PATOLOGIA
MOLECULAR, HUMANA & EXPERIMENTAL

RENAN MONTEIRO DO NASCIMENTO
[ORGANIZADOR]

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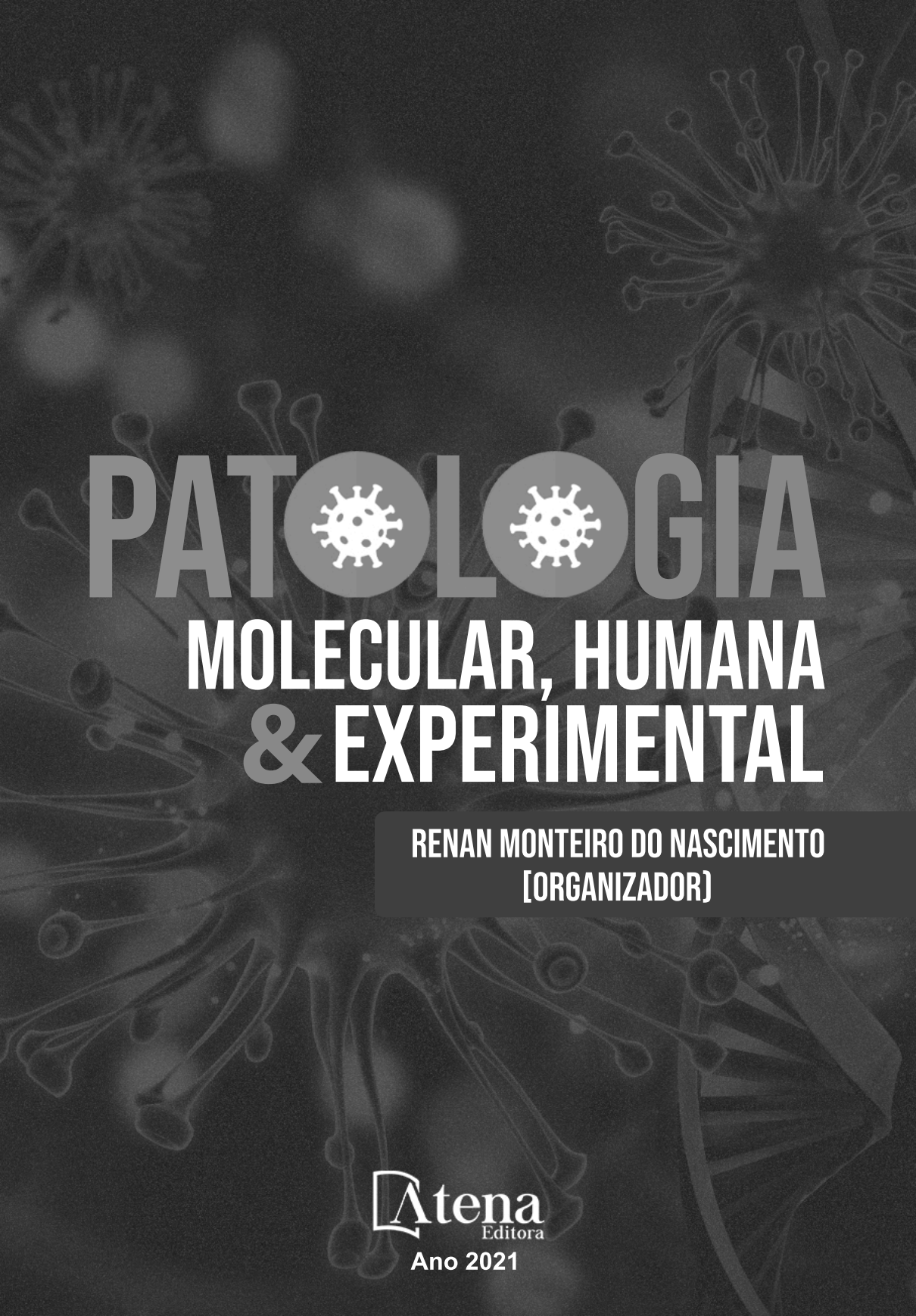
Ano 2021



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APRESENTAÇÃO

Patologia é um ramo da biologia e da medicina primariamente dedicado à análise e estudo de órgãos, tecidos e fluidos corporais, com a finalidade de fazer um diagnóstico das doenças. Nessa perspectiva, apresento a coleção “Patologia: Molecular, Humana e Experimental”, uma obra que apresenta 7 capítulos distribuídos em temáticas que abordam de forma categorizada e interdisciplinar trabalhos e pesquisas que envolvem estudos moleculares, experimentais e com aplicação a saúde humana.

Esse livro é direcionado a todos os acadêmicos, docentes e pesquisadores que desenvolvem estudos sobre as bases patológicas das doenças, respondendo perguntas biológicas com o auxílio de ferramentas da Biologia Celular e Molecular, Bioquímica, Histologia, Embriologia, Genética, Imunologia, Hematologia, Anatomia, Fisiologia, dentre outras áreas correlatas e também a todos aqueles leitores, que de alguma forma se interessam por estudos com aplicação às Ciências da Vida.

Neste contexto, este livro “Patologia: Molecular, Humana e Experimental”, apresenta uma teoria bem fundamentada nos resultados práticos obtidos por vários pesquisadores, professores e acadêmicos que arduamente desenvolveram seus estudos que aqui estão apresentados de maneira concisa e didática. Sabemos o quão importante é a divulgação científica, por isso evidenciamos também a estrutura da Atena Editora, que é capaz de oferecer uma plataforma consolidada e confiável, permitindo que esses pesquisadores exponham e divulguem seus trabalhos científicos.

