BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022000Xe20114

Article

Curcuma longa extract protects against 5-fluorouracil-induced oral mucositis in hamsters

Aurigena Antunes de Araújo[1,2,3*], Edna Maria Silva[4], Cristiane Assunção da Costa Cunha Mafra[1,3], Íris do Céu Clara Costa[4], Amanda Lanna Andrade Barbalho[4], Iolanda Augusta Fernandes de Matos[4], Matheus Araujo dos Santos[4], Maria Luiza Diniz de Sousa Lopes[4], Caroline Addison Carvalho Xavier de Medeiros[3,5,6], Luiz Alberto Lira Soares[7], Janaina Carla Barbosa Machado[7], Magda Rhayanny Assunção Ferreira[7], Raimundo Fernandes de Araújo[8,9], Rubens Maribondo do Nascimento[10], Gerlane Coelho Bernardo Guerra[2,3,5]

[1]Postgraduate Program in Dentistry Science, UFRN, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [2]Postgraduate Program in Pharmaceutical Science, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [3]Department of Biophysics and Pharmacology, UFRN, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [4]Department of Dentistry, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [5]Postgraduate in Biological Science, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [6]Postgraduate Program in RENORBIO, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [7]Postgraduate Program in Pharmaceutical Science/Department of Pharmaceutical Sciences, Federal University of Pernambuco (UFPE), Recife, PE, Brazil, [8]Postgraduate Program in Functional and Structural Biology, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [9]Department of Morphology, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil, [10]Postgraduate Program in Materials Engineer, Federal University of Rio Grande Norte (UFRN), Natal, RN, Brazil

Curcumin, contained at Turmeric (*Curcuma longa*), can exert many beneficial pleiotropic activities in the gastrointestinal tract. This study evaluated the antioxidant and anti-inflammatory activity of *C. longa* on 5-fluorouracil (5-FU)-induced oral mucositis (OM) in hamsters. Phytochemical analysis of crude *C. longa* extract (CLE) was performed to detect the presence of curcumin by TLC and HPLC. Golden Syrian hamsters were orally pre-treated with CLE (5, 50, or 100mg/kg). Cheek pouch samples were subjected to macroscopic and histopathological evaluation. ELISA was performed to quantify the inflammatory cytokines IL-1β and TNF-α. Superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) levels were assessed by ultraviolet-*visible* spectroscopy analysis. Behavior analysis was conducted by the open field test. Curcumin content in the CLE was 0.55%m/m ± 0.0161 (2.84%). The group treated with 5mg/kg CLE showed healing evidence with macroscopic absence of ulceration ($p<0.05$) and microscopic aspect of re-epithelialization, discrete inflammatory infiltrate and absence of edema. Treatment with 5mg/kg CLE significantly increased GSH levels, and reduced MDA levels and SOD activity ($p<0.05$), and decreased IL-1β ($p<0.05$) and TNF-α ($p<0.01$) levels. A significant reduction in walking distance, ambulation, speed, and rearing was observed for motor activity. Curcumin reduced oxidative stress, inflammation, and motor activity in hamsters with 5-FU-induced OM.

Keywords: 5-fluorouracil. Oral mucositis. Curcumin. Oxidative stress. Inflammation.

***Correspondence:** A. A. de Araújo. Departamento de Biofísica e Farmacologia. Centro de Biociências – UFRN. Av. Senador Salgado Filho, S/N, Campus Universitário. Lagoa Nova, 59072-970, Natal, RN, Brasil. Phone/Fax: +55843215-3419. E-mail: aurigena@ufrnet.br. ORCID: https://orcid.org/0000-0001-9264-4695

INTRODUCTION

Mucositis, an inflammatory condition, is one of the most frequent adverse effect of anticancer therapies. The onset of mucositis lesions and duration are thought to be related to treatment factors such as irradiated tissue volume, daily and total doses of radiotherapy and chemotherapy (Al-Ansari *et al*., 2015; Sonis, 2010). Methotrexate, irinotecan, and 5-fluorouracil (5-FU) are chemotherapeutic agents associated with a high prevalence of oral and intestinal mucositis, as well as anorexia, nausea and diarrhea (Boussios *et al*., 2012).

The epithelium of the oral mucosa suffers most of the damage caused by antineoplastic agents due to its high mitotic activity, especially the non-keratinized mucosa of the lip, buccal mucosa, soft palate and floor of the mouth (Leão, Gomes, Porter, 2007). Oral mucositis (OM) presents as erythematous and ulcerated lesions, associated to a higher risk for local or systemic infectious and to severe pain, which may impair food intake and compromise the quality of life of the oncologic patient (Leão, Gomes, Porter, 2007; Normando *et al*., 2019; Panghal *et al*., 2012; Sonis, 2010).

The pathogenesis of OM involves sequential biologic events that include reactive oxygen species (ROS) generation by pro-inflammatory pathways (Sonis, 2010). Clinical management largely relies on symptom management and prevention of complications. Specific therapeutic strategies include antioxidant agents (amifostine and GC4419), low intensity laser/photobiomodulation and granulocyte-macrophage colony-stimulating factor (Al-Ansari *et al*., 2015; Sonis, 2010). However, these therapies face limitations both in their cost and delivery in specialized health services.

In this sense, the use natural products is deemed as an accessible and more affordable alternative to treat many human diseases, with a growing interest by phytochemical and pharmacological studies on new drugs and herbal medicines (Stanic, 2017). Turmeric (*Curcuma longa*) is a rhizomatous herb that belongs to ginger Zingiberaceae family, which is frequently found in the southern and tropical western Asia. Turmeric has been traditionally used as a culinary spice and is also known for its use as medical treatment of illnesses for centuries, especially in India and China (Siviero *et al*., 2015; Stanic, 2017). Among *C. longa* compounds, a variety of pharmacological properties is attributed to curcumin (diferuloyl methane), including antimicrobial, antioxidant, anti-inflammatory, antihyperlipidemic, anticarcinogenic and hypoglycemic activity (Siviero *et al*., 2015; Stanic, 2017; Willenbacher *et al*., 2019).

The protective role of curcumin on chemotherapy-induced toxicity is well-stablished in the literature. Promising results from preclinical studies support the antibacterial, antioxidant and anti-inflammatory properties of curcumin on OM (Dos Santos Filho *et al*., 2018; Luer, Troller, Aebi, 2012; Luer *et al*., 2011; Schmidt, Curra, 2019), and a recent systematic review reported its clinical effect on reducing pain, erythema intensity, ulceration area, and severity degree of OM in oncologic patients undergoing radio and/or chemotherapy (Normando *et al*., 2019). In view of the aforementioned evidence, the aim of this study was to evaluate the antioxidant, anti-inflammatory and behavioral effect of *C. longa* on an *in vivo* experimental OM model.

MATERIAL AND METHODS

Plant material

Specie: *Curcuma longa* L. Family: Zingiberaceae. Crude *Curcuma longa L.* extract (CLE) (95%, imported from China, lot number of the material/extract: CJH-A-706624, Supplementary information) was obtained from the *Companhia da Fórmula* handling compounding pharmacy (Natal, RN, Brazil). The certification of the analysis for use was performed by *Iberoquímica Magistral* (São Paulo, Brazil). The detailed methods of extraction and the yield of extracts should be clearly indicated in the manuscript.

Botanical origin: Curcuma longa L. (root). Solvents: water and ethanol. The following physicochemical information has been described by the *Iberoquímica Magistral*: Appearance - fine orange powder with characteristic odor; particle size - 100% passes through 80 mesh; water - 3%, total ash - 1.5%; bulk density 4-60 g/100mL; packed density 60-90 g/100mL; lead - maximum 2 mg/kg; arsenic at most 1mg/kg; cadmium

- maximum 1mg/kg; mercury - maximum 1mg/kg (Supplementary information).

