Evaluation of bergenin in amylase enzyme inhibition and lipid uptake in liver cells

Avaliação da bergenina na inibição da enzima amilase e captação de lipídeos em células hepáticas

Received: 2023-08-10 | Accepted: 2023-09-11 | Published: 2023-09-17

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ABSTRACT

Changes in the global economy contribute to a sedentary lifestyle and higher caloric intake, increasing the incidence of metabolic diseases. It is estimated that the number of individuals diagnosed with metabolic diseases will increase by 25% by 2030 and 51% by 2045. Natural products have been tested in metabolic diseases with positive results, as shown by bergenin. Thus, this work aimed to evaluate the possible effects of bergenin in models of metabolic disease through in silico and in vitro experimental procedures. Bergenin showed the ability to inhibit protein glycation by 19.88 ± 4.22% in the oxidative pathway and 8.32 ± 1.85% in the non-oxidative pathway. This study showed for the first time that bergenin has the potential to inhibit the α-amylase enzyme (33.89 ± 0.96%) using in silico and in vitro assays. Bergenin was not able to significantly inhibit lipid uptake by the Oil Red method. After these results, it is understood that bergenin has the potential to be better studied as a therapeutic option in metabolic diseases. However, additional in
vivo studies are needed to better elucidate the possible pharmacological effects of bergenin in metabolic diseases.

**Keywords**: Bergenin; Metabolic disease; Liver damage; Antioxidant

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**RESUMO**

As mudanças na economia global contribuem para um estilo de vida sedentário e maior ingestão calórica, aumentando a incidência de doenças metabólicas. Estima-se que o número de indivíduos diagnosticados com doenças metabólicas aumentará em 25% até 2030 e 51% até 2045. Produtos naturais têm sido testados em doenças metabólicas com resultados positivos, como mostra a bergenina. Assim, este trabalho teve como objetivo avaliar os possíveis efeitos da bergenina em modelos de doença metabólica por meio de procedimentos experimentais in silico e in vitro. A bergenina mostrou capacidade de inibir a glicação de proteínas em 19,88 ± 4,22% na via oxidativa e 8,32 ± 1,85% na via não oxidativa. Este estudo mostrou pela primeira vez que a bergenina tem o potencial de inibir a enzima α-amilase (33,89 ± 0,96%) usando ensaios in silico e in vitro. Bergenin não foi capaz de inibir significativamente a absorção de lipídios pelo método Oil Red. Após esses resultados, entende-se que a bergenina tem potencial para ser melhor estudada como opção terapêutica em doenças metabólicas. No entanto, estudos adicionais in vivo são necessários para melhor elucidar os possíveis efeitos farmacológicos da bergenina em doenças metabólicas.

**Palavras-chave**: Bergenina; Doença metabólica; Doença hepática; Atividade antioxidante

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**INTRODUCTION**

Non-communicable chronic metabolic diseases, such as diabetes mellitus, obesity, non-alcoholic fatty liver disease and hyperlipidemias, have become important public health problems worldwide (COSTELLO; SCHONES, 2018). They have a complex and multifactorial etiology that can include a sedentary lifestyle, unhealthy diets, reduced energy expenditure, intestinal problems and genetic susceptibility (FÄNDRIKS, 2017; BERTHOUD; NEUHUBER, 2019). According to the World Health Organization (WHO), cardiovascular diseases and diabetes, together, are responsible for approximately 19 million deaths annually (MARRERO; ADASHI, 2015).

Metabolic syndrome is diagnosed when a person meets at least three of the proposed variables. These variables include central obesity, increased triglycerides, low high-density lipoprotein cholesterol, increased blood pressure and increased fasting glucose (GOH et al., 2022). Its pathophysiology involves multiple intricate and still not fully elucidated mechanisms (LEE; PARK; CHOI, 2022). The pro-inflammatory state brought about by obesity and insulin resistance remains at the center of the pathogenesis of the metabolic syndrome (GOH et al., 2022; LEE; PARK; CHOI, 2022). Although insulin resistance is the most likely suspect for being the pathophysiological basis of the metabolic syndrome, there is considerable doubt as to its existence in every patient (GOLDEN et al., 2002).

