PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

SUPLEMENTAÇÃO COM L-ARGININA AMENIZA ESCORE DE LESÕES NO INTESTINO DELGADO DE RATOS SUBMETIDOS À QUIMIOTERAPIA COM 5-FLUOROURACIL

CAROLINI ROSSETTI CERVINI

Presidente Prudente - SP
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Dissertação apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de mestre em Ciência animal.

Orientador: Prof. Dr. Luis Souza Lima de Souza Reis

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Dissertação apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal - Área de Concentração: Fisiopatologia Animal

Presidente Prudente, 27 de Setembro de 2017

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DEDICATÓRIA

Dedico primeiramente à minha família que sempre me incentivou, diante de todas as dificuldades, nunca desistiram do meu futuro.

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“Embora ninguém possa voltar atrás para fazer um novo começo, qualquer um pode começar agora a fazer um novo fim”.

(Chico Xavier)

RESUMO

Suplementação com L-arginina ameniza escore de lesões no intestino delgado de ratos submetidos à quimioterapia com 5-Fluorouracil

O 5-Fluorouracil (5-FU) é um quimioterápico utilizado frequentemente no tratamento de tumores, mas pode causar efeitos colaterais, como a mucosite. A L-arginina é um aminoácido que tem utilizado para amenizar os efeitos colaterais da mucosite. No entanto, mas não está totalmente esclarecido uma dose ideal de suplementação. O objetivo desse estudo foi de avaliar o efeito da suplementação com L-arginina no escore de lesão no jejuno (porção média do jejuno) em ratos submetidos a quimioterapia com 5-FU. Utilizou-se 32 ratos Wistar, divididos aleatoriamente em 4 tratamentos (8 ratos/grupo) que foram alimentados com ração (Supralab®, Alisul, Brazil) e água filtrada *ad libitum*. Os grupos receberam: grupo controle (G_C): ração e água; grupo arginina (G_{Arg}): receberam ração e 458 mg de L-arginina adicionada na água, grupo 5-FU ($G_{5\text{-}FU}$) ração, água e aplicou-se uma dose do 5-FU e o grupo Arg+5-FU ($G_{Arg+5\text{-}FU}$) receberam ração, 458 mg de L-arginina e uma dose de 5-FU. A 5-FU (200 mg de 5-FU/kg de peso corporal) foi aplicado para induzir a mucosite nos ratos. 4 dias após a administração do 5-FU, sacrificou-se os ratos para colheita das amostras do jejuno e examinou-se uma área de 2.000-2.500 mm^2 identificando as lesões (dilatação dos vasos linfáticos, enterócitos cúbicos, achatamento dos vilos, fusão dos vilos, edema intersticial e necrose apical dos vilos). As extensões e severidade das lesões foram avaliadas aplicando escore lesional máximo de 39. Os dados foram analisados por meio da ANOVA *one-way* e teste Tukey com significância de 5%. Os ratos do grupo $G_{5\text{-}FU}$ apresentaram maior escore lesional no jejuno (24,2 ± 4,9). O maior escore lesional no jejuno foi observado nos ratos do G5-FU (24,2 ± 4,9; $P < 0,05$). Nos ratos suplementados com L-arginina ($G_{Arg+5\text{-}FU}$) observou-se redução de 42,2% no escore lesional no jejuno (10,2 ± 4,8; $P < 0,05$) e semelhante ao escore lesional do G_c (6,4 ± 2,8; $P > 0,05$). Conclui-se que a suplementação com 458 mg de L-arginina amenizou as lesões no jejuno dos ratos causadas pela quimioterapia com o 5-FU.

Palavras chave: aminoácido, câncer, mucosite, neoplasia, quimioterapia.

ABSTRACT

Supplementation with L-arginine mitigates lesion scores in the small intestine of rats under chemotherapy with 5-Fluorouracil

5-Fluorouracil (5-FU), a chemotherapy drug used in tumors treatment, but it can cause side effects like mucositis. L-arginine is an amino acid that has been tested to prevent this chemotherapy side effect. However, an optimal dose of supplementation is not fully understood. Objective to evaluate the effects of L-arginine supplementation on the lesion scores in the jejunum (middle portion of the small intestine) of rats under chemotherapy with 5-Fluorouracil. 32 Wistar rats were divided into 4 treatments (8 rats/treatment), all the groups were fed commercial balanced feed (Supralab®, Alisul, Brazil) and *ad libitum* filtered water. The groups received: Control group (C_G): feed and water; Arginine group (G_{Arg}): feed and 458 mg L-arginine added to the water; Group 5-FU ($G_{5\text{-}FU}$): feed, water and one 5-FU dose; and Arg+5-FU group ($G_{Arg+5\text{-}FU}$): feed, 458 mg L-arginine added to the water and one 5-FU dose. 5-FU (200 mg kg/body weight) was used to induce mucositis in the rats. Four days after 5-FU administration, jejunum samples were collected from the rats and a 2,000-2,500 mm^2 area examined for lesions (lymph vessel dilatation, cuboidal enterocytes, inflammatory infiltrate, flattening and fusion of villus, interstitial edema and necrosis of apical portion of villi). The extensions and severity of the lesions was assessed by applying a maximum score of 39. Data were analyzed by one-way ANOVA followed by the Tukey test, with significance level set at 0.05. The highest jejunum lesion score was identified $G_{5\text{-}FU}$ (24.2 ± 4.9; $P < 0.05$). It was 42.2% lower (10.2 ± 4.8; $P < 0.05$) in $G_{ARG+5\text{-}FU}$, which was similar to the control group (6.4±2.8; $P > 0.05$). In Wistar rats, daily supplementation with 458 mg of L-arginine mitigates jejunum lesions caused by chemotherapy with 5-FU.