Desejo a todos uma excelente leitura.

Renan Monteiro do Nascimento

SUMÁRIO

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CAPÍTULO 1

CYTOTOXICITY OF RED ONION EXTRACTS (*ALLIUM CEPA*) AND QUERCETIN FLAVONOID IN TUMOR HEp-2 CELL

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ABSTRACT: Red onion (*Allium cepa*) is a vegetable that is a major source of quercetin in the human diet, contributing around 30% of consumed flavonoids, which act as anticarcinogenic, antibacterial, antiviral, anti-allergic and antimutagenic, displaying also, activities on the cardiovascular system. Based on this evidence, this study aimed to evaluate the influence of red onion extracts and QCT (standard) on human laryngeal carcinoma cells (HEp-2). Different solvents were used for red onions extractions, (chloroform, 70% methanol and 70% ethanol) using a Soxhlet extractor. Samples were subjected to gas chromatography test (GC), evaluation of mitochondrial metabolic activity (MTT) and fluorescence microscopy (mitochondria and nucleus). According to the analysis carried out by the GC test, specific peaks that characterize the extracts as flavonoid quercetin can be observed. The results obtained from the MTT assay showed that at a concentration of 5µM extracts and QCT have no loss of mitochondrial activity, while at concentrations of 50µM and 100µM there was a significant reduction in mitochondrial activity. Furthermore, a decrease in mitochondrial membrane potential, morphological changes in the nucleus characteristic of early and late apoptosis, necrosis or completely dead cells were observed. Based on our results, the extracts at concentrations of 50µM and 100µM significantly induce HEp-2 cells cytotoxicity, decreasing cell viability by apoptosis and/or necrosis.

KEYWORDS: *Allium cepa*. Quercetin. HEp-2 Cell Culture.

CITOTOXICIDADE DE EXTRATOS DE CEBOLA ROXA (*ALLIUM CEPA*) E FLAVONÓIDE QUERCETINA EM CÉLULAS TUMORIAS HEp-2

RESUMO: A cebola roxa (*Allium cepa*) é um vegetal com maior fonte de quercetina na dieta humana, contribuindo com cerca de 30% dos flavonóides consumidos, que atuam como

anticarcinogênicos, antibacterianos, antivirais, antialérgicos e antimutagênicos, apresentando também, atividades sobre a sistema cardiovascular. Com base nessas evidências, este estudo teve como objetivo avaliar a influência dos extratos de cebola roxa e Quercetina (QCT - padrão) sobre as células do carcinoma da laringe humana (HEp-2). Diferentes solventes foram usados para as extrações (clorofórmio, methanol 70% e etanol 70%) usando extrator Soxhlet. As amostras foram submetidas a teste de cromatografia gasosa (GC), avaliação da atividade metabólica mitocondrial (MTT) e microscopia de fluorescência (mitocôndria e núcleo). De acordo com a análise realizada pelo teste GC, podem ser observados picos específicos que caracterizam os extratos como flavonóide quercetina. Os resultados obtidos no ensaio de MTT mostraram que na concentração de 5µm dos extratos obtidos e QCT não apresentam queda da atividade mitocondrial, enquanto nas concentrações de 50µM e 100µM houve redução significativa da atividade mitocondrial. Além disso, observou-se diminuição do potencial de membrana mitocondrial, alterações morfológicas do núcleo características de apoptose precoce e tardia, necrose ou células completamente mortas. Com base em nossos resultados, os extratos nas concentrações de 50µM e 100µM induzem significativamente a citotoxicidade das células HEp-2, diminuindo a viabilidade celular por apoptose e/ou necrose.
PALAVRAS - CHAVE: *Allium cepa*. Quercetina. Células HEp-2

1 I INTRODUCTION

Red onion (*Allium cepa*) is a vegetable that is a major source of quercetin in the human diet, contributing around 30% of consumed flavonoids (Hertog, Hollman and Katan, 1992). Quercetin (QCT) extraction from this plant involves separation techniques frequently used in analytical chemistry laboratories for isolating one or more of its components. Technical improvement for quercetin extraction from the red onion is critical because some details can compromise the reliability of the results. To achieve the goal of a well performed extraction with minimal experiments, full factorial design methodologies allow improvement by evaluating their effects and possible factor interactions in the desired responses (Morais *et al*., 2001; Garda-Buffon and Badiale-Furlong, 2008; apud Souza *et al*., 2009).

Flavonoids are substances resulting from secondary metabolites of plants, constituting the largest and most important group of polyphenolic compounds found widely in the Plantae kingdom (Nijveldt *et al*., 2001; Beecher, 2003). These are substances found in natural products, such as in medicinal herbs, apple, onion and wine being widely studied due to their antioxidant properties, which interact with free radicals protecting DNA from oxidative damage (Velioglu *et al*, 1998;. Calliste *et al*., 2003). Furthermore, they act as anticancer, anti-neoplastic, antibacterial, antiviral, antiallergenic, antimutagenic, exhibit affects on the cardiovascular system and cause an increase in capillary permeability and leukocyte migration (Pelzer *et al*., 1998).

Quercetin, 3,5,7,3',4'-pentahydroxyflavone, belongs to a subclass of flavonols, being the most consumed one among flavonoids (26mg to 1 g/day) (Sesso *et al*., 2003). Flavonoid quercetin is one of the most efficient antioxidants, since the greater the number OH groups,

the greater the antioxidant capacity (Cao, Sofic and Prior, 1997).