The inhibition of lipolysis in the adipose tissue of individuals with insulin resistance results in the excessive flux of free fatty acids, which, in turn, alters the insulin signaling cascade in different organs (i.e., reducing glucose uptake in skeletal muscle and promoting glycogenesis
and lipogenesis in the liver), thus creating yet another vicious cycle for insulin resistance (FAHED et al., 2022). Failure to compensate for hyperinsulinemia leads to a decrease in insulin levels that is exacerbated by the lipotoxic effect of free fatty acids on pancreatic beta cells (SAKLAYEN, 2018). A chronic pro-inflammatory state resulting from altered cytokine production and activation of inflammatory signaling pathways, primarily induced by obesity or secondarily by insulin resistance, is also responsible for metabolic syndrome (DE OLIVEIRA DOS SANTOS et al., 2021). There are other suspects for the pathophysiological mechanism of the metabolic syndrome, such as inflammation, obesity and hyperglycemia, all of which, however, require research to prove them (XU et al., 2018). The treatment of the syndrome is no different to the treatment of each of its components (KEREM; LAWSON, 2021).

Medicines based on natural products have been used for thousands of years and are widely used in the treatment of metabolic diseases. Recently, natural products have again attracted the attention of consumers interested in their health benefits (CASTELO BRANCO et al., 2018; LÓPEZ et al., 2009). A myriad of studies have indicated the importance of these compounds and their pharmacotherapeutic potential, in addition to demarcating successful examples of identification and synthesis of new drugs of natural origin (JAYAKODY et al., 2018).

Among the numerous natural products studied for the prevention or treatment of metabolic syndromes, we highlight bergenin. In 1881, bergenin was isolated for the first time, however, its structure was confirmed only in 1958 and was classified as a hydrolyzable tannin, which is derived from dihydroisocoumarin and has three hydroxyl groups (OH) and two phenolic groups (HAY; HAYNES, 1958). It is a polyphenolic compound that is present in many medicinal plants such as Berenia crassifolia, Ficus racemosa, Mallotus japonicus, and M. philippinensis, among others (QIAO et al., 2019). Multiple bioactivities are attributed to it, including antioxidant, anti-inflammatory, hepatoprotective, anti-diabetic, antimicrobial, antiviral and anticancer properties (BAJRACHARYA, 2015; WANG et al., 2017). Theses kind of products has gained attention in the scientific community for its pharmacological properties, which provide the possibility of application in clinical research. Its phenolic compounds are described as having a significant effect on oxidative stress, lipid metabolism, insulin resistance and inflammation (FAN; PEDERSEN, 2021; SRIVASTAVA; APOVIAN, 2018).

Thus, based on the promising beneficial role of bergenin due to its properties mentioned above, and considering the pharmacological limitations for the treatment of metabolic diseases, bergenin has become a strong candidate to be incorporated into the therapy of these diseases due to its significant results in vitro and in vivo studies. Therefore, the present study proposes to investigate bergenin’s activities related to chronic diseases in a pre-clinical setting, using in silico and in vitro models of lipid uptake and enzymatic inhibition assays.
MATERIAL AND METHODS

Acquisition of bergenin and evaluation of the degree of purity

Bergenin of 98% purity was purchased from the CN Lab Nutrition Asia Group. The degree of purity of the molecule was evaluated by injecting a solution of the substance into a high-performance liquid chromatography system (Proeminence, Shimadzu, Japan). The method used was reverse phase chromatography using acetonitrile as mobile phase: water acidified with 0.1% formic acid (70:30) in an isocratic manner and detection at 272 nm.

Amylase Molecular docking protocols

The interaction affinity between bergenin and the alpha-amylase receptor was estimated using molecular docking. The first step involved optimizing the structure of bergenin using Gaussian 09 software with DFT theory, employing the B3LYP functional and 6-311G++ (2d, p) basis set. Subsequently, the structure was converted to pdbqt format using MGLTools 1.5.7 software, which also determined the rotational bonds. The alpha-amylase receptor structure (PDB ID: 4W93) (WILLIAMS et al., 2015) was obtained from X-ray diffraction and prepared using Schrödinger Maestro 2023-1 software. Hydrogen atoms were added, the structure was minimized, and water molecules along with the co-crystallized ligand Montbretin A were removed. In addition, Gasteiger partial charges were assigned to the receptor, and it was then converted to pdbqt format. Additionally, amino acids within 5.0 Å of the binding site were considered flexible. Docking was performed using the AutoDock Vina v1.2.5 algorithm (EBERHARDT et al., 2021), with an "exhaustiveness" parameter of 96. The grid box size and coordinates were based on the position of the crystallographic ligand, with center_x = -11.887, center_y = 3.826, center_z = -22.744, and their dimensions were size_x = 28.500 Å, size_y = 24.750 Å, size_z = 21.000 Å, which were automatically determined by AGFR software. The resulting complexes from docking were subjected to re-scoring using the KDeep platform (JIMÉNEZ et al., 2018), which utilizes deep learning algorithm to estimate the experimental binding free energy. Finally, the docking results were visualized using Discovery Studio 2021, enabling the generation of a chemical interaction diagram.