Keywords: amino acid, cancer, mucositis, neoplasia, chemotherapy.

LISTA DE SIGLAS

OMS - Organização Mundial de Saúde

INCA- Instituto Nacional de Câncer “José Alencar Gomes da Silva”

G_{C-} Grupo Controle

$G_{5\text{-}FU}$ – Grupo 5-Fluorouracil

G_{Arg} - Grupo L-arginina

$G_{Arg+5\text{-}FU}$ - Grupo L-arginina + 5-FU

SUMÁRIO

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Supplementation with L-arginine mitigates lesion scores in the small intestine of rats under chemotherapy with 5-Fluorouracil

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Abstract

5-Fluorouracil (5-FU), a chemotherapy drug used in neoplasm treatment, can cause mucositis. L-arginine is an amino acid that has been tested to prevent this chemotherapy side effect. Objective to evaluate the effects of L-arginine supplementation on the lesion scores in the jejunum (middle portion of the small intestine) of rats under chemotherapy with 5-Fluorouracil. 32 Wistar rats were divided into 4 treatments (N = 8 per treatment), all the groups were fed commercial balanced feed (Supralab®, Alisul, Brazil) and *ad libitum* filtered water. The groups received: Control group (C_G): feed and water; Arginine group (G_{Arg}): feed and 458 mg L-arginine added to the water; Group 5-FU ($G_{5\text{-}FU}$): feed, water and one 5-FU dose; and Arg+5-FU group ($G_{Arg+5\text{-}FU}$): feed, 458 mg L-arginine added to the water and one 5-FU dose. 5-FU (200 mg kg/body weight) was used to induce mucositis in the rats. Four days after 5-FU administration, jejunum samples were collected from the rats and a 2,000-2,500 mm^2 area examined for lesions (lymph vessel dilatation, cuboidal enterocytes, inflammatory infiltrate, flattening and fusion of villus, interstitial edema and necrosis of apical portion of villi). The extensions and severity of the lesions was assessed by applying a maximum score of 39. Data were analyzed by ANOVA followed by the Tukey test, with significance level set at 0.05. The highest jejunum lesion score was identified $G_{5\text{-}FU}$ (24.2 ± 4.9; $P < 0.05$). It was 42.2% lower (10.2 ± 4.8; $P < 0.05$) in $G_{ARG+5\text{-}FU}$, which was similar to the control group (6.4±2.8; $P > 0.05$). In Wistar rats, daily supplementation with 458 mg of L-arginine mitigates jejunum lesions caused by chemotherapy with 5-FU.

Keywords: amino acid, chemotherapy, mucositis, cancer, neoplasia.

Introduction

The chemotherapy drug 5-fluorouracil (5-FU) has been used for more the 40 years in the treatment against cancer. However, because of its low specificity for tumor cells, 5-FU also attacks healthy body cells [1], producing side effects in 80 percent of the cases [2].

5-FU acts by inhibiting thymidylate synthesis which is an essential precursor for the formation of thymidine triphosphate which is a deoxyribonucleotide required for the synthesis and repair of DNA and also incorporates fluorouridine triphosphate in RNA that compromises RNA functions, thereby interfering with DNA replication, repair and transcription [3] may induce apoptosis in tumor cells [2] and also as healthy intestinal cells, resulting in mucositis.

Mucositis is one of the main adverse effects 5-FU treatment, affecting almost all the patients subjected to high chemotherapy levels [4]. The clinical symptoms of mucositis are gastrointestinal disorders such as esophagitis, nausea, vomiting, edema, abdominal pain, constipation and diarrhea [5,6]. Mucositis compromises the health of the patients [1] to the point of leading to chemotherapy interruption because of patient weakness and vulnerability to infections [2].