Phytochemical Analysis

Phytochemical profile consisted of using silica gel plates $60F_{254}$ (10-12 µm particle size, Macherey-Nagel®) using semi-automated equipment (Linomat V, Camag®) controlled by WinCats® software (Camag®). First, 20 µL of methanolic *C. longa* crude extract solution (50 µg/mL) and 20 µL of the standard solution (Curcumin; 10 µg/mL) were applied in bands of 10 mm of width and 5 mm space between them. The chromatogram was developed in a twin trough vertical glass chamber (10 cm x 10 cm, Camag®) after saturation (30 min) with the mobile phase chloroform: ethanol: glacial acetic acid (95:5:1, v/v/v). The plate was observed under UV light at 365 nm after drying.

The sample and standard were prepared at the concentration of 0.5 mg/mL ECL, using ethanol as solvent (Cinética®). The homogenization was performed on ultrasound (Ultracleaner 1600A, Unique®) without heating for 30 minutes. Next, aliquots were transferred to 10 mL volumetric flasks and the volume was checked with ethanol 50% (v/v). The sample and standard solutions were subsequently filtered through a PVDF membrane (0.45 µm, Macherey-Nagel®) directly into the vials.

The analyses were carried out in a HPLC (Ultimate 3000, Thermo Fisher Scientific®) equipped with diode array detector (DAD–3000 (RS); Thermo Fisher Scientific®), with a binary pump (HPG3x00RS, Thermo Fisher Scientific®), a degasser and an autosampler with a 20 µL loop (ACC 3000, Thermo Fisher Scientific®). Chromeleon software (Dionex, Thermo Fisher Scientific®) was used for data analysis and processing. The chromatographic separation was performed using a C_{18} column (250 mm × 4.6 mm i.d., particle size 5 µm, Dionex®) equipped with a pre-column of the (4 mm x 3.9 µm, Phenomenex®) at a temperature of 25 ± 2 °C. Ultrapure water (A) and methanol (B) both acidified with trifluoroacetic acid (0.05%) were used as the mobile phase. The elution gradient was performed as following: 10-20% B (0-10.0 min), 20-25% B (10.0-13.5 min), 25-40% B (13.5-18.0 min), 40-80% B (18.0-25.0 min), 80% B (25.0-30.0 min), 80-10% B (30.0-34.0 min) and 10% B (34.0-36.0 min) at a flow rate of 0.9 mL/min. Wavelengths of 210 nm, 270 nm and 350 nm were used for detection, according to the maximum absorption measured by the detector.

The results in contents were expressed as %m/m of curcumin (Primary Reference Standard - HWI group, purchased from Sigma-Aldrich®).

Experimental Design and Induction of Oral Mucositis

Male Golden (Syrian) Hamster (140-180 grams), aged 8-10 weeks, reproduced and were kept during the experimental period in the Department of Biophysics and Pharmacology, under standard conditions of temperature (22 °C) and light/dark cycle of 12 hours. The animals were housed in individual cages with free access to water and feed. The research project was approved by the UFRN Animal Research Ethics Committee with the number 043.022/2017.

Hamsters were randomized into 09 groups (n=05/group), divided in 03 controls and 06 experimental groups (03 groups treated with CLE but without oral mucositis - CLE NOM; and 03 groups with OM treated with CLE - CLE OM). The three CLE-NOM groups served to control the toxicological and behavioral effects of curcumin without OM, while CLE OM-treated groups underwent all analyzes: toxicological, behavioral, macroscopic, histopathological, and oxidative stress marker dosages and inflammatory cytokine quantification. The control and experimental groups are described in the Table I.

On the 1st and 2nd days of the experiment, the vehicle or CLE at the doses of 5mg/kg, 50 mg/kg and 100mg/kg were orally administered. Thirty minutes after this oral administration, 5-FU was administered intraperitoneally at the dose of 60 mg/kg (first day) and 40 mg/kg (second day). In control groups that did not receive 5-FU, the corresponding saline volume was administered intraperitoneally. On the 3rd day of the experiment, only oral administration of different treatments with vehicle or CLE was performed. On the 4th day of the experiment, gavage and then injury to the buccal mucosa of the "trauma" groups animals (MT or 5FUT/MT or CLE OM treated groups, all doses) was performed. Trauma was induced by a 0.7 x 25 mm blunt needle under anesthesia with 80mg/kg 10%

ketamine, and 10mg/kg 2% xylazine. From the 5th to the 10th days of the experiment, only daily oral administration of the vehicle or CLE was performed. On the 11th day, the animals were euthanized with thiopental overdose (100 mg/kg) and the buccal mucosa was removed and submitted to the evaluation of malonaldehyde (MDA) and glutathione (GSH) dosage, superoxide dismutase (SOD) activity and cytokines quantification. The samples were fixed in formaldehyde for the studies.

TABLE I - Description of the control and experimental groups.

Groups	Description
Control	
N	Animals without MT and OM and treated with saline solution by gavage.
MT	Animals without OM submitted to MT and treated with saline solution by gavage.
5FUT/MT	Animals submitted to MT, treated with saline by gavage, and intraperitoneal injection of 5-FU.
Experimental	
5 mg/kg CLE NOM	Animals without oral mucositis treated with oral administration of crude *Curcuma longa* extract at a dose of 5 mg/kg.
50 mg/kg CLE NOM	Animals without oral mucositis treated with oral administration of crude *C. longa* extract at a dose of 50 mg/kg.
100 mg/kg CLE NOM	Animals without oral mucositis treated with oral administration of crude *C. longa* extract at a dose of 100 mg/kg.
5 mg/kg CLE OM	Animals with oral mucositis treated with oral administration of crude *C. longa* extract at a dose of 5 mg/kg.
50 mg/kg CLE OM	Animals with oral mucositis treated with oral administration of crude *C. longa* extract at a dose of 50 mg/kg.
100 mg/kg CLE OM	Animals with oral mucositis treated with oral administration of crude *C. longa* extract at a dose of 100 mg/kg.

N, Normal. MT, Mechanical trauma. 5-FU, 5-fluorouracil. CLE, Crude *Curcuma longa* extract. NOM, normal oral mucosa. OM, Oral mucositis.

Biochemical analysis

Serum (n=5/group) was obtained by centrifuging total blood without anticoagulants at 2,500 rpm for 15 min. Serum levels of alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined with standardised diagnostic kits (LABTEST®) and spectrophotometry. For leucocyte counts, 20-µl samples of total blood were combined with 380 µl of Turk solution. Total leukocyte counts per mm^3 were determined by standard manual light microscopy procedures (De Souza, Ferreira, 1985).

Macroscopic analysis

The animals were euthanized on the 11th day of the experimental model and their buccal mucosa were photographed for macroscopic analysis. The evaluated parameters were the presence and intensity of erythema, hyperemia, hemorrhage, ulcers and abscesses, classified according to standardized scores: *Score 0*, completely healthy mucosa, without erosion or vasodilation; *Score 1*, presence of erythema but no evidence of mucosal erosion; *Score 2*, severe erythema, vasodilation and superficial erosion; *Score 3*, ulcer formation on one or more faces, but not affecting more than 25% of the surface area of the pouch, severe erythema and vasodilation; *Score 4*, cumulative ulcer formation of about 50% of the pouch surface area; *Score 5,* virtually complete ulceration of the pouch mucosa.