Several studies have shown that quercetin may be effective in treating cancer due to reduced cell viability in different tumor cell lines such as breast cancer (Duo *et al.*, 2012), prostate (Vijayababu *et al.*, 2005), lung (Robaszkiewicz, Balcerczyk and Bartosz, 2007) and liver (Granado-Serrano *et a*l, 2006), and presents benefits in platelet aggregation inhibition (Tzeng, Ko and Ko, 1991), in the detoxification of various enzymes (Uda *et al.*, 1997) and ability to induce apoptosis (Wen-Fu *et al.*, 2003).

Regarding toxicity, quercetin is known as tolerable and safe for humans and can be consumed orally at doses up to 1g/day or at a dosage of 756 mg/day intravenously (Harwood *et al.*, 2007). Concerning tissue distribution, studies observed quercetin present in the lungs, testicles, kidneys, thymus, heart, liver, being excreted via urine and by breathing, and a substantial part of the metabolites can be excreted by bile (Murota; Terao, 2003).

In addition to its antioxidant effects already reported, studies show that quercetin can act as a pro-oxidant in higher concentrations (Robaszkiewicz; Balcerczyk and Bartosz, 2007; Duo *et al,* 2012), which might be indicative of its hermetic properties, in which it is described as a dose-dependent response, with beneficial effects at lower doses and toxic at high doses (Vargas; Burd, 2010; Serra, 2011). Rodrigues *et al.* (2014) reported that the ability of quercetin to prevent and / or delay tumor progression may be due to the result of the modulation of various pathways related to cell growth and proliferation, which is highly desirable in optimizing anti-tumor therapy.

This study aimed to evaluate the influence of different concentrations (0, 5, 50 and 100 µM) of red onion extracts (*Allium cepa*), carried out with chloroform solvents, methanol 70% and ethanol 70%, and QCT standard in human larynx carcinoma cells (HEp-2).

2 I MATERIALS AND METHODS

2.1 Extraction processes and Rotavapor

To obtain red onion (*Allium cepa*) extracts, we used the Soxhlet extractor, in which the solid material (15g of onion bulb) wrapped in filter paper was added. For each extraction, 150 mL of different solvents (Chloroform, Ethanol 70% and Methanol 70%) were separately added to different flasks and heated by electric mantle. Each extraction process lasted 2h. After the extraction process, a rotavapor (Buchi R-114 model) was used at 60°C, for solvent evaporation. The extracts were diluted with DMSO (Dimethyl sulfoxide) (Sigma-Aldrich, Saint Louis, Canada) and kept in a freezer at -80 °C until analysis.

2.2 Gas Chromatography (GC) analysis

For the analysis performed with onion extracts, QCT and solvents (chloroform, 70% methanol and 70% ethanol), a GC system (PerkinElmer Precisely Clarus 600) was used under the following conditions: Elite column 1 fused silica capillary column consisting of

poly dimethyl siloxane. Hydrogen gas, nitrogen gas and synthetic air were used as carrier gas at a constant fluid rate of 1.5 ml/min, where the split ratio was 1:80, the injection volume of 1 µL, the reason being employed 1:10 division and injector temperature 300 °C, and the detector (ion source), at 280 °C. Samples were diluted in the respective solvents which were extracted and stocked in Eppendorf tubes, where the derivatizer was added N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) (Sigma-Aldrich, Saint Louis, Canada). The derivatisation step promotes a silanization reaction of the phenolic groups with trimethyl silicon, converting the phenolic compounds into volatile substances. After this process, samples were taken to the GC apparatus, at an initial temperature programmed at 60 °C (isothermal for 2 min) with an increase of 10 °C/min up to 150 °C (kept for 1 minute). Finally, it was increased 30 °C/min to attain a final isothermal temperature of 210 °C, which was maintained for 1 min.

2.3 Cell culture

The cell lineage HEp-2 (human laryngeal carcinoma) was acquired from the Bank of cells (BCRJ, Rio de Janeiro, BR) and cultured with DMEM (Dulbecco's Modified Eagle's Medium) (Thermo Fisher Scientific, Waltham, USA), where the formulation consists of a bicarbonate buffering system and modified concentrations of amino acids and vitamins necessary for the stimulation of cell growth. The medium was supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, USA), required for presenting growth factors in composition to accelerate cell growth, and 1% antibiotic- antimycotic (Thermo Fisher Scientific, Waltham, USA). Cells were grown in 25 cm² culture flasks (Corning, New York, USA), maintained in a incubator (Forma Scientific) with automatic controlled temperature (37 °C) and atmosphere (5% CO_2). Cell growth was accompanied by observation on an inverted microscope (Olympus CK40).

2.3.1 Growth, maintenance and preparation of cells for experimentation

When cells obtained 70-80% confluence, they were tripsinized. For this, cells were maintained with trypsin (Thermo Fisher Scientific, Waltham, USA) for 2 minutes. Then, cells were transferred to a 15 mL tube (Corning, New York, USA), 1mL of DMEM was added and centrifuged for 5 minutes, at 2500 rpm. The supernatant was discarded and the precipitate (cells) resuspended in DMEM. For the experiments, cells were transferred to 96-wells cell culture plates (TPP®, Trasadigen, Switzerland), at $5x10^4$ cells per well, being supplemented with DMEM (89%), FBS (10%) and antibiotic-antimycotic (1%), with a final volume of 200 µl per well. Once plated, cells remained incubated at 37°C and an atmosphere of 5% CO_2 for 24 hours for adhesion.