Studies on nutrition have gained a great interest in the search for treatments that minimize chemotherapy effects. Some nutrient in fact can ameliorate undesirable side-effects of the treatment and improve the health and quality of life of cancer patients [7]. L-arginine, for instance, is an amino acid that can reduce apoptosis [8,9,10], benefit the immune response [11] and anti-tumor defense [12,13], protect the intestinal mucosa, promote the recovery of the intestinal barrier [12], prevent the increase in intestinal permeability and bacterial translocation [14] resulting from 5-FU-caused lesions the intestinal mucosa, thus favoring wound healing [15].

Despite the evidences of L-arginine efficiency, the optimal dose and administration time to benefit cancer patients is yet to be determined, as is the limitations of this supplement during chemotherapy. In this context, the study tested the hypothesis that supplementation with 458 mg of L-arginine attenuates the lesions caused by 5-FU in small intestine mucosa of Wistar rats.

Materials and Methods

According to the National Council for Control of Animal Experimentation – CONCEA [16], the experiment was approved by the Committee on Animal Use Ethics of UNOESTE (protocol 2755). The experimental procedures followed the Guide for the Care and Use of Laboratory Animals [19].

Delineamento Experimental

Thirty two male Wistar rats, *Rattus novergicus*, 60 days old, with 161.5 ± 9.0 g mean body were used. The rats were randomly assigned to 4 experimental groups (8 rats/group) held under the same environmental conditions, with 12/12 h light/dark cycle, nearly 23°C room temperature [19]. All the groups were fed commercial balanced feed (Supralab®, Alisul, Brazil) and *ad libitum* filtered water. The groups received: Control group (C_G): feed and water; Arginine group (G_{Arg}): feed and 458 mg L-arginine added to the water; Group 5-FU ($G_{5\text{-}FU}$): feed, water and one 5-FU dose; and Arg+5-FU group ($G_{Arg+5\text{-}FU}$): feed, 458 mg L-arginine added to the water and one 5-FU dose.

In the first 7 days of the experiment the rats were allowed to adapt to the experimental conditions and supplementing L-arginine. The arginine dose provide was based on the daily recommendation of 10 g arginine for athletes [17] and

extrapolated allometrically for Wistar rats [18]. L-arginine aspartate, supplied as effervescent tablet without vitamin C (Targifor®, Sanofi, Brazil), was diluted in the rat drinking water.

After 7-day adaptation, the experiment onset (Day 0) was determined by the 5-FU application. A single 5-FU dose of 200 mg kg/body weight was administered intraperitoneally, as recommended by Leocádio et al. [9] for mucositis induction in rats.

The consumption of L-arginine was determined daily throughout the experiment by measuring water intake. To that end, water volume was before and after 24-h being placed in the cage. Mean daily water consumption was determined for each experimental group and then divided by the number of rats in each group to determine individual water intake.

Similarly, daily feed intake was determined by weight difference between provided and rejected food after it remained for 24 h in the feeder. Mean daily food consumption was determined for each experimental group and then divided by the number of rats in each group to determine individual water intake.

The commercial diet provided contained (guaranteed levels per feed kilogram): 22.0% crude protein; 2.5% ether extract; 6.0% fiber; 10% mineral matter; 1.2% calcium; 0.7% phosphorus; 3,000 mg methionine; 7,000 IU vitamin A; 50 mg vitamin C; 2,000 IU vitamin D_3; 15 IU vitamin E; 1.0 mg vitamin K_3; 2.0 mg vitamin B_1; 6.0 mg vitamin B_2; 3.0 mg vitamin B_6; 9.0 mg vitamin B_{12}; 1.0 mg folic acid; 12.0 mg pantothenic acid; 0.5 mg biotin; 500.0 mg choline; 20.0 mg niacin; 9.0 mg copper; 40.0 mg iron; 0.7 mg iodine; 90.0 mg manganese; 0.4 mg selenium; 50.0 mg zinc.

Small intestine sampling and istological assay

Four days after 5-FU application, the rats were killed with sodium thiopental (Thiopentax®, Cristália, Brazil) at a dose of 100 mg kg^{-1} body weight [19] to collect small intestine samples, in the middle jejunum.

The intestine samples were fixed in 10% buffered formaldehyde, dehydrated by immersion in a graded series of alcohol and embedded in paraffin wax. Using a precision microtome, the paraffin blocks were cut into 3-µm thick sections, placed on glass slides and stained with hematoxylin-eosin. An area of 2,000-2,500 mm2 was observed in each sections under optical microscope (Nikon), at 400x magnification, for lesion identification. The score of each lesion was obtained by multiplying the severity factor by lesion extension, and the score per organ provided by the sum of individual lesions in a section. The lesions considered were lymph vessel dilatation, cuboidal enterocytes, flattening and fusion of villus, interstitial edema and necrosis of apical portion of villi). The severity factor (or degree of severity) was considered 1 for mild lesions, 2 for moderate lesions and 3 for severe lesions. The extent of each lesion (intensity or frequency observed) was assessed and scored as 0 = no lesion, 1 = low measure, 2 = intermediate extent, 3 = large extent. Maximum lesion score was set at 39, as recommended by Gerez et al.[20].

