**In silico pharmacokinetic and toxicological study of Flavone analogues**

*Estudo farmacocinético e toxicológico in silico de análogos de Flavonas*

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**ABSTRACT**
Flavone analogs are natural compounds of the flavonoid class that have a wide range of biological activities. The present study aimed to predict, with the aid of in silico methodologies, the oral bioavailability and pharmacokinetic and toxicological analyzes for three flavone analogues (apigenin, chrysin and luteolin). The study revealed that the analogues have good oral availability, favorable pharmacokinetic and toxicological parameters. The Virtual Screening performed to predict oral bioavailability revealed that all analogues did not violate Lipinski's Rule. The in silico pharmacokinetic study revealed that all analogues have high intestinal absorption, do not cross the blood-brain barrier, are permeable by Caco-2 cells and do not inhibit P-glycoprotein. The in silico ADME study showed that all analogues inhibit the enzymes of the cytochrome P450 complex (CYP4501A2, CYP4502C9, CYP4502C19, CYP4503A4) and not only the CYP4502D6 enzyme. The in silico Toxicology study indicated that the analogues do not show toxicity by the AMES Test and are not carcinogenic. Apigenin and chrysin have low toxicity, while luteolin has moderate toxicity.
Keywords: natural products, Flavonoids, Flavones, chemoinformatics, in silico ADME, in silico-Toxicology.

RESUMO
Os análogos de flavonas são compostos naturais da classe dos flavonoides que apresentam ampla gama de atividades biológicas. O presente estudo objetivou predizer com auxílio de metodologias in silico a biodisponibilidade oral e análises farmacocinéticas e toxicológicas para três análogos de flavonas (apigenina, crisina e luteolina). O estudo revelou que os análogos apresentam boa disponibilidade oral, parâmetros farmacocinéticos e toxicológicos favoráveis. O Screening Virtual realizado para predição da biodisponibilidade oral revelou que todos os análogos não violaram a Regra de Lipinski. O estudo farmacocinético in silico revelou que todos os análogos possuem elevada absorção intestinal, não atravessam a barreira hematoencefálica, são permeáveis pelas células Caco-2 e não inibem a glicoproteína P. O estudo ADME in silico mostrou que todos os análogos inibem as enzimas do complexo citocromo P450 (CYP4501A2, CYP4502C9, CYP4502C19, CYP4503A4) e que apenas a enzima CYP4502D6 não sofre inibição. O estudo Toxicológico in silico indicou que os análogos não apresentam toxicidade pelo Teste de AMES e não são carcinogênicos. A apigenina e a crisina apresentam baixa toxicidade, enquanto a luteolina possui toxicidade moderada.

Palavras-chave: produtos naturais, Flavonoides, Flavonas, quimioinformática, ADME in silico, toxicologia in silico.

1 INTRODUCTION
Since the beginning of time, natural products have been used as a source of medicines for humans and animals. As an example, there is the infusion of parts of the plants used in the treatment of pathologies. (SHAOHAIB et al., 2011). Natural products are chemical derivatives produced by microorganisms, plants, marine organisms and animals that serve as: protective barriers, host defense against microbial infections, ecological niche protection, coenzymes and cofactors, pigments, cell signaling, gene expression and organisms homeostasis (JOHN, 2010).

Countries with industrial resources are increasingly investing in the therapeutic potential coming from plants. Between 1981 and 2002, new drugs were developed from natural products, with about 60% being used to fight cancer and 75% being used against various infectious diseases. Numerous reports in the literature reveal the development of new drugs coming from natural products in the therapy of other pathologies, such as diabetes Mellitus, dyslipidemia, bacterial infections, fungal and Alzheimer's disease (COWAN, 1999), (FLAMBÓ, 2015).

Flavonoids are natural pigments present in most plants with the following primary functions: protection against oxidizing agents, participation in plant growth, development
and defense against pathogen attacks (FONSECA, 2016). Generally, the flavonoids found in the leaves differ from those present in flowers, twigs, roots, and fruits. The same compound (analog) can still be found in different concentrations depending on the plant organ (SIMÕES et al., 2007). It is important to mention that natural abiotic factors such as UV rays, periods of drought or rain, nutrients and seasons influence considerably the metabolism and biosynthesis of derivatives (CATHERINE and PACKER, 2003) and (MACHADO et al., 2008). Currently, 8,000 more flavonoids are found in nature, among which can be mentioned the subcategory classified as flavone (SIMÕES et al., 2007). Flavone derivatives act as natural pesticides in plants providing protection against insects, fungal diseases and plant signaling (HARBONE and WILLINAMS, 2000).

Apigenin is a flavone often found in common vegetables, fruits, and herbs such as thyme, celery, onion, pepper, orange, chamomile, and parsley. There are reports that apigenin exhibits the following biological activities: anti-inflammatory, anti-neoplastic, antiviral, purgative and ability to remove free radicals (SHUKLA and GUPTA, 2010) and (ZHOU, et al., 2017). Chrysin is a flavone analog possessing biological antioxidant, anti-inflammatory, anti-hyperlipidaemic and anti-tumor activity (BAE et al., 2011), (PUSHPAVALLI et al., 2010), (ZARZECKI et al., 2014) and (LORENZONI, 2015). Luteoline, on the other hand, is a flavone derivative found abundantly in celery, green pepper, parsley, pear leaf and chamomile tea, which plays a relevant role in the human organism as an antioxidant agent, in the prevention of inflammatory processes, acts on the metabolism of carbohydrates, exercises function as a modulator of the immune system and in the prevention of cancer (SHIMOI et al., 1998).