Histopathological analysis

Histopathological analysis of the buccal mucosa was performed under optical microscopy (x40). The specimens were fixed in 10% buffered formaldehyde solution for 24 hours, and then the samples were embedded in paraffin. Next, 4-µm thick histological sections were cut from the formalin-fixed paraffin-embedded (FFPE) specimens and stained with hematoxylin-eosin (H&E). Microscopic analysis was performed by evaluating inflammatory aspects such as presence and intensity of cell infiltrate, vascular dilation and engorgement, hemorrhage, edema, ulcers and abscesses, classified according to the following

standardized scores: *Score 0*, epithelium and connective tissue without vasodilation and absent or discrete cellular infiltrate; *Score 1*, mild vascular engorgement; re-epithelialization areas and discrete cellular infiltrate, with higher number of mononuclear leukocytes. *Score 2*, moderate vascular engorgement; hydropic epithelial degeneration (vacuolization); moderate cellular infiltrate, with a predominance of polymorphonuclear leukocytes; *Score 3*: severe vascular engorgement, marked vasodilation, marked cellular infiltrate, with a higher number of polymorphonuclear leukocytes.

Malonaldehyde Dosage (MDA)

The content of MDA a product of lipid peroxidation, in the buccal mucosa samples (n=4/group) was measured by the assay previously described (15). Samples were suspended in Trisma 1:5 (w/v) buffer. The material was incubated for 40 minutes at 45 °C in a water bath, centrifuged at 2500 G for 5 minutes at 4 °C; 300 µL was then removed, read at 586 nm, and interpolated in a standard curve. Supernatants were tested for MDA content and placed in microplates. The absorbance of each sample was measured at 586nm. The results are expressed as nanomoles of MDA per gram of tissue.

Glutathione Dosage (GSH)

GSH levels were measured to verify antioxidant activity. Briefly, 0.02 M EDTA were added to the prepared mucosal tissue, 4 per group, and stored at -80 °C until use. For GSH determination, the samples were thawed and automatically homogenized for 2 min. Samples were then centrifuged at 3000xg for 15 min at 4°C, then the supernatant (400 µl) was removed, and 800 µl of 0.4 M Tris buffer (pH 8.9) and 20 µl of 5,5'-dithiobis-(2-nitrobenzoic acid) were added. Absorbance was measured at 420 nm, and results were reported as GSH units per milligram of tissue.

Estimation of superoxide dismutase (SOD)

Estimation of SOD was carried out following the assay described (Shirane *et al*., 2010). A sample of buccal mucosa tissue (100mg) in 1mL of 0.4M phosphate buffer, pH 7.0, was centrifuged at 10,000 rpm for 15 minutes. The plates labeled as TEST (0,25mL Methionine, 0.03 mL Riboflavin, 0.01mL NBT, 0.01 mL liver homogenate), STANDARD (0,25mL Methionine, 0.03 mL Riboflavin, 0.01mL NBT, 0.01 mL phosphate buffer) and CONTROL (0,25mL Methionine, 0.03mL Riboflavin, 0.01mL phosphate buffer (pH7.8), 0.01mL liver homogenate) were subjected to illumination for 10 minutes in an illumination chamber lined with aluminum foil, and fitted with a 15W fluorescent lamp. Following illumination, the optical density of all the reaction mixtures were immediately read at 560nm. Enzyme units present in the sample were calculated and expressed as U/mg protein (oral mucosa homogenate).

Cytokine immunoassays

Samples of oral mucosa (n=4/group) were homogenized and processed. Briefly, interleukin (IL)-1β (detection range, 62.5–4000 pg/mL; minimum detection limit, 12.5 ng/mL) and tumour necrosis factor (TNF)-α (detection range, 62.5–4000 pg/mL; minimum detection limit, 50 ng/ml) levels were determined by commercial enzyme-linked immunosorbent assay kits (R & D Systems, Minneapolis, MN), according to the manufacturer's instructions. The results were expressed as pg/mL.

Open Field test

Open field test was conducted in a detachable crystal acrylic monitoring box with the following dimensions in mm (C x A x P): 500 x 480 x 500 mm. Weight: 17 kg. Supply voltage 127/220 AC, 1A. Bar with 16 infrared sensors. Sensor bar positioning adjustment. Computer connection via USB (Insight, São Paulo, Brazil). Measurement parameters: Total Distance traveled (cm), ambulation score (registered when an animal crossed a line with all *four paws* resulting in the number of squares traversed), running speed: the mean running speed (cm/s) and rearing or vertical activity: the number of times an animal stood upon its hind legs with forelegs in the air or against the wall.

Statistical analysis

Analysis of variance (ANOVA) followed by Bonferroni's test was used to compare mean values across groups. Kruskal-Wallis test followed by Dunn's test was used to compare medians. The statistical analyses were conducted in Prism 5.0 software (GraphPad, La Jolla, CA). A p-value <0.05 indicated a statistically significant difference.

RESULTS

Phytochemical analysis of C. longa

Analysis of Turmeric extract by TLC demonstrated the presence of curcumin (Rf = 0.38), which showed greenish fluorescence when observed under UV light at 365 nm. In addition, other bands of the same color with Rf = 0.15 and 0.25 were also observed in the sample (Figure 1A).

Chromatogram from HPLC analysis at 350 nm showed the presence of curcumin at retention time (Rt) 29.55 min. Curcumin content in the *Curcuma longa* extract was 0.55 % m/m ± 0.0161 (2.84%). The peak was identified by the retention time, the UV spectra and co-injection of *C. longa* extract with the standard curcumin (Figure 1B).

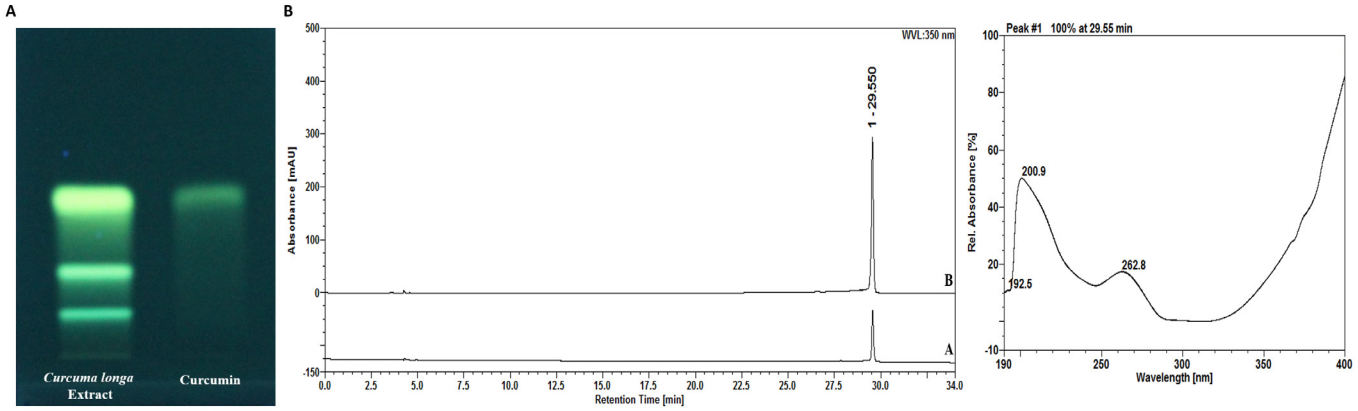

FIGURE 1 - TLC analysis of Turmeric extract showed the presence of curcumin (Rf = 0.38), which when observed under UV light at 365 nm, showed greenish fluorescence (A). The chromatogram from HPLC analysis shown at 350 nm the presence of curcumin at retention time (Rt) 29.55 min (B).

Biochemical, macroscopical, and histopathological features

Table II shows the general animal health conditions, data on weight, death count, leukocyte count, AST, and ALT. The results showed death in 2 groups: 5FUT/MT and 50mg/kg CLE OM. There were no significant hepatic changes between groups (p>0.05). There was significant leukocytosis in the 5FUT/MT group (p<0.05).

TABLE II - Number of deaths, weight, leukocyte count, AST and ALT values.