Statistical analysis

After confirming normality of data distribution (Shapiro Wilk test), body weight of the rats, feed and water intake and intestine lesion results were analyzed by one-way ANOVA followed by the Tuckey test to contrast the means. The tests were performed by the ERE software. Significance level was set at 0.05.

Results

On experiment onset, the test and control groups showed similar weight. At the end of the experiment, however, rats from $G_{5\text{-}FU}$ and $G_{Arg+5\text{-}FU}$ were lighter than those from G_{Arg} ($P < 0.05$).

Feed intake was also similar among the groups on experiment onset, but after chemotherapy it decreased in $G_{Arg+5\text{-}FU}$ ($P < 0.05$). With respect to water intake, it was higher in G_{Arg} and $G_{Arg+5\text{-}FU}$ before and after chemotherapy ($P < 0.05$).

$G_{5\text{-}FU}$ rats, which were subjected to 5-FU chemotherapy without L-arginine, showed the highest lesion score in middle jejunum ($P < 0.05$) (Figure 1). In contrast, $G_{Arg+5\text{-}FU}$ lesions were 42.2% lower than in $G_{5\text{-}FU}$ ($P < 0.05$) and similar to the control group ($P > 0.05$). Lesion score in G_{Arg} was also similar to the control group ($P > 0.05$).

Discussion

Figure 2 illustrates jejunum lesions in Wistar rats from $G_{5\text{-}FU}$ and $G_{Arg+5\text{-}FU}$ and the respective degrees of severity. It also shows an intact jejunum sample from the G_{Arg}.

Daily supplementation with 458 mg L-arginine decreased the scores of middle jejunum lesion caused in the rats by chemotherapy (Figure 1). This result is positive for cancer patients under chemotherapy because suggests a treatment that can reduce the risk of intestinal infections and improve their quality of life.

The increased water intake by the G_{Arg} and $G_{Arg+5\text{-}FU}$ rats before and after chemotherapy was probably due to palatability improvement caused by the addition of the L-arginine tablet in the water. However, it did not affect feed intake or body weight, which were similar between GArg and the control group. Therefore, the

reduction in body weight and feed intake observed in $G_{5\text{-}FU}$ and $G_{Arg+5\text{-}FU}$ rats probably resulted from the mucositis caused by 5-FU treatment. The disorder likely caused rat discomfort during feeding.

According to Sonis et al.[22], mucositis is a complex process of cell and molecular changes that can be divided into five phases: initiation, up-regulation, signaling and amplification, ulceration and healing. The initiation phase begins after application of 5-FU, with direct DNA damage in the basal epithelial cells and in signaling and amplification, proinflammatory cytokines exert a direct detrimental effect on mucosal target cells and also play an indirect role in the amplification of mucosal injury initiated by chemotherapy. This fact may have occurred in the $G_{5\text{-}Fu}$ rats by 5-FU-induced enterocyte apoptosis and caused the highest lesional score in the middle portion of the jejunum (Figure 1) and mononuclear inflammatory infiltrate in the submucosa (Figure 2) were similar to those described by Costa et al.[10] and Fukui et al.[6]. The most severe mucositis cases they can lead to bacterial translocation and sepsis [4,21]. A fact that can put patients' lives at risk during chemotherapy.

In the present study, the physiological processes underlying the protective effect of L-arginine on the intestinal mucosa of $G_{Arg+5\text{-}FU}$ rats was not investigated. This protective effect, however, is possibly associated with the several actions that L-arginine on the intestinal mucosa, such as preservation of cell DNA integrity after 5-FU application [23], which may decrease apoptosis induction [24]. L-arginine also stimulates the production of nitric oxide and, combined to that, it maintains the integrity of the intestinal mucosa by increasing blood and nutrient flow in the injured tissues [23], can stimulate the proliferation of enterocytes and increase the height of the villi and intestinal crypts [24], reducing the inflammatory process in the intestinal

mucosa caused by 5-FU [9,25,26,27] and the risk of bacteremia and sepsis [23]. According to Jhon et al. [28], arginine supplementation can be used to reduce the severity of retinoid-induced hypertriglyceridemia [29]. According to Ma et al.[30] colorectal tumors decrease significantly after treatment with L-arginine.

Although supplementation of L-arginine may ameliorate intestinal lesions caused by chemotherapy with 5-FU, thereby promoting patient's health during chemotherapy, decreasing the costs and days of hospitalization, other studies are necessary to confirm and make clearer the physiological effects of this nutrient that are associated with neoplasms with chemotherapy.

In conclusion, supplementation with 458 mg of L-arginine ameliorates the small intestinal lesions caused by 5-FU chemotherapy.