2.3.2 Concentrations of the extracts and QCT in Cells

For *in vitro* assays, extracts and standard QCT were first diluted in DMSO at a concentration of 1 mM, samples were stored under light protection at -80°C. These samples were diluted at 5µM, 50µM and 100µM. These final concentrations were selected for all *in vitro* tests carried out in this study.

2.3.3 Plating and addition of extracts and QCT

Cell suspensions were plated at the density of $5x10^4$cells per well in 96-well cell culture plates (TPP®, Trasadingen, Switzerland). The plates were incubated for 24 hours at 37 °C and 5% CO_2 for adhesion. 24 hours after plating, the different concentrations of the extracts and QCT were added to the wells. For the mitochondrial metabolic activity assay, MTT; and fluorescence microscopy, cells were incubated with extracts and QCT for 4 hours.

2.4 Metabolic mitochondrial activity assay – MTT

MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) (Sigma-Aldrich, Saint Louis, Canada) assay was performed 4 hours after cells treatment. After the incubation period, medium was removed and cells were incubated with MTT (5 mg/mL), for 1 hour, under light protection. After that, MTT was removed and organic solvent DMSO was added to the wells. The plate was stirred for 15 minutes to solubilize the formazan crystals. Plate reading was performed by a spectrophotometer (Spectracount, Packard, USA) at a wavelength of 570 nm.

2.5 Fluorescent labels - Mitochondria and nucleus

Cells were incubated for 4 hours with the extracts and QCT, the culture medium discarded and the wells, washed with phosphate buffered saline (PBS). For mitochondria label, Mito Tracker dye (Thermo Fisher Scientific, Waltham, USA) was added to the wells at a concentration of 79 nM, and incubated for 45 minutes under light protection. Then, the solution was discarded and cells were washed with PBS. For nucleus label, DAPI (4'6-diamidino-2-phenylindole, dihydrochloride) (Thermo Fisher Scientific, Waltham, USA) was added in the wells, diluted in PBS at a concentration of 300 nM, and incubated for 10 minutes at room temperature under light protection. The solution was discarded and cells were washed with PBS. All samples were plated in triplicate. Fluorescent microscopy was carried out by an inverted fluorescence microscope (Leica DMLB) with images captured via digital video camera (Leica DFC 300FX) and analyzed by a program (Leica Application Suite V3).

2.6 Fluorescent labeling - Apoptosis/Necrosis

After 4 hours of incubation, with the addition of different concentrations of extracts obtained with the chloroform solvent, culture medium was discarded and cells were washed

with PBS. In order to evaluate qualitative and quantitative apoptotis and necrosis, the kit Alexa Fluor 488 Annexin V-FITC and Propidium Iodide (PI) of InvitrogenTM (Sigma-Aldrich, Steinheim, Germany) were used for fluorescent labeling. Then, a solution containing PBS (94%), Annexin V-FITC (5%) and PI (1%) was added to the wells. After 15 minutes at room temperature, the solution was discarded and wells were washed with binding buffer solution and deionized water. Analyses were carried out with a fluorescence microscope Leica DMLB with images captured via digital video camera (Leica DFC 300FX) and analyzed by a software (Leica Application Suite V3). Samples were evaluated in triplicate and the process was performed under light.

2.7 Statistical analysis

MTT data was analyzed with the software Origin 9.0 SRO (Origin Lab Corporation). Statistical significance was accessed by ANOVA followed by Bonferroni test, considering $p < 0.05$ compared to the control group.

3 I RESULTS

3.1 Analysis of samples (extracts, QCT and solvents) by Gas Chromatography Test

According to the results obtained from the GC test, extracts made with solvents: chloroform, methanol (70%) and ethanol (70%) showed specific flavonoid quercetin peaks presented by the retention times: 6.9 min, 2.5 min and 2.6 min, respectively (Figure 1). Commercial quercetin and solvents used in the extraction were analyzed by default.

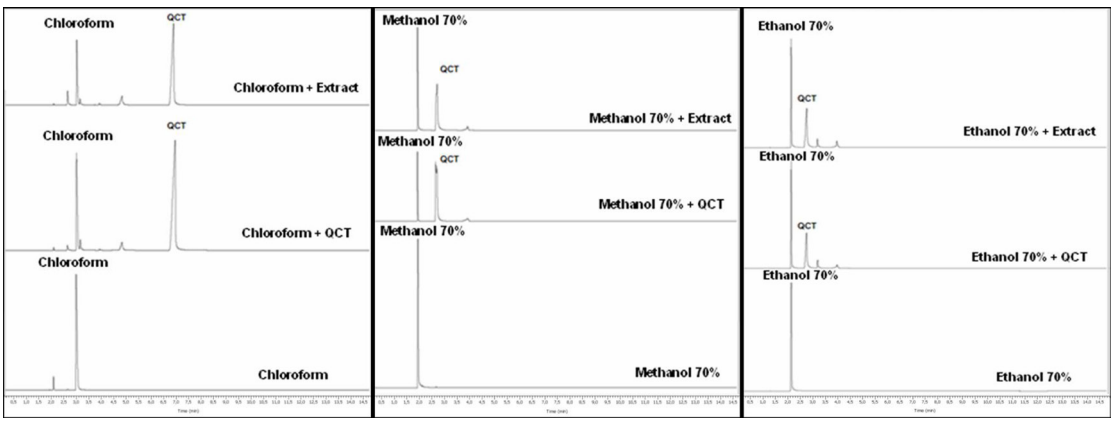

Figure 01. Gas Chromatography of extracts with different solvents. Specific flavonoid quercetin peaks were identified.