Groups	Weight (grams)	Number of deaths /group	Leukocyte count (blood) mm^3	AST	ALT
N	139,66	0	6900	62,5	43
TM	189,25	0	6385	74,75	38,5
5FUT/TM	173	1	14050*(a)	69,6	39,6
CLE OM 5 mg/kg	171,8	0	7533	42,75	39,57
CLE OM 50 mg/kg	166	2	13925	48,5	44,17
CLE OM 100 mg/kg	183,8	0	18647	62,33	52,8

*Statistically significant result (0.05) – (a) compared with N group. Analysis of variance (ANOVA) followed by Bonferroni's test. ALT, alanine amino transferase. AST, aspartate amino transferase. N, Normal. MT, Mechanical trauma. CLE, Crude *Curcuma longa* extract. NOM, normal oral mucosa. OM, Oral mucositis.

Administration of 5-FU followed by MT to the buccal mucosa (5FUT/MT) caused an inflammatory process with the appearance of macroscopic lesions, score of 4.0 (3.0-4.0), severe erythema, vasodilation, and cumulative formation of ulcers in about 50% of the surface area of the jugal mucosa (Figure 2). On the other hand, the preventive effect of CLE on oral mucositis was observed at a dose of 5 mg/kg and 50 mg/kg, scores of 2.0 (0.5-2.75; $p<0.05$) and 3.0 (2.0-3.0; $p<0.05$), respectively, when compared to the 5FUT/MT group (Figure 2). These CLE groups showed the presence of severe erythema, vasodilation, and surface erosion, but absence of ulceration in the buccal mucosa of the animals. The 100 mg/kg CLE OM group presented a score of 3.5 (3.0-4.0, $p>0.05$), and showed severe erythema and vasodilatation with ulcers in one or more faces of the mucosa affecting at least 25–50% of the surface area of the cheek pouch (Figure 2).

Comparison of the histopathological scores revealed a statistical difference between groups (Figure 3). Mucosal tissue from the 5FUT/TM group (Figure 3C) showed marked inflammatory infiltration composed of a mixture of polymorphonuclear and mononuclear cells, associated with vasodilatation, with a score of 3 (2-3). Compared to the 5FUT/TM group, normal control specimens exhibited a significantly lower score (median score = 0, $p<0.001$) for the studied parameters, since no inflammatory feature was observed (Figure 3A). Animals treated with 5 mg/kg CLE, score of 0.5 (0.5-1, $p <0.01$), and 50 mg/kg CLE, score of 1.0 (0.5-1.5, $p<0.05$) (Figure 3D and E, respectively) revealed a significant decrease in the parameters compared to the 5FUT/MT group, presenting mild or absent leukocyte infiltration. The group treated with 100 mg/kg CLE OM (Figure 3F) revealed slight improvement in the analyzed parameters, score of 1.0 (1.0-2.5, $p>0.05$), but still showed moderate inflammatory infiltration. In summary, macroscopical and histopathological results suggested that CLE improved the lesions of the buccal mucosa of hamsters with 5-FU-induced OM.

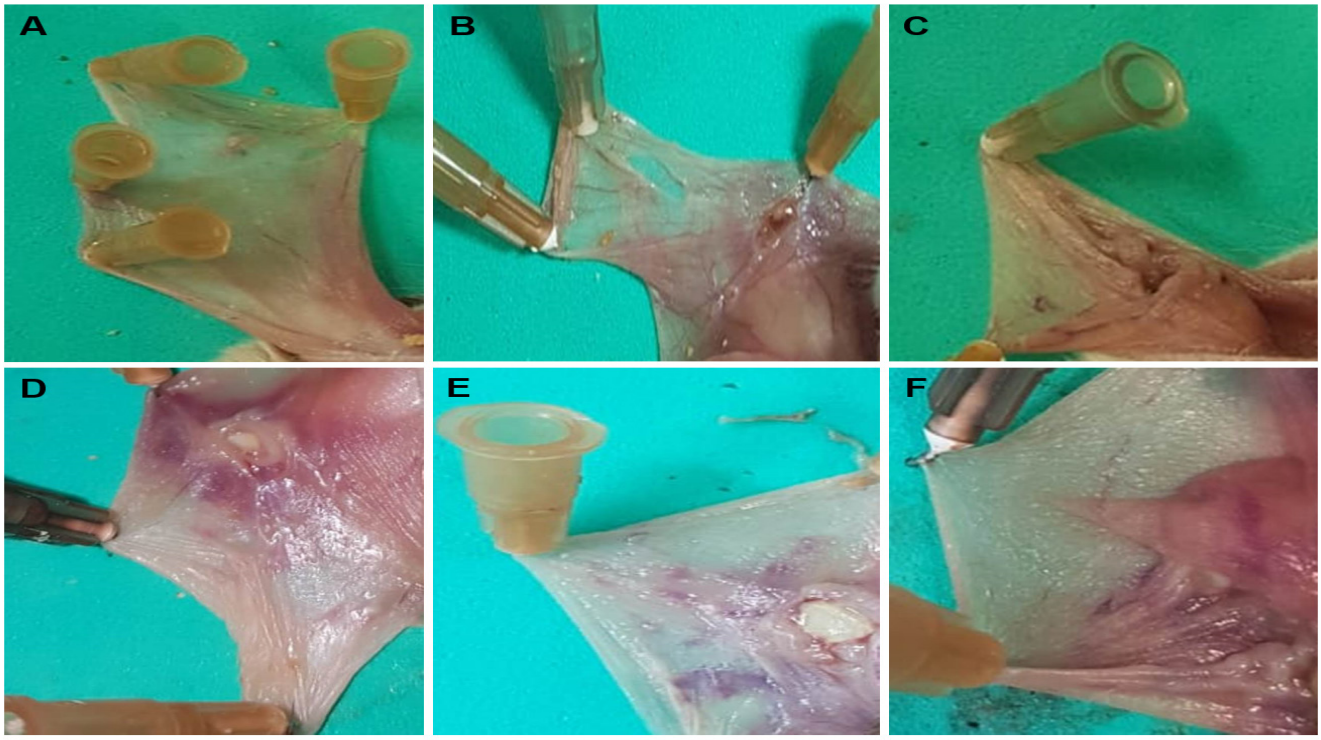

FIGURE 2 - Macroscopic analysis (n=5/group) of normal group (A), tissue subjected to mechanical trauma group (B), 5FUT/TM group (C), 5 mg/kg CLE (D), 50 mg/kg CLE (E), and 100 mg/kg CLE (F). (*p <0.05, **p < 0.01, ***p<0.001). Analysis of variance (ANOVA) followed by Bonferroni's test.