Antiglycation activity: oxidative pathway

The assay was performed according to (KIHO et al., 2004a), with modifications. The reaction was carried out in triplicate with concentrations of albumin (BSA) 8 mg/mL, glyoxal 30 mM, and bergenin 100 µM. The glyoxal and BSA solutions were diluted in phosphate buffer (200 mM, pH 7.4), containing 3 mM sodium azide as an antimicrobial agent. The 300 µL of the total reaction mixture were composed of BSA (135 µL), glyoxal (135 µL) and DMSO or bergenin (30 µL) and was incubated at 37 °C for 24 hours (in sterile conditions and in the dark). After
incubation, each sample was analyzed in the microplate reader using fluorescence intensity (emission λ330 nm and excitation λ420 nm). Quercetin (100 μM) was used as the standard and DMSO as the negative control. The results were expressed as percentage of inhibition, which was calculated as follows: (% inhibition = 100 - [ Fluor A/Fluor C] x100). Where: Fluor A = sample fluorescence; Light C = control fluorescence.

Non-oxidative pathway

Antiglyclicant activity was determined according to (KIHO et al., 2004b), with modifications. The steps used in the antiglycation activity via the oxidative pathway were followed, with the exception of glyoxal, which was replaced by fructose (100 mM) and the incubation time was increased to 120 hours.

Lipase inhibitory activity assay

Lipase inhibitory activity was determined according to (SLANC et al., 2008), with slight modifications. Porcine pancreatic lipase type II (SIGMA, code L3126-25G) was diluted in TRISMA-HCL 75 mM buffer pH 8.5 and, to prepare the substrate 4-nitrophenyl palmitate (NPP) (code N2752-50G), it was diluted first in acetonitrile, then in ethanol at a 1:4 ratio. The standard used was orlistat and the readings were done in a microplate reader (Beckman Coulter DTX 800 multimode detector) at 450 nm. Then, 30 µL of the samples, standard and/or control (DMSO) were added to the different wells of the microplate in triplicate, with 250 µL of the enzyme (0.8 mg/mL) and incubated for 5 minutes at 37 °C (protected from light). We added 20 µL of NPP (4 mg/mL), incubated it again for 10 minutes or until the control reading was 1.000±0.1. Then, calculations of the percentage of inhibition were performed, as well as the determination of the samples that gave an inhibition greater than 50% in order to make serial dilutions and obtain the IC₅₀.

Glucosidase inhibitory activity

The glucosidase inhibitory activity was determined according to (ANDRADE-CETTO; BECERRA-JIMÉNEZ; CÁRDENAS-VÁZQUEZ, 2008), with slight modifications. First, the enzyme was prepared at a concentration of 3 mg/mL using intestinal acetone extract from rat, under agitation during 5 minutes. This was centrifuged at 3,600 rpm for 10 minutes and the supernatant that contained the α-glucosidase enzyme was extracted. Both the substrate 4-nitrophenyl-D-glucopyranoside (4-NPGP) and the enzyme were diluted in 10 mM of phosphate buffer pH 6.9. Then, 30 µL of samples, standard and/or control (DMSO) were placed in different wells of the microplate, in triplicate. A sample of 170 µL of the extracted enzyme was incubated for 5 minutes at 37 °C (protected from light), 100 µL of 4-NPGP (5 mg/ml) was added and this was then incubated for 20 minutes or until the control reading reached 405 nm of 1,000± 0.1. The
percentage of inhibition was calculated using the following equation: \( \% \text{ inhibition} = 100 - \frac{\text{Abs } a / p}{\text{Abs } C} \times 100. \)

\( \alpha \)-Amylase inhibition

The \( \alpha \)-amylase enzyme test was performed according to (SUBRAMANIAN; ASMAWI; SADIKUN, 2008a), adapted for microplates. Initially, 30 \( \mu \text{L} \) of the sample, standard and/or control (DMSO) were added at a concentration of 10 mg/mL. Subsequently, the mixture was incubated for 5 minutes at 37 °C with 100 \( \mu \text{L} \) of enzyme (diluted in 10 mM phosphate buffer pH 6.9), then 170 \( \mu \text{L} \) of substrate (Amylase CNPG Liquiform) was added and the first reading was immediately performed with the microplate reader at 405nm. Afterwards, it was incubated at 37 °C for 20-40 minutes, until the final absorbance of the control reached 0.8-1.00 ± 0.1. The results are expressed as percentage of inhibition and the IC50 was calculated using the statistical program GraphPad Prism 6.0. The standard used was acarbose. The percentage of inhibition was calculated using the following equation: \( \% \text{ inhibition} = 100 - \frac{\text{Abs } a / p}{\text{Abs } C} \times 100. \)