3.2 Action of extracts and QCT about HEp-2 cells

The use of extracts and QCT at 5µM showed no statistic significance compared to the control group. On the other hand, treatment with concentrations of 50µM and 100µM significantly decreased mitochondrial metabolic activity of the cells compared to the control group (Figure 2).

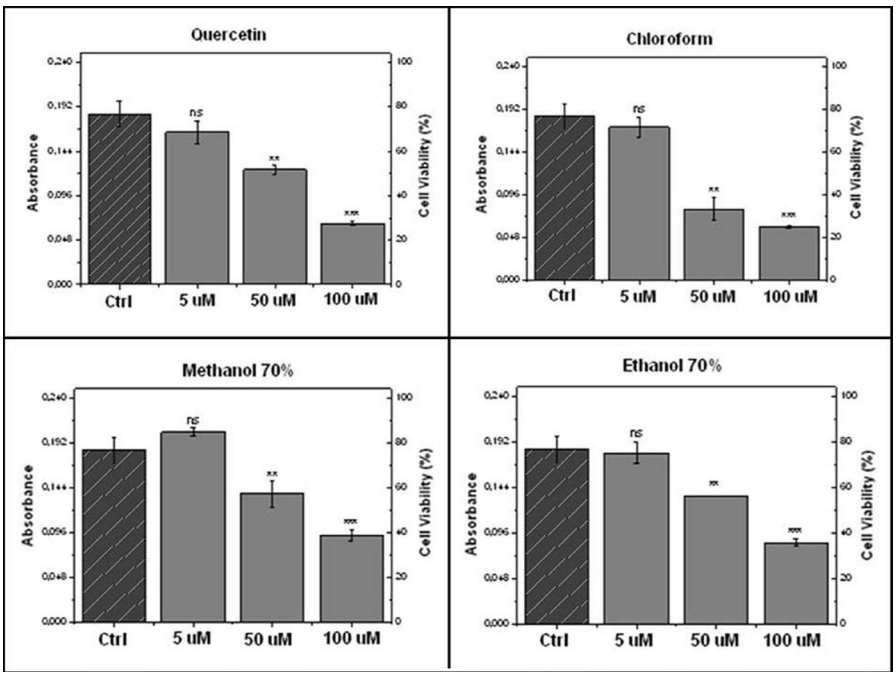

Figure 02. Metabolic mitochondrial activity assay – MTT of different extract on HEp-2 cells lineage. *p <0,05, **p <0,001 and *** p <0,0001 compared to control (ANOVA and Bonferroni test).

3.3 Extracts and QCT action in mitochondrial membrane potential and nucleus

Fluorescence microscopy test was performed to qualitatively assess the mitochondrial membrane potential and morphological changes of the nucleus. The results were evaluated 4 hours after treatment with the extracts and QCT in comparison with the untreated group (control). Cells present in the control group showed a homogeneous distribution of mitochondria throughout the cytoplasm, characterizing the potential normal mitochondrial membrane. For the cells treated with 5, 50 and 100µM of the different extracts and QCT, fluorescence indicated that the higher the concentration applied, the stronger the decrease of the pattern of mitochondrial metabolic activity, occurring also, morphological changes of the nuclei in the analyzed groups (Figures 3, 4, 5 and 6).

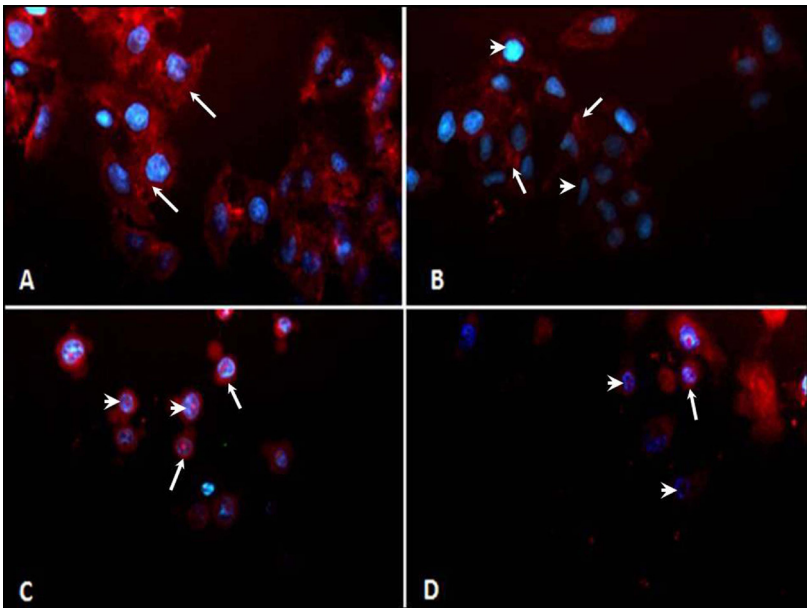

Figure 03 - Fluorescence microscopy. The image highlights the nuclei in blue (DAPI) and mitochondria in red (Mito Tracker) of HEp-2 cell lineage after treatment with Chloroform extract. A) Control group. B) Treatment with 5μM extract. C) Treatment with 50μM extract. D) Treatment with 100μM extract. Arrows indicate mitochondrial alterations and arrowhead nuclei alterations. Original magnification 1000x.