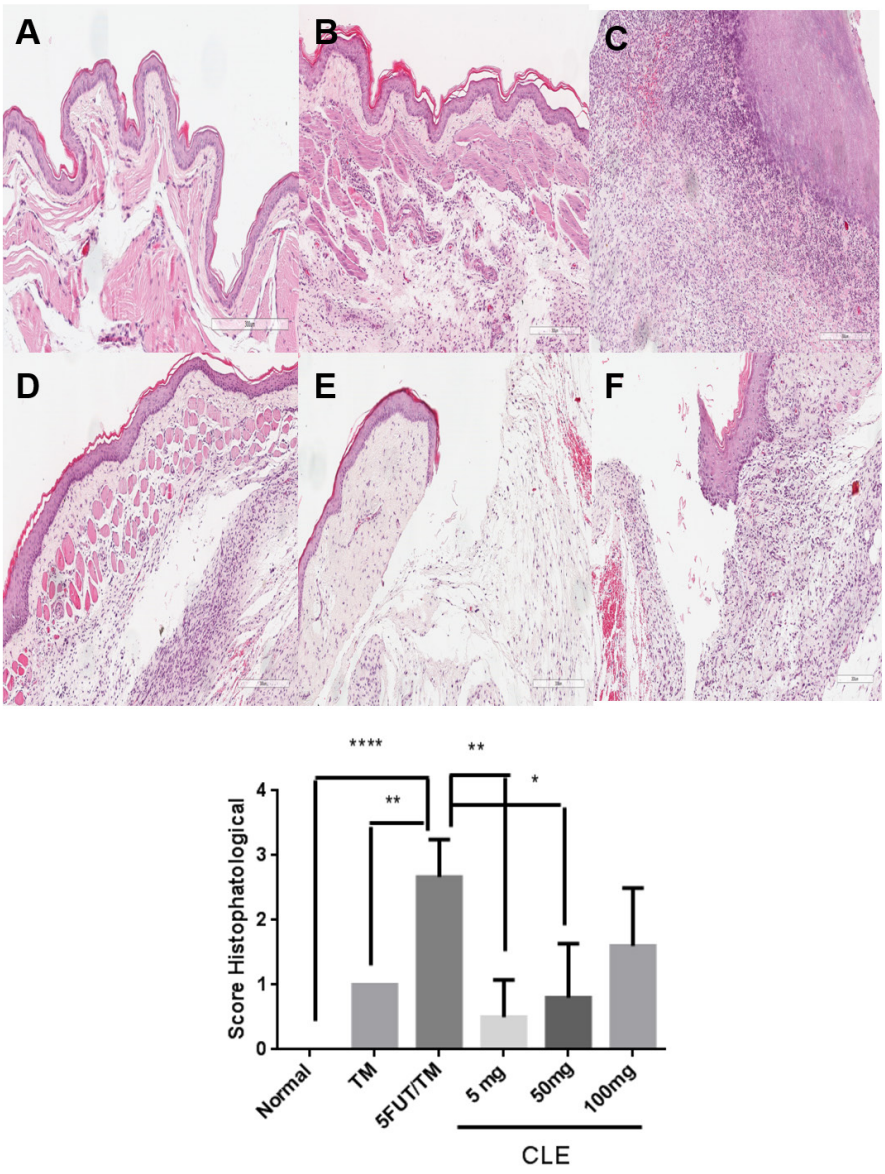

FIGURE 3 - Histopathological analysis of the buccal mucosa of the hamsters. Normal group showed no mucosal alterations (A). Mechanical trauma (MT) group revealed discrete inflammatory infiltrate (star) (B). The 5FUT/TM group presented many tissue alterations, and intense inflammatory infiltrate (arrowhead). The group treated with CLE at the dose of 5 mg/kg (D) and 50 mg/kg CLE (F) revealed mild to moderate inflammatory infiltrate (arrows). Animals treated with 100 mg/kg CLE showed sparse inflammatory cells (arrows) (E) (H&E; Bars indicate 300µm). (*p<0.05, **p< 0.01, ***p<0.001). Kruskal-Wallis test followed by Dunn's test.

Antioxidant activity

In the present study, oxidative stress evaluation revealed a significant reduction in MDA in the CLE OM groups treated with doses of 5 mg/kg ($p<0.05$), 50 mg/kg ($p<0.05$) and 100mg/kg ($p<0.001$; Figure 4). GSH levels showed a significant difference when comparing 5 mg/kg CLE OM with 5FUT/MT group ($p<0.05$). Moreover, SOD levels were significantly reduced in the CLE OM treated groups at doses of 5 mg/kg ($p<0.05$), 50 mg/kg ($p<0.05$) and 100mg/kg ($p<0.05$). In summary, our data indicate an antioxidant effect and prevention of free radical formation, since there was no increase in the antioxidant enzyme SOD, while GSH was maintained at high/unchanged level, and finally there was no significant formation of lipid peroxidation product MDA.

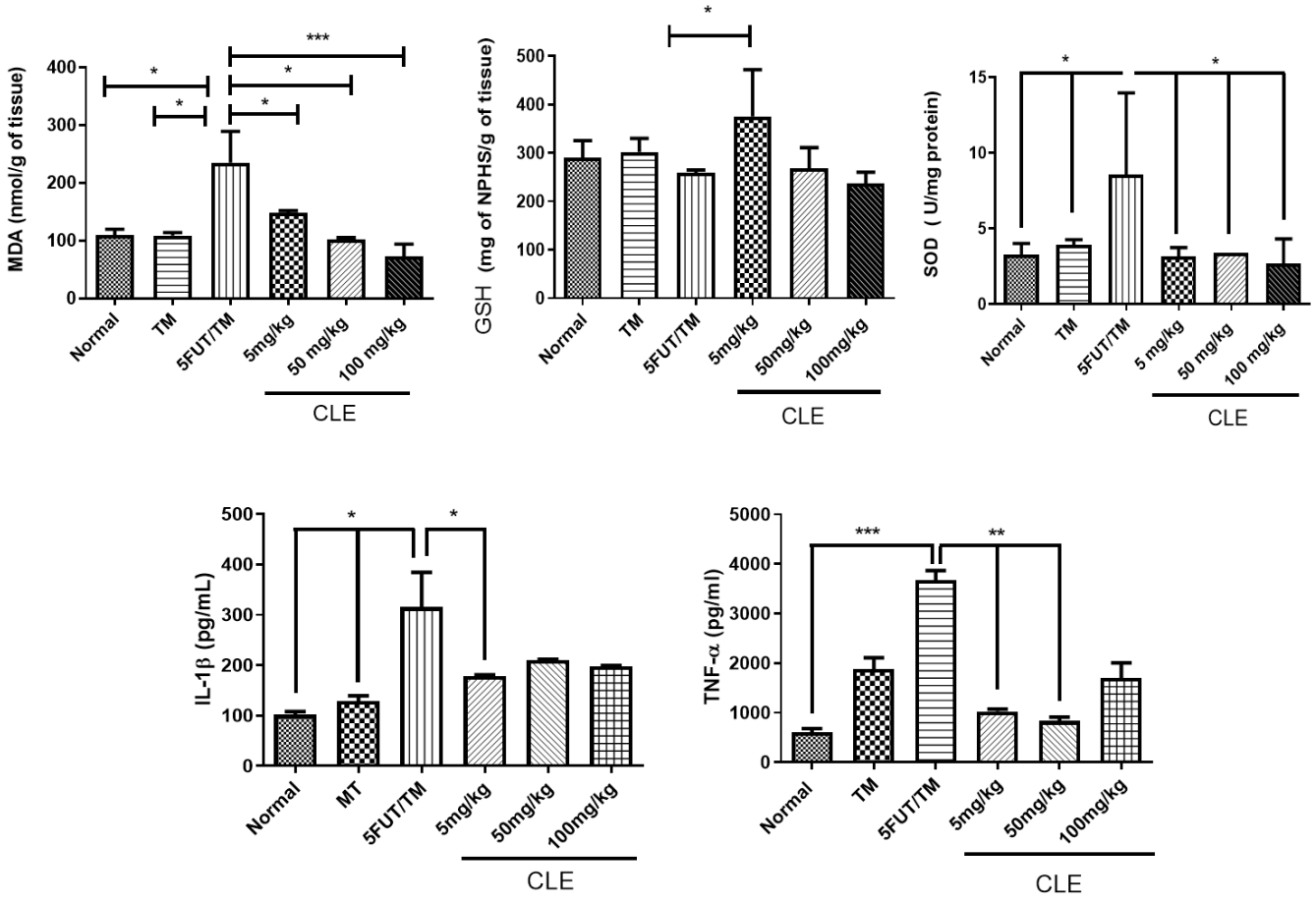

FIGURE 4 - MDA activity, GSH and SOD levels, IL-1β and TNF- α in Normal, MT, 5FUT/TM, 5 mg/kg CLE, 50 mg/kg CLE and 100 mg/kg CLE groups (n=4/group; *p<0.05, **p< 0.01, ***p<0.001). Analysis of variance (ANOVA) followed by Bonferroni's test.