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STATEMENT OF INTEREST

All of the authors contributed in the planning of this study, interpretation of the data, writing, review and approval of the manuscript. All of the authors declare that there is no conflict of interest.

References

1. Zhang X, Sun B, Lu Z. Evaluation of Clinical Value of Single Nucleotide Polymorphisms of Dihydropyrimidine Dehydrogenase Gene to Predict 5- Fluorouracil Toxicity in 60 Colorectal Cancer Patients in China. *Int J Med Sci*. 2013;10:894-902.

2. Chung TC, Tin YH, Ho L, Ji AL, Hui CH. 5-Fluorouracil Induced Intestinal Mucositis via Nuclear Factor-κB Activation by Transcriptomic Analysis and In Vivo Bioluminescence Imaging. *Plos one*. 2012;7:e31808.

3. Skeel T. *Handbock of Cancer Chemotherapy*. 5th ed. Lippincott Williams & Wilkins, 1999.

4. Van Vliet MJ, Harmsen HJM, Bont ESJM, Tissing WJE. The role of intestinal microbiota in the development and severity of chemotherapy induced mucositis. *PLOS Pathog*, 2010;6:e.1000879.

5. Tecza K, Pamula-Pilat J, Lanuszewska J, Grzybowska E. Pharmacogenetics of FAC chemotherapy side effects in breast cancer patients. *Hered Cancer Clin Pract.* 2015;13(suppl.2):A10.

6. Fukui T, Suzuki K, Ichida K, Takayamay Y, Kakizawa N, Muto Y et al. Sequential administration of xelox and xeliri is effective, feasible and well tolerated by patients with metastatic colorectal. *Oncol Lett.* 2017;13:4947-4952.

7. Vasconcelos DM e Duarte AJS. Avaliação dos efeitos da nutrição na resposta imunológica. In: Waitzberg DL. *Dieta, nutrição e câncer*. São Paulo: Atheneu, 2006, 148-55.

8. Quirino IEP, Cardoso VN, Santos RGC, Evangelista WP, Arantes RME, Fiúza JA et al. The role of L-arginine and inducible nitric oxid synthase in intestinal

permeability and bacterial translocation. *JPEN J Parenter Enteral Nutr.* 2013;37:392-400.

9. Leocadio L, Antunes M, Teixeira L, Leonel A, Alvarez-Leite J, Machado DC et al. L-Arginine pretreatment reduces intestinal mucositis as induced by 5-FU in mice. *Nutr Cancer.* 2015;67, 486-93.

10. Costa A, Soares N, Wanner P, Santos GC, Fernandes FA, Martins F dos S et al. L-Arginine supplementation prevents increases in intestinal permeability and bacterial translocation in male Swiss mice subjected to physical exercise under environmental heat stress. *J Nutr.* 2013;144:218-23.

11. Xia Y, Li Y, Chen Y, Zhang J, Liu Q, Deng JY et al. A phase II study of concurrent chemoradiotherapy combined with a weekly paclitaxel and 5-fluorouracil regimen to treat patients with advanced oesophageal carcinoma. *Radiat Oncol.* 2017;12:47.

12. Koppelmann T, Pollak Y, Mogilner J, Bejar J, Coran AG, Sukhotnik I. Dietary L-arginine supplementation reduces methotrexate-induced intestinal mucosal injury in rat. *BMC Gastroenterol.* 2012;12:41.

13. Araujo CV, Lazzarotto CR, Aquino CC, Figueiredo IL, Costa TB, Alves LA et al. Alanyl-glutamine attenuates 5-fluorouracil-induced intestinal mucositis in apolipoprotein E-deficient mice. *Braz J Med Biol Res.* 2015;48:493-501.

14. Cetin Al, Atasoy B, Cilaker S, Alicikus ZA, Karaman M, Ersoy N et al. A Diet Containing beta-hydroxy-beta-methylbutyrate, L-Glutamine and L-Arginine ameliorates chemoradiation-induced gastrointestinal injury in rats. *Radiat Res.* 2015;184:411-21.

15. Jahani M, Azadbakht M, Norooznezhad F and Mansouri, K. L-arginine alters the effect of 5-fluorouracil on breast cancer cells in favor of apoptosis. *Biomed. Pharmacother.* 2017;88:114-23.

16. Ministério da Ciência, Tecnologia e Inovação, Conselho Nacional de Controle de Experimentação Animal – CONCEA. *Diretrizes da prática de eutanásia do CONCEA*, 2013.

17. Mahan LK, Scott-Stump S, Raymond JL. *Krause alimentos, nutrição e dietoterapia*. Rio de Janeiro: Elservier, 2013.

18. Cubas ZS, Joppert AM. Terapêutica dos animais selvagens. In: Andrade SMCF (editor). *Manual de terapêutica veterinária*. São Paulo: Roca, 2008.