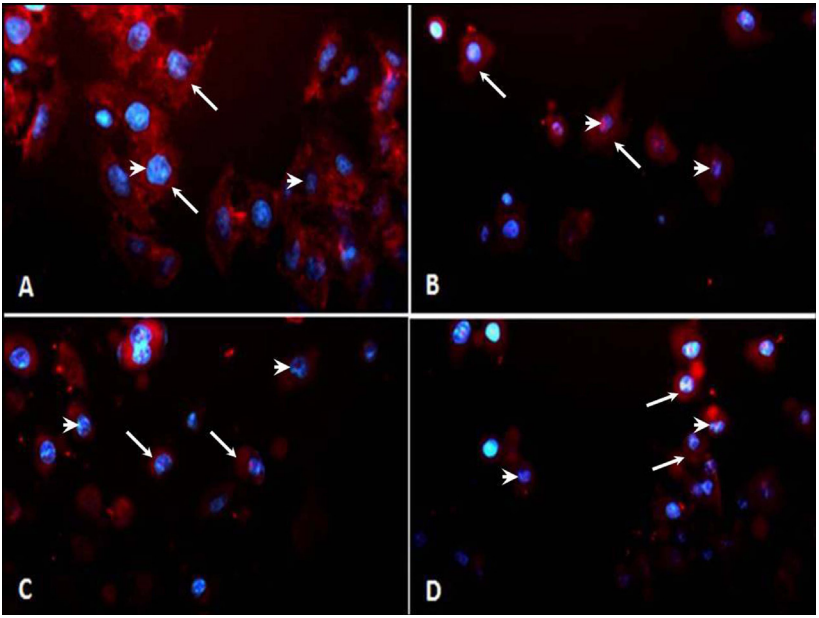

Figure 04 - Fluorescence microscopy. The image highlights the nuclei in blue (DAPI) and mitochondria in red (Mito Tracker) of HEp-2 lineage after treatment with Methanol extract. A) Control group. B) Treatment with 5μM extract. C) treatment with 50μM extract. D) Treatment with 100μM extract. Arrows indicate mitochondrial alterations and arrowhead nuclei alterations. Original magnification 1000x.

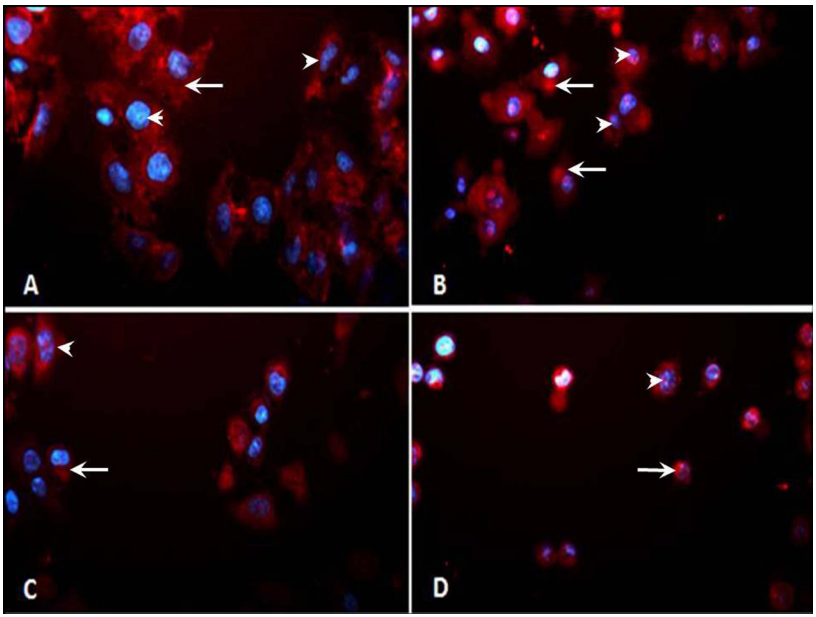

Figure 05 - Fluorescence microscopy. The image highlights the nuclei in blue (DAPI) and mitochondria in red (Mito Tracker) of HEp-2 lineage after treatment with Ethanol extract. A) Control group. B) Treatment with 5μM extract. C) Treatment with 50μM extract. D) Treatment with 100μM extract. Arrows indicate mitochondrial alterations and arrowhead nuclei alterations. Original magnification 1000x.

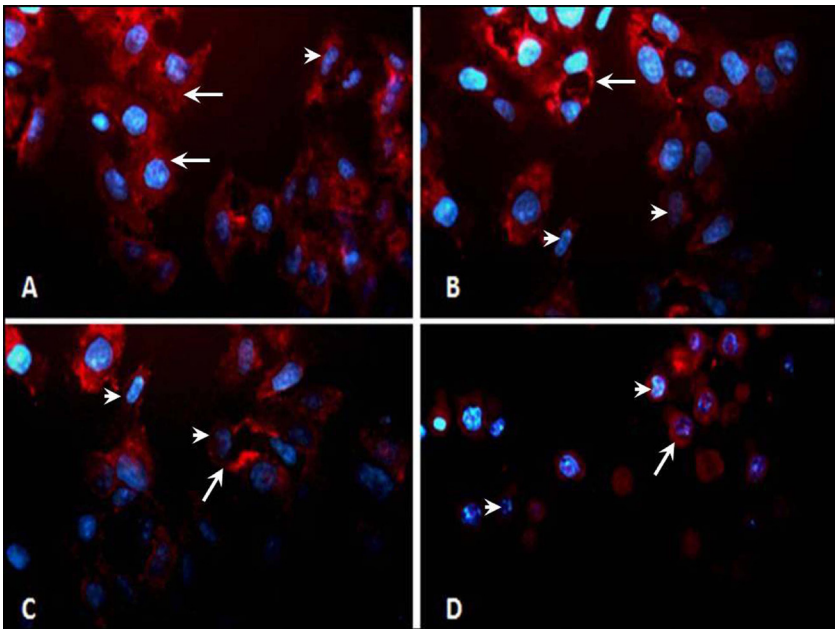

Figure 06 - Fluorescence microscopy. The image highlights the nuclei in blue (DAPI) and mitochondria in red (Mito Tracker) of HEp-2 lineage after treatment with QCT. A) Control group. B) Treatment with 5μM. C) Treatment with 50μM. D) Treatment with 100μM. Arrows indicate mitochondrial alterations and arrowhead nuclei alterations. Original magnification 1000x.

