Barbosa, Renata de Britto Mari

- **Jheniffer Rayane de Lima Duarte**
  STUDY OF THE SHORT-CHAIN FATTY ACID RECEPTOR GPR41 IN THE ENTERIC NEURONS OF MICE SUBMITTED TO EXPERIMENTAL ULCERATIVE COLITIS. Jheniffer Rayane de Lima Duarte, Laura Barbosa da Conceicao, Marcos Antônio Ferreira Caetano, Patricia Castelucci

# Abstracts Presented as Posters

## Master students' category

### 1st place, in master students category

**Maysa Pacheco Alvarez da Silva**, for the work entitled NITRITE CONCENTRATION AND 3-NT EXPRESSION IN NITRERGIC NEURONS IN THE JEJUNUM OF RATS INDUCED TO COLORECTAL CARCINOGENESIS TREATED WITH MICROENCAPSULATED QUERCETIN AND PROBIOTIC - Maysa Pacheco Alvarez da Silva, Lucas Casagrande, Sabrina Silva Sestak, Carla Cristina de Oliveira Bernardo, Matheus Ferreira Zambonini, Marcos Yudi Nagaoka Godoy, João Victor Kuller, Lucas Sala Bellettini, Giovana Emanuele Derio De Lemos, Andressa Felipe Lima, Larissa Celine Violin, Mariana Sanches, Juliana Vanessa Colombo Martins Perles, Jacqueline Nelisis Zanoni.

### 2nd place, in master students category

**Sabrina Silva Sestak,** for the work entitled EFFECTS OF MICROENCAPSULATED QUERCETIN AND Bifidobacterium animalis ON ENTERIC GLIAL CELLS IN THE ILEUM MUCOSA OF RATS WITH CHEMICALLY INDUCED COLORECTAL CARCINOGENESIS - Sabrina Silva Sestak, Lucas Casagrande, Carla Cristina de Oliveira Bernardo, Mariana Sanches, Giovana Emanuele Derio de Lemos, Andressa Felipe Lima, Marcos Yudi Nagaoka Godoy, Lucas Sala Bellettini, Juliana Vanessa Colombo Martins Perles, Jacqueline Nelisis Zanoni.

### 3rd place, in master students category

**João Victor Kuller**, for the work entitled EFFECTS OF QUERCETIN-LOADED MICROCAPSULES AND Bifidobacterium animalis IN THE VIP IMUNOREACTIVES VARICOSITIES OF JEJUNUM MUCOSA IN EXPERIMENTAL MODEL OF COLERECTAL CARCINOGENESIS - Sabrina Silva Sestak, João Victor Kuller, Isadora Goulart Garcia, Lucas Casagrande, Carla Cristina de Oliveira Bernardo, Abygail Karlla Donadelli Damico, Larissa Celine Violin, Julia Miwa Komagome, Profa. Juliana Vanessa Colombo Martins Perles, Jacqueline Nelisis Zanoni

# Abstracts Presented as Posters

## PhD students' category

### 1st place, in PhD students category

**Roberta Figueiroa de Souza,** for the work entitled ANTI-TNF ALPHA TREATMENT IS EFFICIENT IN THE RECOVERY OF MYENTERIC NEURONS EXPRESSING TUMOR NECROSIS FACTOR-ALPHA 2 RECEPTOR (TNFR2) FOLLOWING EXPERIMENTAL ULCERATIVE COLITIS – Roberta Figueiroa de Souza, Felipe Alexandre Machado, Patricia Castelucci.

### 2nd place, in PhD students category

**Roberta Figueiroa de Souza**, for the work entitled STUDY OF ENTERIC NEURONS AND ENTERIC GLIAL CELLS IN CHRONIC EXPERIMENTAL ULCERATIVE COLITIS IN MICE DEFICIENT FOR THE P2X7 RECEPTOR (P2X7R -/-) – Roberta Figueiroa de Souza, Marcos Antônio Ferreira Caetano, Patricia Castelucci.

### 3rd place, in PhD students category

**Camila Cristina Alves Machado**, for the work entitled EXCESS PROTEIN INTAKE INDUCES COLONIC NEURONAL ALTERATIONS IN A DEXTRAN SULFATE SODIUM-INDUCED ULCERATIVE COLITIS MODEL AND AGGRAVATES THE CLINICAL SIGNS OF DISEASE – Camila Cristina Alves Machado, Vinicius Balan Ramos Coronado, Larissa Da Silva Bonassa, Yasmin Gonçalves de Oliveira Santos, Giovanni Bruno Clivati Sodré, Stephanie Salgado, Alexandre Oba, Eduardo José de Almeida Araújo.

# ORGANIZING AND SCIENTIFIC COMMITTEES

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