Cell culture

Human hepatocellular carcinoma (HepG2) and human fibroblast (MRC-5) cell lines were purchased from the Rio de Janeiro Cell Bank (BCRJ). At the Cell Culture Laboratory of the Faculty of Pharmaceutical Sciences of the Federal University of Amazonas (FCF/UFAM), cells were cultured in high-glucose Dulbecco's Modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution, in an oven at 37 °C under an atmosphere of 5% CO2.

Cytotoxicity Assay:

Cells from the lineages HepG2 and human fibroblast (MRC5) were used. Cells were cultured at a concentration of 5 x 103 cells/well in 96-well plates (WOLFE; LIU, 2007). After 24 hours of incubation and adherence, the cells were treated with bergenin at concentrations of 3.12; 6.25; 12.5; 25; 50; 100 and 200 \( \mu \text{M} \). As a negative death control, the cytotoxicity of bergenin with the culture medium and the DMSO diluent was evaluated. After the treatment period of 24, 48 and 72 hours, 10 \( \mu \text{L} \) of 0.4% resazurin (diluted at 1:20) were added. After the standardized resazurin metabolism time, (2 hours), the fluorescence reading was performed. Viability was calculated according to the formula below, where \( \text{Ft} = (\text{cell fluorescence + medium + substance + resazurin}) \) and \( \Delta \text{Fb} = (\text{cell fluorescence + medium + resazurin}) \).

Uptake of fatty acids

This test was carried out according to the technique described by GAO et al., (2015), with modifications. HepG2 cells were grown in high-glucose DMEM at a concentration of 1x105
cells/well in 24-well plates in an oven at 37 °C under an atmosphere of 5% CO2. After 24 hours, steatosis was induced by adding 1 mM of oleic acid and bergenin at concentrations of 50, 25 and 12 µM. As a positive control, cells were treated with oleic acid only. In the negative control, the cells did not receive oleic acid, but DMSO, and resveratrol (50, 25 and 12 µM) was used as the standard substance to inhibit lipid uptake. Inhibition was measured using the OIL RED O method, which is a dye that stains the fat inside the cell red. After removing the culture medium, the cells were washed with PBS and, subsequently, 250 µL of 4% formaldehyde were added to each well, and the plate was then incubated for 45 minutes in an oven at 37 °C. Then, the formaldehyde was removed, the wells were washed in PBS. 250 µL of OIL RED O stain 0.5% (0.05 g/mL in 60% isopropyl alcohol) were added, and the plate was incubated for another 30 minutes. Subsequently, the dye was removed, the wells washed again in PBS and photographed, the images were obtained using a microscope (Zeiss). Afterwards, 250 µL of isopropyl alcohol were added to dilute the intracellular dye present. The plate was shaken for 10 minutes on a plate shaker and, finally, the absorbance was measured in a spectrophotometer at 510 nm.

Statistical analysis

Results were expressed as mean ± SD (standard deviation. Means were analyzed by ANOVA followed by multiple comparisons test (Dunnett’s test with a significance level of p < 0.05) using GraphPad Prism® software version 6.0.

RESULTS AND DISCUSSION

Bergenin purity analysis

With the aim of verifying the degree of purity of the bergenin, we carried out analyses using high-performance liquid chromatography (HPLC), shown in Figure 1. As only one chromatographic peak appeared (illustrated in graph B), we were able confirm what was informed by the manufacturer, i.e., the bergenin presents 98% purity. This degree of purity seems to be acceptable when we compare it other studies in the literature (ANDRADE-CETTO; BECERRA-JIMÉNEZ; CÁRDENAS-VÁZQUEZ, 2008; SUBRAMANIAN; ASMAWI; SADIKUN, 2008b).