3.4 Extract (Chloroform) action in cell death process

After labeling, it was notable that, in comparison with the control group (Figure 7A and 7B), cells subjected to treatment with 5µM of the extract showed no higher expression in Annexin V-FITC and PI expression, as shown in Figure 7C and 7D. The group of cells subjected to the treatment with 50µM of the extract (chloroform) (Figure 7E and 7F) showed early apoptosis characteristics due to the intense positive binding Annexin V-FITC and late apoptosis, necrosis, or totally dead cells due to the positive tack IP. The cells treated with 100µM of the extract showed strong positive PI label, characterized in late apoptosis, necrosis or totally dead cells, as shown in Figure 7G and 7H.

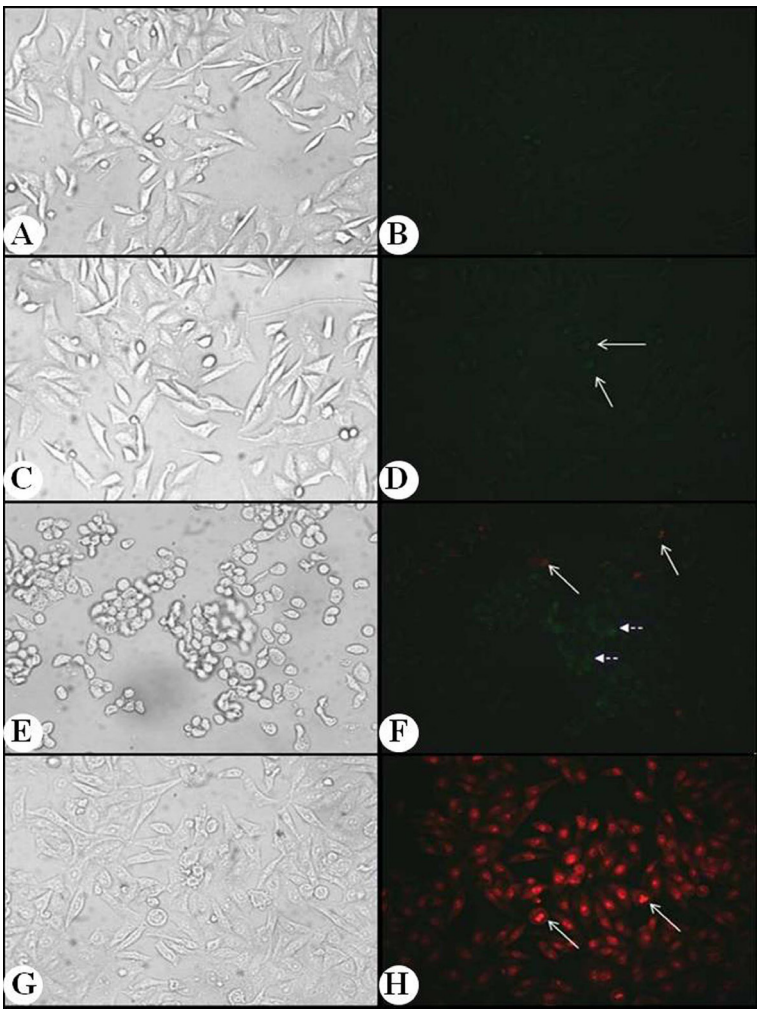

Figure 07 – Fluorescence microscopy - HEp-2 cell lineage after treatment with Chloroform extract in order to evaluate apoptotis (Annexin V) and necrosis (Propidium Iodide). Control group (A and B). Group treated with 5μM extract (C and D). Group treated with 50μM extract (E and F). Group treated with 100μM extract (G and H). Brightfield microscopy (A, C, E, G) and Fluorescence microscopy (B, D, F, H). Original magnification 1000x.

4 | DISCUSSION

Quercetin belongs to the subclass of flavonoids, being the most consumed one among flavonoids (Sesso *et al*., 2003). Its distribution in plants depends on several factors, according to the variation of each plant species. In many situations, the flavonoids present in the leaves may not be the same one found in other parts of the plant. The same compound can also have different concentrations, which will depend on which part of plant it is located (Zuanazzi and Mountain, 2003). Albuquerque (2013), assessed the efficiency of tea (*Camellia sinensis*) and infusion of onion skin (*Allium cepa*) and natural dye, analyzing the content of total flavonoids in QCT, showing that the higher QCT concentration is present in the infusion of onion skin with respect to the dye, which is consistent with the published studies designating the same as the main flavonoid of the constitution of the onion skin, responsible for its golden yellow color.

In our extracts obtained with chloroform, 70% methanol and 70% ethanol, the presence of QCT was observed by GC test, it was also observed a golden yellow color in all the extracts, which is a typical QCT characteristic.

In the present study, the GC test was performed with extracts obtained with the following solvents: chloroform, methanol (70%) and ethanol (70%), which showed specific flavonoid QCT peaks in 6.9 min retention time, 2.5 min and 2.6 min respectively. Dias (2010) held the High Performance Liquid Chromatography test (HPLC), in order to characterize a possible presence of gallic acid, QCT, resveratrol, catechin, and maldivina in Brazilian wine, which included a liquid-liquid extraction of the sample stage assisted by ultrasound for the pre-concentration of phenolic compounds in wines produced in *Vale do Sião*. After their analysis, it was observed that QCT had a retention time of 5.7 min.