Anti-inflammatory activity

In our study, IL-1β levels were significantly lower after treatment with 5mg/kg CLE OM (p<0.05). There was also a significant reduction of TNF-α levels in the CLE OM groups at a dose of 5mg/kg and 50mg/kg (p<0.01), compared with 5FUT/MT group (Figure 4).

Open field evaluation

Analyzing the open field activity data, we have focused on four selected measures assess the activity level which generally reflects locomotor function. These parameters included: total distance traveled, ambulatory movement, speed, and rearing. Animals with reduced locomotor activity were generally less active and therefore travelled shorter distances with reduced ambulatory activity and speed (Figure 5, p<0.05). A significant reduction in ambulatory movement was observed in the 5FUT/MT 100mg/kg CLE (NO/OM group) and in the other CLE OM treated groups at all doses with oral mucositis at T1 compared to the 5FUT/MT group at T1 (p<0.05) (Figure 5).

The open field also provided us information about emotionality. A significant increase in the 5FUT/MT 100mg/kg CLE (NO/OM group) and in the other CLE OM treated groups at all doses with oral mucositis at T1 was found for rearing compared to the 5FUT/MT group at T1 (p<0.05), which indicates a possible reduction of anxiety/fear in the animal.

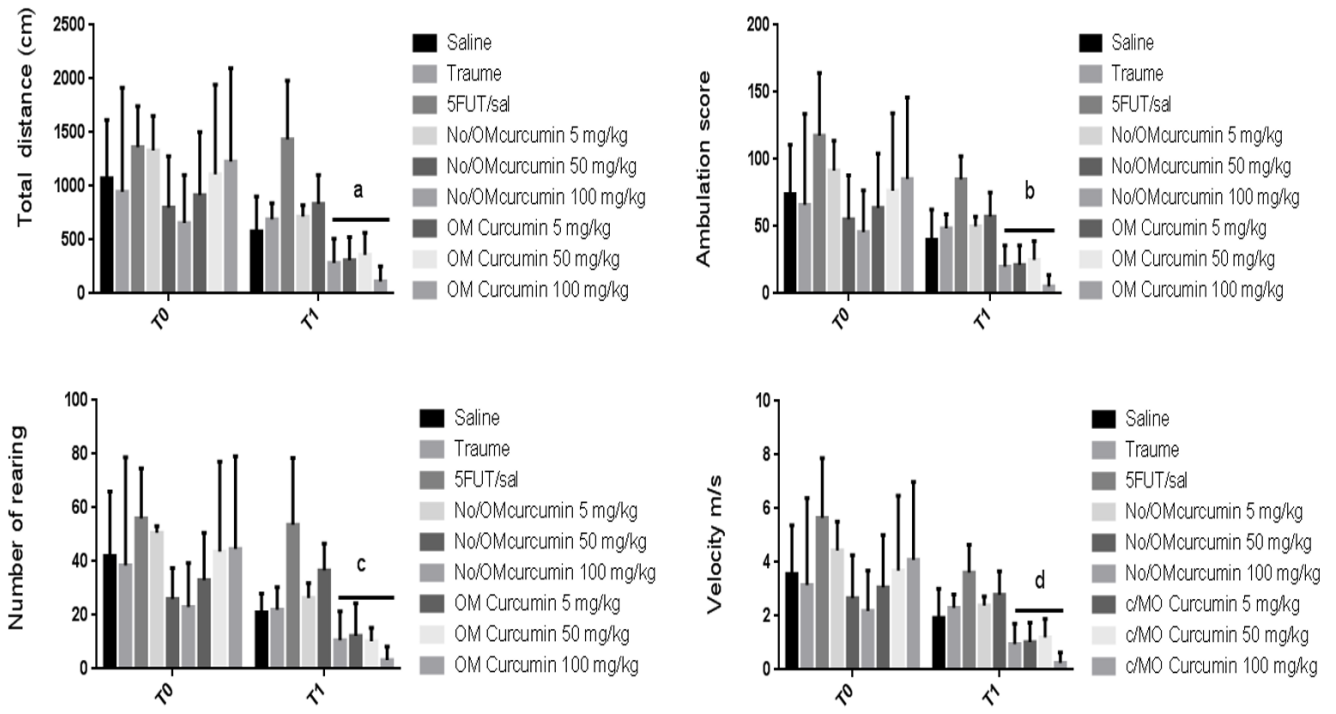

FIGURE 5 - Total distance traveled, ambulation, speed and rearing (Open field): Normal, MT, 5FUT/TM, 5 mg/kg CLE, 50 mg/kg CLE and 100 mg/kg CLE groups (a, b, c, d $*p < 0.05$, compared with 5FUT/TM group at T1). Analysis of variance (ANOVA) followed by Bonferroni's test.

DISCUSSION

Curcumin is an important active compound of Turmeric, with many pharmacological effects related to different functional groups in its chemical structure, such as phenolic hydroxyl group. The pleiotropic molecule of curcumin can interact with many molecules inside the cell, including proteins, enzymes, nucleic acids, and carrier molecules (Siviero *et al*., 2015).

Beneficial properties of curcumin have been investigated in the treatment of a wide variety diseases, including cancer (Willenbacher *et al*., 2019) and inflammatory diseases (Funk *et al*., 2006; Normando *et al*., 2019; Schmidt, Curra, 2019). A gastroprotective effect against reflux esophagitis and gastric mucosal damage induced by non-steroidal anti-inflammatory drugs (NSAIDs) and necrotizing agents was also reported (Kwiecien, Magierowski, 2019).

Chemoprotective effects of curcumin on OM has been demonstrated *in vitro* and *in vivo* (Dos Santos Filho *et al*., 2018; Luer, Troller, Aebi, 2012; Luer *et al*., 2011; Schmidt, Curra, 2019). Moreover, clinical studies have supported that Turmeric topically applied as a gel, as a mouthwash, or in the form of nanomicelle orally administered has significant benefits in preventing and reducing the severity of OM (Charantimath, 2019; Delavarian *et al*., 2019; Patil *et al*., 2015; Rao *et al*., 2014).

Despite the protective properties of curcumin, it has poor bioavailability mostly due to low solubility in water, which has encouraged new strategies investigation to overcome this limitation (Stanic, 2017). In this sense, a recent *in vivo* study evaluated a topical mucoadhesive formulation of curcumin and showed promising preventive effects on OM in hamsters (Schmidt, Curra, 2019).

Several studies have demonstrated the curcumin potential to improve OM lesions in cancer patients. Rao *et al*. (2014) findings showed that turmeric gargle delayed and reduced the severity of radiation-induced OM in head and neck cancer patients. Patil *et al*. (2015) showed that mouthwash with curcumin controlled the signs and

symptoms of OM in 10 cancer patients undergoing radiotherapy and chemotherapy. Charantimath (2019) reported that treatment with curcumin gel significantly decreased erythema and size of the ulcers in oral cancer patients with OM induced by radiochemotherapy, when compared to control. Additionally, a recent clinical trial tested curcumin in the form of nanomicelle in head and neck cancer patients and demonstrated effective results in preventing OM or decreasing its severity (Delavarian *et al*., 2019).

Jagetia, Rajanikant (2004) found that curcumin pretreatment significantly enhanced wound contraction rate and reduced wound healing time in wounds created on the dorsum of whole-body irradiated mice. Results of Lim *et al*. (2016) showed that topical application of curcumin improved the wound healing process of oral ulcers in a rabbit model, while Shmidt, Curra (2019) revealed that treatment with a mucoadhesive formulation containing curcuminoid from *C. longa* extract accelerated healing of 5-FU-induced OM lesions in hamsters, verified by re-epithelialization and a decrease of the inflammatory process clinically and microscopically. Panchatcharam *et al*. (2006) performed a histopathological examination of cutaneous wounds in rats topically treated by curcumin and found an improvement in epithelialization and wound contraction, as well as a decrease in the lipid peroxides levels, and increase in antioxidant enzymes. The beneficial effect of curcumin in promoting wound healing is thought to occur through augmentation of collagen synthesis and modulation, neovascularization, anti-inflammatory and antioxidant properties (Jagetia, Rajanikant, 2004; Panchatcharam *et al*., 2006; Schmidt, Curra, 2019).