Computational analysis of bergenin inhibiting alpha-amylase

The docking results revealed an affinity of -7.454 kcal/mol for bergenin interacting with the alpha-amylase receptor in its most favorable conformation (Figure 2). In contrast, the reference ligand, Montbretin A, exhibited a redocking affinity of -9.436 kcal/mol. This disparity may be attributed to the higher number of atoms in Montbretin A (a total of 150) compared to bergenin (a total of 39), allowing for an increased number of binding sites.
While the initial docking protocol assumed a rigid receptor, the introduction of partial flexibility altered the results. The affinity of bergenin slightly decreased to -6.564 kcal/mol, whereas the reference ligand significantly reduced to -6.183 kcal/mol. Re-scoring with the AI-powered KDeep platform confirmed bergenin's affinity at -6.23 kcal/mol, validating its proximity to the molecular docking results with the flexible receptor. Regarding its mechanism of action, the formation of four hydrogen bonds between bergenin's hydroxyl group and the amino acids Asp197 and Asp300, as well as a carbonyl group on the residue Gln63, was observed. Other interactions of lesser intensity, yet essential, include pi-sigma type binding with His305 and pi-pi stacking with Trp58 and Trp59. Consequently, there is substantial evidence supporting bergenin's potential to inhibit the alpha-amylase enzyme, even when compared to the flavonoid Montbretin A, a reference drug. Thus, based on the docking results, bergenin exhibits a possible future adjunctive treatment for Type 2 Diabetes.

Oxidative and non-oxidative antiglycation activity.

Bergenin was subjected to antiglycation analysis via oxidative and non-oxidative pathways, and the results are shown in Figure 3. Aminoguanidine was used as the standard drug for the antiglycation test of the non-oxidative pathway and quercetin as the standard drug for the oxidative pathway. Both standard drugs, as well as bergenin, were tested at a single concentration of 100 µM. As shown in Figure 3, in graph A, bergenin presents a percentage of inhibition of the oxidative pathway of 19.88 ± 4.22% and 8.32 ± 1.85% in the non-oxidative pathway (illustrated in graph B). The drugs used as an inhibition standard for both the oxidative (quercetin) and non-oxidative (aminoguanidine) pathways showed 55% (55.46 ± 2.35) and 42% (42.68 ± 3.78) inhibition, respectively.
Figure 2: Three-dimensional visualization of the bergenin or montbretin A-Amylase complexes resulting from molecular docking, the diagram of chemical interactions formed, and the molecular docking affinity values in kcal/mol.

Glycation is a very common irreversible process in diabetic patients, and it leads to the development of complications because it directly affects protein activity. The accumulation of protein glycation reaction products in living organisms induces structural and functional modifications of tissue proteins (KHALIFAH; BAYNES; HUDSON, 1999). Thus, antiglycants can contribute positively to the treatment of diabetic patients.

From the analysis of the graphs, it is understood that bergenin did not present antiglycation activity via the non-oxidative pathway (graph B) and that the effect against glyoxal could have been due to the antioxidant properties (graph A). This indicates that bergenin inhibited almost 20% (19.88 ± 4.22) of the oxidative pathway and quercetin (standard) inhibited 55% (55.46 ± 2.35).
Figure 3 – Inhibitory activity of bergenin. Graph (A) refers to the inhibitory activity of bergenin and quercetin (standard) of the oxidative pathway. Graph (B) represents the inhibitory effect of bergenin and aminoguanidine (standard) of the non-oxidative pathway. Values represent percentage of inhibition. Bergenin, as well as standard drugs, were tested at a concentration of 100 µM. Different letters indicate a statistically significant difference by the one-way ANOVA followed by Dunnet’s test, *p <0.05.

In a study developed by Vijaya Kumar et al., (2011), bergenin showed moderate antiglycation activity (IC50 = 186.73 µM); however, a series of bergenin derivatives were synthesized and tested at a concentration of 200 µM. All exhibited better antiglycation activity than bergenin when tested using the non-oxidative pathway (IC50 = 60.75 and 12.28 µM).

Corroborating the present study, (LEE; CHOI, 2017) observed that bergenin prevented glycation induced by methylglyoxal and provided protection against reactive carbonyl damage in osteoblastic cells. The results indicate that the antioxidant mechanisms, i.e., the oxidative pathway, contributed to its protective effect against the oxidative stress induced by methylglyoxal.

The glyoxalase-1 enzyme decreases the levels of AGEs caused by hyperglycemia and oxidative stress (BROUWERS et al., 2011). Thus, glyoxalase-1-mediated methylglyoxal clearance plays an important role in the pathogenesis of diabetic complications, and glyoxalase-1 may be a preventive and/or therapeutic target.