19. National Institutes of Health. *Guide for the care and use of laboratory animals*. 8th ed. Washinton, DC: The National Academic Press, 2011.

20. Gerez JR, Pinton P, Callu P, Grosjean F, Oswald IP, Bracarense AP. Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. *Exp Toxicol Pathol.* 2014;67:89-98.

21. Wu Z, Han X, Qin S, Sheng Q, Wang Z, Xiang D et al. Interleukin 1 receptor antagonist reduces lethality and intestinal toxicity of 5-Fluorouracil in a mouse mucositis model. *Biomed Pharmacother.* 2011;65:339–44.

22. Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M et al. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer.* 2004;100;1995-2025.

23. Andrade RME, Santos GC, Soares ADN Costa KA, Fernandes SOA, Souza CM et al. Pretreatment and treatment with L-arginine attenuate weight loss and bacterial translocation in dextran sulfate sodium colitis. *JPEN J Parenter Enteral Nutr.* 2015;40:1131-9.

24. Jiang X, Zhu W, Li N, Tan L, Li J. Effects of continuous enteral L-Arginine in a rat model of the short bowel syndrome. *Asia Pac J Clin Nutr.* 2007;16:554-60.

25. Tan B, Yin Y, Kon X, Li P, Li X, Huang R et al. L-Arginine stimulates proliferation and prevents endotoxin-induced death of intestinal cells. *Amino Acids.* 2010;38:1227-35.

26. Yavas C, Yavas G, Acar H, Toy H, Yuce D, Akurek S et al. Amelioration of radiation-induced acute inflammation and mucosal atrophy by beta-hydroxy-beta-methylbutyrate, L-glutamıne, and L-argınıne: results of an experimental study. *Support Care Cancer.* 2013;21:883-8.

27. Cintra AESU, Martins SL, Patricio NFR, Higa E.M.S, Monteiro EFS. Nitric oxide levels in the intestines of mice submitted to ischemia and reperfusion: L-Arginine effects. *Transplante Proc.* 2008;40:830-5.

28. John D, Radcliffe, Dorice M, Narins C. Use of Arginine to reduce the severity of retinoidinduced hypertriglyceridemia. *Nutr Cancer.* 2015;36:200-6.

29. Synakiewiczt A, Stencel TS, Drozynska EA. The role of arginine and the modified arginine deiminase enzyme ADI-PEG 20 in cancer therapy with special emphasis on phase I/II clinical trials. *Expert Opin Investig Drugs.* 2014;23:1517-29.

30. Ma Q, Wang Y, Gao X. Ma Z, Song Z. L-arginine reduces cell proliferation and ornithine decarboxylase activity in patients with colorectal adenoma and adenocarcinoma. *Clin Cancer Res.* 2007;13:7407-12.

Chapter of figures and tables

TABLE 1. Feed and water intake (mean ± sd) in Wistar rats subjected to 5-FU treatment and dietary L-arginine supplementation.

Experimental groups	Body weight (g)		Feed intake (g/day)		Water intake (mL/day)	
	Before 5-FU treatment	After 5-FU treatment	Before 5-FU treatment	After 5-FU treatment	Before 5-FU treatment	After 5-FU treatment
Control	163,9±19,0^{AB}	242,8±7,4^{A}	66,3±3.7^{AB}	62,3±3,7^{A}	33,5±0,3^{C}	33,4±0,5^{C}
G_{Arg}	152,1±14,9^{A}	227,4±7,3^{A}	69,3±3,3^{A}	65,9±4,1^{A}	57,5±3,8^{A}	57,8±2,7^{A}
$G_{5\text{-}FU}$	156,5±22,3^{AB}	172,2±7,0^{B}	66,2±2,4^{AB}	61,5±0,5^{A}	36,4±0,6^{B}	31,3±3,1^{C}
$G_{Arg+\ 5\text{-}FU}$	175,4±14,9^{B}	192,3±7,4^{B}	63,0±0,5^{B}	58,2±0,3^{B}	58,8±1,1^{A}	48.1±0.3^{B}

$^{A,B-}$ Different uppercase letters in a column indicate statistical difference between groups.

Control = commercial balanced feed + water. $G_{5\text{-}Fu}$ = commercial balanced feed + water + 5-FU application. GArg = commercial balanced feed + water with 458 mg L-arginine + 5-FU application.