The participation of oxidative stress in OM pathogenesis is well-known. The biological sequence of OM begins with direct cell injury mediated by chemotherapy or radiation and evolves as a complex cascade of events described in a five-phase process: initiation, signaling, amplification, ulceration and healing (Sonis, 2010). In the initiation stage, the release of ROS and lipid peroxidation induced by radio or chemotherapy results on direct damage to DNA, which activate many submucosal and epithelial signaling pathways of the next stages (Al-Ansari *et al*., 2015).

Normal cells use enzymatic and non-enzymatic antioxidant mechanisms as a protection against oxidative stress (Kesarwala, Krishna, Mitchell, 2016). SOD scavenging activity by turning superoxide radical anion to H_2O_2 is part of the enzymatic antioxidant system, known as the first enzymatic line of defense against oxidative stress (Kesarwala, Krishna, Mitchell, 2016; Pisoschi, Pop, 2015). On the other hand, the reduced form of glutathione, GSH, is a low-molecular-mass metabolic compound found within cells, which plays a major role in the "second line of defense" by neutralizing toxic reactive species. GSH acts as effective electron donor, as free thiol groups are oxidized to disulfide bonds (Pisoschi, Pop, 2015).

Our data indicate an antioxidant effect and prevention of free radical formation, since no increase in the antioxidant enzyme SOD was observed, while GSH levels were maintained high/unchanged, and finally there was no significant formation of lipid peroxidation product MDA.

Antioxidant property of curcumin was demonstrated by inhibition of lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (Reddy, Lokesh, 1994). Tawfik, Abouelella, Shahein (2013) reported that curcumin protected mouse liver against the radiation-induced decline in GSH concentration and lipid peroxidation. A protective effect was also demonstrated by curcuminoids from *C. longa* on 5-FU-induced intestinal mucositis in mice (Bastos *et al*., 2016; Dos Santos Filho *et al*., 2016).

Curcumin is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals, which are important in initiating lipid peroxidation. The phenolic and the methoxy groups on the phenyl ring and the 1,3-diketone system seem to contribute to these scavenger effects (Sreejayan, Rao, 1994). Ak and Gulcin (2008) performed various *in vitro* antioxidant assays to investigate the antioxidant activity of curcumin, and found that scavenging of reactive radicals and antioxidant activity was associated to H-atom abstraction from the free hydroxyl group.

Chemotherapy and radiotherapy direct damage to the mucosa induced by oxidative stress and the activation of transcription factors, such as nuclear factor kappa B

(NF-κB), increases the expression of proinflammatory mediators, such as TNF-α, IL-1β, IL-6, COX-2 and iNOS. The production of inflammatory markers, together with increased inflammatory infiltrate is responsible for ulceration of the mucosa (Al-Ansari *et al*., 2015). Cytokines IL-1β and TNF-α are known pro-inflammatory markers that play an important role in amplifying the tissue damage mechanisms involved in 5-FU-induced oral mucositis (Kwon, 2016).

Anti-inflammatory mechanisms induced by curcumin comprise regulation of numerous transcription factors, cytokines, protein kinases, adhesion molecules, redox status, and enzymes (Aggarwal, Harikumar, 2009). Evidence shows that curcumin can inhibit production of inflammatory cytokines by peripheral blood monocytes, as it penetrates into the cytoplasm of cells, accumulating in membranous structures such as plasma membrane, endoplasmic reticulum and nuclear envelope (Jaruga *et al*., 1998).

In vitro experiments have shown that curcumin can inhibit NF-κB and several pro-inflammatory cytokines, including TNF-α, and also has the potential to shift macrophage polarization towards anti-inflammatory phenotype M2 (Gao *et al*., 2015; Land *et al*., 2003). Recently, Yin *et al*. (2018) demonstrated that curcumin can inhibit lipopolysaccharide (LPS)-induced pro–IL-1β expression and TNF-α production, and further suppress the inflammasome activation by strong inhibition of NOD-like receptor NLRP3 in mouse bone marrow-derived macrophages.

In addition, it is well-known that cancer patients undergoing chemo or radiotherapy are at higher risk of infection because of neutropenia and OM, with this risk being proportional to the severity of mucositis (Panghal *et al*., 2012). Loss of mucosal integrity followed by microbiological colonization by oral bacteria trigger the host-inflammatory cell responses via activation of NF-κB pathway, with increased release of pro-inflammatory cytokines (Al-Ansari *et al*., 2015). Curcumin has shown a strong antibacterial effect on oropharyngeal cells *in vitro*, which is associated to the inhibition of the release of many inflammatory mediators, such as TNF-α, IL-6, IL-8 (Luer, Troller, Aebi, 2012; Luer *et al*., 2011).

Santos Filho *et al*. (2018) showed that treatment a mucoadhesive formulation containing curcuminoids from *C. longa* and *Bidens Pilosa* L. extract, protected against 5-FU-induced cytotoxicity on an *in vitro* model based on epithelial cells. This effect was related to the decreased levels of pro-inflammatory cytokines TNF, IL-1β, IL-6 and IL-8. Our study corroborates the anti-inflammatory potential of curcumin, which was confirmed by the decreased levels of pro-inflammatory markers such as TNF-α, IL-1β in the buccal mucosa of hamsters after treatment with CLE, as well as the reduction in neutrophils infiltration in the microscopic analysis.

Our findings also draw attention to the effect of curcumin on both motor mobility and emotionality. The open field test presented as a good model of generalized anxiety, since it is sensitive to classical anxiolytics. Curcumin recovered neurochemical abnormalities and single prolonged stress and decreased 5-HT tissue levels in the hippocampus, amygdala, and striatum of rats, indicating anxiolytic-like effects on biochemical and behavioral symptoms associated with anxiety (Lee, Lee, 2018). Variables related to motor activity such as total distance traveled, ambulation and speed were reduced, while the ability to explore the environment by rearing were reduced, possibly indicating reduced anxiety in the animal.

There was a significant reduction in ambulatory movement in the 5FUT/MT 100mg/kg CLE (NO/OM group) and in the other CLE OM treated groups at all doses with oral mucositis at T1 compared to the 5FUT/MT group at T1. A significant increase in the 5FUT/MT 100mg/kg CLE (NO/OM group) and in the other CLE OM treated groups at all doses with oral mucositis at T1 was found for rearing compared to the 5FUT/MT group at T1, which indicates a possible reduction of anxiety/fear in the animal.

The results have shown an antioxidant and anti-inflammatory effect of curcumin on OM induced by 5-FU in hamsters. In addition, the CLE reduced the locomotory activity with a possible anxiolytic-like effect on the emotionality. These findings add to the perspective for the exploitation of curcumin in the developing therapeutic strategies for OM and other inflammatory conditions in oncology.

ACKNOWLEDGMENTS

This study was partially financed by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)* - Finance Code 001.

REFERENCES

Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol. 2009;41(1):40-59.

Ak T, Gulcin I. Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact. 2008;174(1):27-37.

Al-Ansari S, Zecha JA, Barasch A, de Lange J, Rozema FR, Raber-Durlacher JE. Oral Mucositis Induced By Anticancer Therapies. Curr Oral Health Rep. 2015;2(4):202-11.