Inhibition of digestive enzymes lipase, α-amylase and α-glucosidase

The bergenin was subjected to analysis of lipase, α-amylase and α-glucosidase inhibition (Figure 4). Graphs A and B show the standard substance used for the lipase inhibition test, namely orlistat, and the concentration used for both the standard and bergenin was 50 µM. Graph C shows the standard substance used for the α-amylase inhibition test (1,000 µM acarbose) and 50 µM bergenin. Graph D shows the inhibitory activity of bergenin on the α glucosidase enzyme. The standard substance used was acarbose, at a concentration of 100 µM and 50 µM bergenin.
**Figure 4** – Evaluation of the inhibitory effect of bergenin on lipase at 50 μM, α-amylase 1,000 μM and α-glucosidase 100 μM. Graph (A) is for bergenin compared to the effects of the standard drug orlistat. Graph (B) represents the IC$_{50}$ of orlistat. Graph (C) is for bergenin compared to the standard drug acarbose. Graph (D) represents the effect of bergenin compared to acarbose (standard drug). Data are expressed as percentage of lipase, α-amylase and α-glucosidase inhibition with mean ± standard deviation (in relation to controls, the standard drugs) and analyzed using two-way ANOVA followed by Dunnett’s test. *p < 0.05.

In the lipase inhibition test (Figure 4, graph A), it was noted that bergenin did not show significant inhibitory activity on this enzyme (5.48±1.41). It was reported by Ivanov et al., (2011) that crude extracts of Bergenia rhizomes crassifolia can efficiently suppress human pancreatic lipase activity (IC$_{50}$ = 3.4 g/mL) in vitro.

Gustavo et al., (2017) reported that Endopleura extracts uchi has an inhibitory effect of pancreatic lipase. The authors also proposed that this effect is due to the phenolic content present in the extract. Several studies have indicated the relationship between the in vitro inhibition of pancreatic lipase and levels of phenolic derivatives and flavonoids. (MORENO et al., 2006) demonstrated that the polyphenol content present in the ethanolic extracts of Mangifera indica L had a strong relationship with the inhibition of pancreatic lipase activity, which was 75%.

Zhang et al., (2008) Taraxacum official extract, which has a high flavonoid content, inhibited pancreatic lipase by 86.3%. It has been reported in the literature that phenolic compounds and flavonoids inhibit both lipase activity and amylase activity (MENDOZA MEZA; MEDINA VALDÉS, 2015).
Observing graph C, it is understood that bergenin had a reasonable inhibitory effect on α-amylase when compared with the standard drug. The inhibitory activity of acarbose (standard) is 55.5% (55.03 ±1.06) against 33.9% (33.89 ±0.96) for bergenin. This study infers for the first time that bergenin has the potential to inhibit the α-amylase enzyme.

The inhibition of α-amylase decreases starch degradation and, thus, the breakdown of complex carbohydrates into absorbable simple sugars (TADERA et al., 2006). Naveen et al., (2014) showed the antihyperglycemic effect of Eucalyptus tereticornis bark, which can be attributed to the presence of several important phytochemicals, such as bergenin, which is present in large amounts in the extract of the bark.

A-Amylase and α-glucosidase are carbohydrate-hydrolyzing enzymes that break the glycosidic bond and release glucose, which increases the glucose concentration in the body and has a negative impact on diabetic patients. In a study on the crude extract of Bergenia pacumbis from Nepal, whose main bioactive constituent is bergenin, inhibitions of α-amylase and α-glucosidase enzymes comparable to those of the standard drug (acarbose) were observed.

In a study carried out by Silva; Teixeira, (2015), the inhibitory effect of the α-glucosidase enzyme was tested for the first time, for both Endopleura uchi hydroethanolic extract and bark infusion, with IC50 = 2.2 µg/mL and IC50 = 2.4 µg/mL for the hydroethanolic extract and bark infusion, respectively. On the other hand, these results obtained by the authors are quite inferior when compared with the positive control acarbose (IC50 = 284 µg/mL). It is worth noting that the main constituent of Endopleura uchi is bergenin.

These descriptions are also corroborated by other studies. Similar data were described by (KASHIMA et al., 2013), who, in a study based on bergenin derivatives, showed that bergenin (IC50 22.7 ± 0.6% at 300 µM) has a lower inhibitory activity of the α-glucosidase enzyme than its derivatives. The 50% inhibition (IC50) of enzymatic activity was calculated as being 24.6 µM; which is considerably lower than the concentrations of the reference drugs, acarbose (907.5 µM) and 1-deoxynojirimycin (278.0 µM). Similarly, San et al., (2020) did not observe a significant effect of bergenin against this enzyme.

Enzymes, such as α-amylase, α-glucosidase, sucrase and lactase, play a crucial role in the complete digestion of polysaccharides into smaller units such as monosaccharides. Monosaccharides diffuse to intestinal epithelial cells, where they cross the membrane by passive diffusion or by facilitated diffusion through transporters called Glut and by co-transport with other ions, mainly sodium ions (MUECKLER, 1994a).