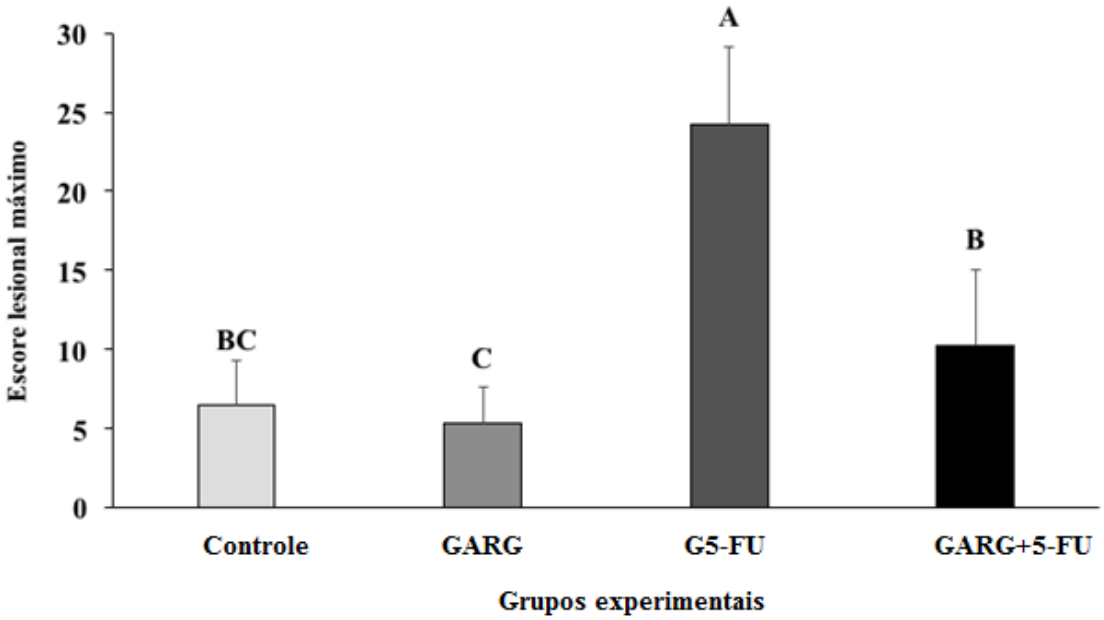

FIG 1- Lesion score in middle jejunum in Wistar rats subjected to 5-FU treatment and dietary L-arginine supplementation.

[A,B-] Different uppercase letters in a bar indicate statistical difference between groups.

Control = commercial balanced feed + water. $G_{5\text{-}Fu}$ = commercial balanced feed + water + 5-FU application. GArg = commercial balanced feed + water with 458 mg L-arginine + 5-FU application.