In the present study, the effects of red onion extracts and QCT inferred to be toxic to human laryngeal carcinoma cells (HEp-2), confirming the study of Bianchi and Antunes (1999), which demonstrated that high and/or prolonged use of QCT can interfere with cellular mechanisms, causing damage. In addition to the QCT, the cyanogenic glycosides, rutin, ricin, alkaloids such as Coniine, alkaloids such as vinca vincristine and vinblastine, taxol and terpenoids such as sesquiterpene lactones are among the main plant metabolites that can cause damage to the organism (Mengue, Mentz and Schenkel, 2001).

Beutler *et al.* (1998) evaluated the cytotoxic activity of 79 flavones and their actions in the process of polymerization of cellular microtubules. This study showed that the compounds with the grouping 3-methoxy are inhibitors of tubulin polymerization. Sonoda *et al.* (2004) demonstrated that 17 flavonoids were tested in human leukemia cells (HL60) and 10 of them were cytotoxic.

MTT assay is one of the most used tasks to determine the cytotoxicity of materials of various types of cells in culture and could be also related to molecules which, when released from a stimulus, can cause tissue damage (Stained *et al*., 2008). This assay quantifies the

mitochondrial metabolic activity by means of analysis based on the formation of formazan crystals (artificial colorants) by the reduction of tetrazolium salt. This salt is highly soluble in water, being reduced by dehydrogenase activities in cells to obtain a formazan dye. The amount of dye is directly proportional to the number of living cells. According to our MTT results it was possible to observe after cell incubation with the extracts and QCT, a considerable decrease in mitochondrial activity, specially at 50μM and 100μM, after 4 hours of incubation.

In this study, fluorescent mycroscopy labels of mitochondria and nucleus were performed in order to verify the cytotoxic action of the extracts and QCT, generating morphological changes in these structures. The labeling showed that the higher the concentration of the extracts and QCT applied, the lower the standard mitochondrial activity, and some morphological changes in the nucleus also occurred. The effects involved with the mitochondria can be explained by the possible presence of alkaloids or large amounts of carbohydrates in addition to the presence of flavonoids. Studies indicate the presence of these substances but do not point to the presence of cytotoxic derivatives of these compounds (Lorenzi and Matos, 2002). In a recent study by Rodrigues *et al.* (2014), the fluorescence microscopy was used for measurement of mitochondrial membrane potential and morphological changes in the nucleus, being performed after 24h of photodynamic therapy (PDT), complemented with QCT. The mitochondria of the control group cells were distributed evenly throughout the cytoplasm, while cells treated with PDT and PDT supplemented with 5μM of QCT obtained heterogeneous labeling in the cytoplasm and perinuclear region. Already the groups of cells treated with PDT and supplemented with 50μM and 100μM QCT severely obtained a reduction in mitochondrial activity, accumulation of mitochondria in the perinuclear region, and probably cytoplasmic retraction, significant morphological changes not occurring in the nucleus in the groups analyzed.

FITC-Annexin V binds to cells early in apoptosis, which remains connected during the process of cell death thereby giving a green fluorescence. On the other hand, propidium iodide (PI) is used to assess the membrane integrity marking the necrotic cells, late apoptotic or completely dead, thereby providing red fluorescence (Martin *et al*., 1995). Rodrigues *et al.* (2014) examined the induction of apoptosis and / or necrosis in HEp-2 cells labeled with a combination of Annexin V-FITC - PI and verified by fluorescence microscopy 24 hours after PDT complemented by QCT. Their studies showed that, compared with the control group cells (untreated), cells undergoing TPD showed initial characteristics of apoptosis, being positively stained with Annexin V-FITC and negatively with PI. Apoptotic cells were also observed in the group treated with PDT with 5μM of QCT, showing also late apoptosis characteristics due to positive labeling with the IP. Groups of cells treated with PDT with 50μM and 100μM of QCT showed late apoptosis characteristics and/or necrosis due to positive staining of Annexin V-FITC and PI, concluding the efficiency of that treatment in those concentrations.

Our results, carried out with the extract obtained from the chloroform solvent, showed that HEp-2 cells, subjected to treatment with 5 µM of the extract, did not show an increase in the label with Annexin V-FITC and PI compared to the control group. On the other hand, cells subjected to treatment with 50µM of the extract showed early apoptosis characteristics due to the intense positive binding of Annexin V-FITC and late apoptosis, necrosis or totally dead cells due to the positive PI label. The group of cells treated with 100µM of the extract (chloroform) showed strong positive binding of PI, characterizing late apoptosis, necrosis or completely dead cells.

5 | CONCLUSIONS

After the analysis with different concentrations of red onion extracts and QCT on cell line HEp-2, the results indicated that there was flavonoid QCT in red onion extracts made with the solvents: chloroform, methanol (70%), ethanol (70%).

Red onion extracts and QCT at low concentration were not cytotoxic, whereas at concentrations equal to or higher than 50µM, they reduced mitochondrial metabolic activity in tumor cells HEp-2. The most significant result was obtained with the extract using chloroform solvent. Fluorescent microscopy demonstrated qualitatively severe reduction in the mitochondrial activity, also noting, morphological changes of the nucleus at higher doses. Apoptosis and/or necrosis are evident after treatment with high concentrations of extract (chloroform).

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