Components that interfere with one of the above important processes can act as antihyperglycemic agents, thus preventing glucose from entering the bloodstream. Despite
searches of the literature, there are still no studies reporting the inhibitory effect of isolated bergenin against the α-amylase enzyme. Thus, this study may provide a basis for future investigations into the potential therapeutic role of bergenin in the treatment of metabolic diseases.

Effects of bergenin on HepG2 and MRC-5 cell viability

The measurement of cell viability is defined as the number of viable cells in a sample and is generally used to track substances by observing whether the molecules interfere with cell proliferation or exhibit direct cytotoxic effects (DENG et al., 2020). Thus, the non-toxicity of drugs is a precondition for their application (XIANG et al., 2020).

Initially, the bergenin compound was tested at concentrations of 3.12; 6.25; 12.5; 25; 50; 100 and 200 µM in MRC-5 and HepG2 cell lines, with the aim of evaluating the ability of this substance to interfere with cell viability or show a potential cytotoxic effect when using the resazurin method.

Figure 5 shows that, within 24 hours, the HepG2 cell line (graph A) treated with Bergenin exhibited a percentage of 77.5±4.36 of viable cells at a concentration of 200 µM. Within 48 hours, the percentage was 79.7 ±4.87 and within 72 hours, this was 68.2±6.43. The human fibroblast cell line (graph B), within 24 hours, showed 61.5±6.96%, within 48 hours showed 79.8±1.12% and, within 72 hours showed 71.1±1.01% cell viability.

In vitro cytotoxicity tests, in accordance with ISO 10993-5 of 2009, establish quantitative and qualitative parameters (DEVI; DUTTA, 2017). According to these parameters, when the
percentage of cell viability is above 80%, the test substance is not considered cytotoxic and, when this percentage is between 80 and 60%, it is classified as low cytotoxicity.

Given this analysis and the data obtained, it is possible to conclude that bergenin does not present a cytotoxic profile up to a concentration of 200 µM for MRC-5 and HepG2 cell lines. Liang et al., (2014) antiplasmodial effect of bergenin and the results obtained indicated that in vitro the compound effectively inhibited the growth of Plasmodium falciparum (IC50 = 14.1 µg/ml, with 100% inhibition at 50 µg/ml). Moreover, in the study above, there were no morphological changes in uninfected erythrocytes, even after exposure to 50 µg/ml of bergenin for 72 hours at effective concentrations for inhibiting parasite growth. Furthermore, no apparent cytotoxic effect was observed on mammalian HeLa and HepG2 cells at concentrations of (1-5 mg/ml) for 72 hours. The IC50 of bergenin was 12.5 mg/ml in HeLa cells and 19.1 mg/ml in HepG2 cells, which can be defined as non-toxic (IC50 > 30 µg/ml).

The results obtained by Pavan Kumar et al., (2019) demonstrated that most of the synthetic derivatives of bergenin presented greater cytotoxicity than bergenin in the cell lines HepG2, HeLa and lung adenocarcinoma (A549), and some derivatives even presented an IC50 = 1.86 µM and 1.33 µM, which are comparable to the standard drug doxorubicin (IC50 = 1.98 µM and 1.34 µM) in A549 and HeLa cells, respectively; while bergenin, for strains A549, HepG2 and HeLa, presented an IC50 of 4.29±1.59; 60.91±3.96; 22±2.11 µM, respectively.

As described by Newell et al., (2010), bergenin may have cytotoxic potential. The authors identified a toxic effect in tumor lineage. This corroborating with Shi et al., (2019), since their results demonstrated that bergenin exhibits considerable cytotoxic activity with an IC50 of 15 µM against HeLa cancer cells compared to (IC50 of 75 µM) when exposed to normal cervical cells (HCerEpiC). In other words, there is less toxicity for normal cells and, therefore, it represents an interesting candidate for use as a chemotherapeutic agent. As such, the literature portrays bergenin as having a profile of anticancer biological activity (CHEN et al., 2008; LIANG et al., 2014b; PAVAN KUMAR et al., 2019b).