Bastos CCC, Avila PHM, Filho E, Avila RI, Batista AC, Fonseca SG, et al. Use of *Bidens pilosa* L. (Asteraceae) and *Curcuma longa* L. (Zingiberaceae) to treat intestinal mucositis in mice: Toxico-pharmacological evaluations. Toxicol Rep. 2016;3:279-87.

Boussios S, Pentheroudakis G, Katsanos K, Pavlidis N. Systemic treatment-induced gastrointestinal toxicity: incidence, clinical presentation and management. Ann Gastroenterol. 2012;25(2):106-18.

Charantimath S. Use of Curcumin in Radiochemotherapy Induced Oral Mucositis Patients: A Control Trial Study. International Journal of Medical and Health Sciences. 2019;10(3):147-152.

De Souza GE, Ferreira SH. Blockade by antimacrophage serum of the migration of PMN neutrophils into the inflamed peritoneal cavity. Agents Actions. 1985;17(1):97-103.

Delavarian Z, Pakfetrat A, Ghazi A, Jaafari MR, Homaei Shandiz F, Dalirsani Z, et al. Oral administration of nanomicelle curcumin in the prevention of radiotherapy-induced mucositis in head and neck cancers. Spec Care Dentist. 2019;39(2):166-72.

Dos Santos Filho EX, Avila PHM, Bastos CCC, Batista AC, Naves LN, Marreto RN, et al. Curcuminoids from *Curcuma longa* L. reduced intestinal mucositis induced by 5-fluorouracil in mice: Bioadhesive, proliferative, anti-inflammatory and antioxidant effects. Toxicol Rep. 2016;3:55-62.

Dos Santos Filho EX, da Silva ACG, de Avila RI, Batista AC, Marreto RN, Lima EM, et al. Chemopreventive effects of FITOPROT against 5-fluorouracil-induced toxicity in HaCaT cells. Life Sci. 2018;193:300-8.

Funk JL, Oyarzo JN, Frye JB, Chen G, Lantz RC, Jolad SD, et al. Turmeric extracts containing curcuminoids prevent experimental rheumatoid arthritis. J Nat Prod. 2006;69(3):351-5.

Gao S, Zhou J, Liu N, Wang L, Gao Q, Wu Y, et al. Curcumin induces M2 macrophage polarization by secretion IL-4 and/or IL-13. J Mol Cell Cardiol. 2015;85:131-9.

Jagetia GC, Rajanikant GK. Role of curcumin, a naturally occurring phenolic compound of turmeric in accelerating the repair of excision wound, in mice whole-body exposed to various doses of gamma-radiation. J Surg Res. 2004;120(1):127-38.

Jaruga E, Salvioli S, Dobrucki J, Chrul S, Bandorowicz-Pikula J, Sikora E, et al. Apoptosis-like, reversible changes in plasma membrane asymmetry and permeability, and transient modifications in mitochondrial membrane potential induced by curcumin in rat thymocytes. FEBS Letters. 1998;433(3):287-93.

Kesarwala AH, Krishna MC, Mitchell JB. Oxidative stress in oral diseases. Oral Dis. 2016;22(1):9-18.

Kwiecien S, Magierowski M. Curcumin: A potent protectant against esophageal and gastric disorders. Int J Mol Sci. 2019;20(6):1477.

Kwon Y. Mechanism-based management for mucositis: option for treating side effects without compromising the efficacy of cancer therapy. OncoTargets Ther. 2016;9:2007-16.

Land Bvt, Blijlevens NMA, Marteijn J, Timal S, Donnelly JP, Witte TJMd, et al. Role of curcumin and the inhibition of NF- κ B in the onset of chemotherapy-induced mucosal barrier injury. Leukemia. 2003;18(2):276-84.

Leão JC, Gomes VB, Porter S. Ulcerative lesions of the mouth: an update for the general medical practitioner. Clinics (São Paulo). 2007;62(6):769-80.

Lee B, Lee H. Systemic administration of curcumin affect anxiety-related behaviors in a rat model of posttraumatic stress disorder via activation of serotonergic systems. Evid Based Complement Alternat Med. 2018;2018:9041309.

Lim YS, Kwon SK, Park JH, Cho CG, Park SW, Kim WK. Enhanced mucosal healing with curcumin in animal oral ulcer model. Laryngoscope. 2016;126(2):E68-73.

Luer S, Troller R, Aebi C. Antibacterial and antiinflammatory kinetics of curcumin as a potential antimucositis agent in cancer patients. Nutr Cancer. 2012;64(7):975-81.

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

Luer S, Troller R, Jetter M, Spaniol V, Aebi C. Topical curcumin can inhibit deleterious effects of upper respiratory tract bacteria on human oropharyngeal cells in vitro: potential role for patients with cancer therapy induced mucositis? Support Care Cancer. 2011;19(6):799-806.

Normando AGC, de Meneses AG, de Toledo IP, Borges GA, de Lima CL, Dos Reis PED, et al. Effects of turmeric and curcumin on oral mucositis: A systematic review. Phytother Res. 2019;33(5):1318-29.

Panchatcharam M, Miriyala S, Gayathri VS, Suguna L. Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Mol Cell Biochem. 2006;290(1-2):87-96.

Panghal M, Kaushal V, Kadayan S, Yadav JP. Incidence and risk factors for infection in oral cancer patients undergoing different treatments protocols. BMC Oral Health. 2012;12(1):1-12.

Patil K, Guledgud MV, Kulkarni PK, Keshari D, Tayal S. Use of curcumin mouthrinse in radio-chemotherapy induced oral mucositis patients: A pilot study. J Clin Diagn Res. 2015;9(8):59-62.

Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015;97:55-74.

Rao S, Dinkar C, Vaishnav LK, Rao P, Rai MP, Fayad R, et al. The Indian spice turmeric delays and mitigates radiation-induced oral mucositis in patients undergoing treatment for head and neck cancer: An investigational study. Integrative cancer therapies. 2014;13(3):201-10.

Reddy AC, Lokesh BR. Effect of dietary turmeric (*Curcuma longa*) on iron-induced lipid peroxidation in the rat liver. Food Chem Toxicol. 1994;32(3):279-83.

Schmidt TR, Curra M, Wagner VP, Martins MAT, Oliveira AC, Batista AC. Mucoadhesive formulation containing *Curcuma longa* L. reduces oral mucositis induced by 5-fluorouracil in hamsters. 2019,33(4):881-890.

Completar referencia

Shirane HY, Oda DY, Pinheiro TC, Cunha MRd. Implantes de biomateriais em falha óssea produzida na fíbula de ratos. Rev Bras Ortop. 2010;45(5):478-82.

Siviero A, Gallo E, Maggini V, Gori L, Mugelli A, Firenzuoli F, et al. Curcumin, a golden spice with a low bioavailability. J Herbal Med. 2015;5(2):57-70.

Sonis ST. New thoughts on the initiation of mucositis. Oral Diseases. 2010;16(7):597-600.

Sreejayan, Rao MN. Curcuminoids as potent inhibitors of lipid peroxidation. J Pharm Pharmacolo. 1994;46(12):1013-6.

Stanic Z. Curcumin, a compound from natural sources, a true scientific challenge - A review. Plant Foods Hum Nutr. 2017;72(1):1-12.

Tawfik SS, Abouelella AM, Shahein YE. Curcumin protection activities against gamma-rays-induced molecular and biochemical lesions. BMC Res Notes. 2013;6:375.

Willenbacher E, Khan SZ, Mujica SCA, Trapani D, Hussain S, Wolf D, et al. Curcumin: New insights into an ancient ingredient against cancer. Int J Mol Sci. 2019;20(8):1808.

Yin H, Guo Q, Li X, Tang T, Li C, Wang H, et al. Curcumin suppresses IL-1beta secretion and prevents inflammation through inhibition of the NLRP3 inflammasome. J Immunol. 2018;200(8):2835-46.

Received for publication on 09th March 2020
Accepted for publication on 05th October 2020