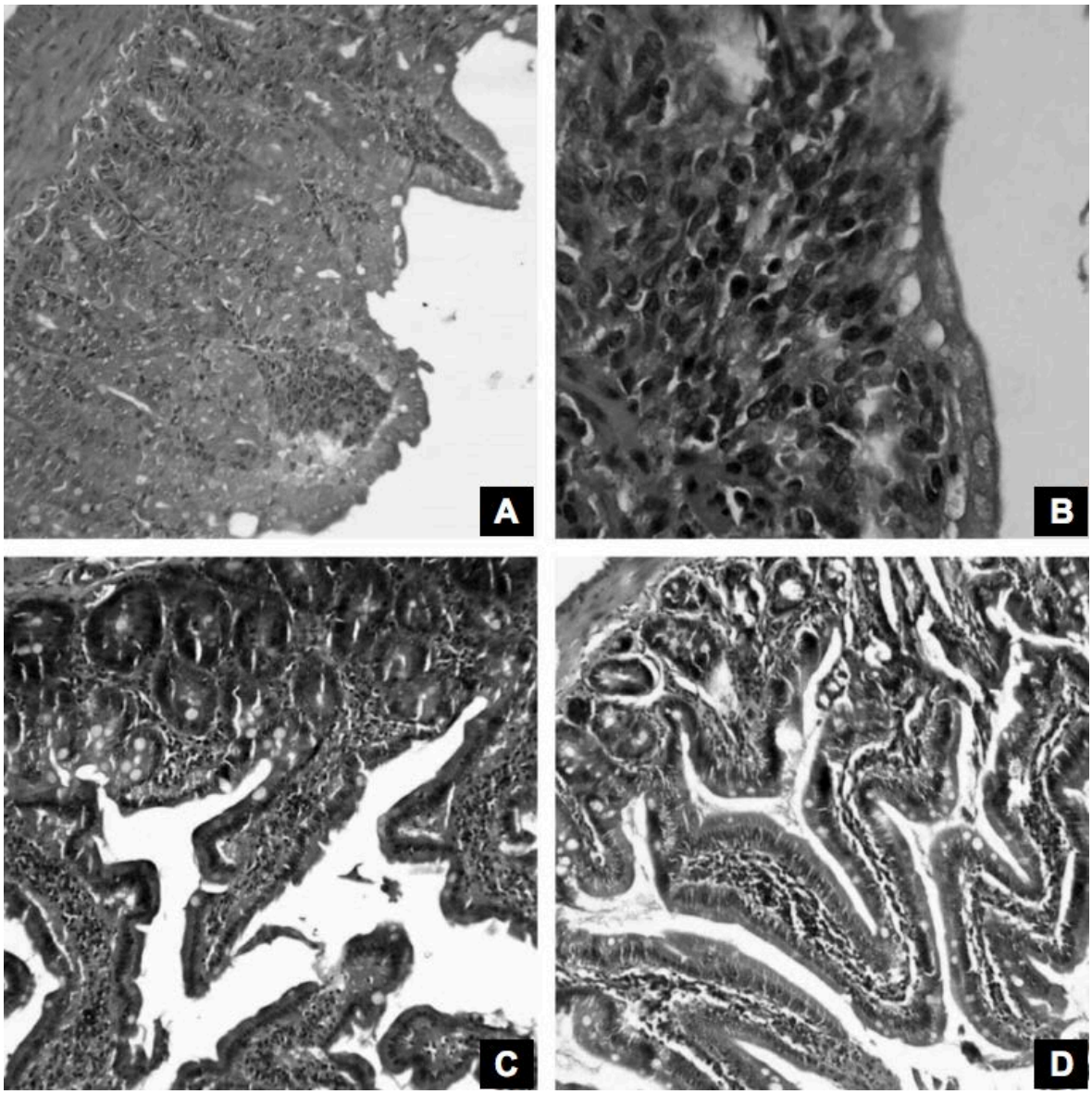

FIG 2- (A) Photomicrography of the middle jejunum of Wistar rats of the $G_{5\text{-}FU}$ group showing apical necrosis and flattening of the villi and mononuclear inflammatory infiltrate in the submucosa (HE, 100x). (B) Photomicrography of the middle jejunum of Wistar rats of the $G_{5\text{-}FU}$ group presenting apical necrosis and mononuclear inflammatory infiltrate in the submucosa (HE, 200x). (C) Photomicrography of the middle jejunum of Wistar rats of the $G_{Arg+5FU}$ group presenting a discrete mononuclear inflammatory infiltrate in the submucosa (HE, 100x). Photomicrography of the middle jejunum of Wistar rats of the G_{Arg} group presenting preserved enteric tissue (HE, 100x).

Control = commercial balanced feed + water. $G_{5\text{-}Fu}$ = commercial balanced feed + water + 5-FU application. GArg = commercial balanced feed + water with 458 mg L-arginine + 5-FU application.

ANEXO A - PARECER DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE DO OESTE PAULISTA – UNOESTE

UNOESTE - Universidade do Oeste Paulista

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PPG - Programa de Pesquisa de Pós-Graduação
PEIC - Programa Especial de Iniciação Científica

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"EFEITO DA SUPLEMENTAÇÃO COM L-ARGININA NO ERITROGRAMA, LEUCOGRAMA E NA PRODUÇÃO DAS IMUNOGLOBULINAS DE RATTUS NOVERGICUS DA LINHAGEM WISTAR SUBMETIDOS À QUIMIOTERAPIA COM 5-FLUOROURACIL"**, cadastrado na Coordenadoria Central de Pesquisa (CCPq) sob o número nº **2755** e tendo como participante(s) **LUÍS SOUZA LIMA DE SOUZA REIS (responsável), CECILIA BRAGA LAPOSY (docente), MARCELO GEORGE MUNGAI CHACUR (docente), ROGERIO GIUFFRIDA (docente), AMANDA BEATRIZ NOVAIS (discente), BIANCA DEPIERI BALMANT (discente), SANDRA CRISTINA GENARO (discente)**, foi avaliado e **APROVADO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) e COMISSÃO DE ÉTICA USO DE ANIMAIS (CEUA)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Presidente Prudente, 25 de Janeiro de 2016.

Prof. Dr. Jair Rodrigues Garcia Jr.
Coordenador Científico da CCPq

Profª Ms. Adriana Falco de Brito
Coordenadora da CEUA - UNOESTE

ANEXO B - INSTRUÇÕES PARA AUTORES: NUTRITION AND CANCER JOURNAL

Nutrition and Cancer: An International Journal uses an online submission and review system, ScholarOne, through which authors submit manuscripts and track their progress up until acceptance for publication. Authors will enter pertinent information into the system and submit the following files: (a) cover letter file (including verification that the article has not been submitted concurrently to any other journals); (b) manuscript file (Word or WordPerfect format [PC compatible]) containing the entire text of the article, including title page, abstract, all text, references, footnotes, and appendixes; (c) figures and tables, which should be submitted as separate files. Please log on to http://mc.manuscriptcentral.com/nc/ for information and instructions regarding registration and manuscript submission.

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• 300 dpi or higher

• Sized to fit on journal page

• EPS, TIFF, or PSD format only

• Submitted as separate files, not embedded in text files

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Please include 3-5 "key words" after the abstract, including words such as 'experimental', 'clinical', and 'epidemiological' to aid in searches.

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