Inhibition of lipid uptake

In order to investigate the hypolipidemic effect of bergenin, a lipid uptake test was performed in HepG2 cells (Figure 6). Bergenin, as well as the standard inhibition drug resveratrol, were tested at concentrations of 12.5; 25 and 50 µM. Oleic acid was tested at 1 mM. Observing graph A, in Figure 6, it is understood that there was no significant inhibition of lipid uptake. The same can be seen in graph G, since the standard substance (resveratrol) did not show the expected effect.
Figure 6 – Effects of bergenin on lipid uptake in cells HepG2. Both bergenin and the standard substance (resveratrol), were tested at concentrations of 12.5, 25 e 50 μM, in the time of 24 hours. Oleic acid was tested at the concentration of 1mM. The graphic (A) refers to treatment with bergenin and oleic acid. The image (B) refers to control (-) for lipid uptake, with culture medium DMEM and DMSO. The image (C) represents control (+) of lipid uptake, where it contains oleic acid. The images (D), (E) e (F) represent treatment with oleic acid and bergenin. The graphic (G) refers to treatment with resveratrol. The image (H) refers to control (-) of capture. The image (I) represents control (+) of lipid uptake, containing oleic acid. The images (J), (K) e (L) represent treatment with oleic acid and resveratrol. Data are expressed in percentage of lipid uptake with mean ± standard deviation (in relation to control (+) oleic acid and analyzed by two-way ANOVA, followed by the test of Dunnett. *p < 0.05.

Excess fat leads to hyperlipidemia and other important metabolic disorders related to obesity. Hyperlipidemia is characterized by increased levels of lipoprotein or cholesterol in the blood, and abnormalities in circulating plasma lipoproteins are responsible for increased deposits of triglycerides in the liver (AMBIKA; SARAVANAN, 2016a). Under appropriate conditions, insulin activates the lipase enzyme that hydrolyzes triglycerides. However, insulin deficiency results in failure to activate enzymes, thus resulting in hypertriglyceridemia (MEDRIKOVA et al., 2012a).
In Figure 6, graph G shows that the standard substance (resveratrol) used in this study did not show inhibition of lipid uptake at any of the tested concentrations. There is no difference between the highest and lowest concentration. Studies indicate that bergenin can significantly reduce lipid levels (MEDRIKOVA et al., 2012b; MUECKLER, 1994b; SILVA; TEIXEIRA, 2015b). However, there are reports that this effect may occur at a lower level in terms of total cholesterol and triglycerides levels (AMBIKA; SARAVANAN, 2016b). In the present study, it was observed that bergenin showed low inhibition of lipid uptake (26.71 ± 4.6) at 50 µM. Although bergenin has a hypolipidemic characteristic, it may be that this effect does not occur through inhibition of hepatic cholesterol uptake, but through another mechanism different from the one evaluated in this study.

Ambika; Saravanan, (2016a) report that bergenin reduced liver lipid levels. (SHIKOV et al., 2012), using Bergenia crassifolia extract, whose main substance is bergenin, observed a reasonable reduction of 45% in the level of triglycerides. On the other hand, in the work of Kumar et al., (2012), it is reported that bergenin reduced the lipid profile of diabetic rats, though not total cholesterol and triglycerides.

Likewise, it was shown by Jahromi; Chansouria; Ray, (1992) that, via administration of bergenin for 14 consecutive days, it was possible to observe a significant reduction in the levels of total lipids. However, the same did not happen with the levels of total cholesterol and triglycerides in the group treated with bergenin when compared to the hyperlipidemic control group.

Numerous structural groups have been linked to the reduction of lipids and cholesterol levels. Simple acetylenic fatty acids alphapyrones, furanochromones, flavonoids and also steroids are examples of this group of bioactive agents. Like the structural characteristics, the mechanisms by which these molecules trigger their activity are also abundant (SRIKANTHAN et al., 2016).

Although the structure of bergenin does not strictly fit a pattern of hypolipidemic and antihypercholesterolemic agents, it has many characteristics of these groups. The role of the different functional groups present in bergenin can be better understood once its mechanism of action is known, which is outside the scope of the present work.

The pathogenesis of metabolic disease and its associated components are still not fully understood; however, central obesity and insulin resistance are recognized as causal factors. It is a clinical condition that has a great impact on the life of individuals and society (ESLAM, 2020; SRIKANTHAN et al., 2016).

Thus, the search for new therapeutic modalities is challenging when one considers the complexity of metabolic diseases. This work confirmed what already exists in the literature.
regarding bergenin and envisions a new possibility for future studies with bergenin, though now
with the expectation of a potential inhibitory effect on the α-amylase enzyme.

The present research provides a basis for the possibility of potentiating this effect through
bergenin derivatives. However, further investigation is needed to provide evidence for the
implications of bergenin as a candidate for a new compound in the management of metabolic
diseases.

ACKNOWLEDGMENTS

The authors would like to thank Coordination for the Improvement of Higher Education
Personnel – CAPES, Research Support Foundation of the State of Amazonas (FAPEAM) and
National Council for Scientific and Technological Development (CNPq) for financial support and
fellowships. We are also grateful to the staff at the Analytical Center at UFAM for making their
infrastructure available.

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