The development of new herbal drugs is now considered to be a promising strategy because of the many proven therapeutic purposes published in the literature. However, such a process requires high cost and highly technological laboratories, as the compounds need to be tested in biological cultures (in vitro) and in animals (in vivo) to check the efficacy and safety of the drug (GONÇALVES, 2020), (SOUZAA-et al., 2021) and (RODRIGUES, 2016).

Faced with such a context, the methodologies in silico for predicting pharmacokinetic and toxicological parameters based on the chemical structure of bioactive compounds have emerged (FIALHO, 2017). Through Chemoinformatics, this work determined molecular descriptors with the help of the online databases of the international platforms: Molinspiration Cheminformatics® (https://www.molinspiration.com) (GROB, 1986) and admetSAR® (https://www.admet-sar.com).
The objective of this study was to analyze oral bioavailability and predict pharmacokinetic and toxicological parameters in silico for three flavone analogs: apigenin, chrysin and luteoline. Figure 1 and Table 1 indicate the two-dimensional (2D) chemical structure of the flavonoid prototype and the subcategory of flavone analogs.

Figure 1: Two-dimensional (2D) chemical structure of the flavonoid nucleus and its flavone analogs.

Table 1: Fundamental nucleus of flavonoids - flavones (R=H) and flavonols (R=OH) and their respective flavone analogs under study.

<table>
<thead>
<tr>
<th>Flavones</th>
<th>Replacers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>5,7,4'-tri-OH</td>
</tr>
<tr>
<td>Chrysin</td>
<td>5,7-di-OH</td>
</tr>
<tr>
<td>Luteoline</td>
<td>5,7,3',4'-teta-OH</td>
</tr>
</tbody>
</table>

Source: SIMÕES et al., 2007.

2 MATERIAL AND METHODS

For the performance of this research, we used computational programs and online databases of platforms to determine molecular properties of the chemical structures of three (03) flavone analogs: apigenin, chrysin and luteoline.

2.1 MOLECULAR MODELING

Initially, the chemical structures of the molecules of the flavone analogs were drawn two-dimensional (2D), visualized three-dimensional (3D) with the aid of the program ACD/ChemSketch® Freeware version 2021 (Advanced Chemistry Development, Inc., 2021) and the energies (E1) of each chemical structure of the analogs.
were tabulated. Subsequently, Energy Minimization for analogs was performed using the semi-empirical Quantum Method PM3 (Parametric Method 3) with the aid of the Arguslab® Freeware program version 4.0 (Thompson and Planaria software LLC, Inc., 1997) and the steric energies (E2) of the chemical structures were tabulated. To obtain the local minima of the chemical structures of derivatives, geometric optimization was performed using Density Functional Theory (DFT), using the hybrid BLYP method (Becke, Lee, Yang and Parr) using the base 6-31G (d,p) function simultaneously with the Simplex Method to simulate the chemical structure of analogs in aqueous, dielectric constant (ε = 78.4), with the aid of Molecular Modeling Pro Plus® (MMP Plus®) program version 8.0 (ChemSW, Inc., 2017). Finally, all optimized chemical structures were saved in MDL-type molfiles (.mol) and the steric energies (E3) of all chemical structures were tabulated.

2.2 PHARMACOKINETIC STUDY IN SILICO HUMAN FOR ORAL BIOAVAILABILITY

After carrying out Molecular Modeling, the code SMILES (Simplified Molecular Input Line Entry Specification) was obtained and exported to the database platforms. Through Chemoinformatics, a human in silico pharmacokinetic study was conducted to evaluate the oral bioavailability profile of flavone-derived analogs and consisted of obtaining molecular descriptors through the Molinspiration Cheminformatics® database (https://www.molinspiration.com) (GROB, 1986). The database predicts molecular properties to evaluate the oral bioavailability of flavone analog molecules based on Lipinski's Rule, also known as the Rule of Five.

2.3 PHARMACOKINETIC STUDY IN HUMAN SILICO (ADME IN SILICO)

Initially, the ADME in silico study (ADME) was conducted to predict the following molecular descriptors: Human Intestinal Absorption (HIA), Hematoencephalic Barrier Permeability (BBB), Inhibition of P-glycoprotein, Permeability by Caco-2 epithelial cells, and Cell Distribution of the human intestinal absorption (HIA in the human body). Subsequently, the ADME in silico study was performed to predict inhibition and interaction with hepatic cytochrome P450 complex enzymes (CYP450) by flavone analogs. The ADME in silico study was conducted with the assistance of the Chinese online platform admetSAR® (http://lmmd.ecust.edu.cn/admetsar2/), coordinated by Professor Dr. Yun Tang, Leader of the Molecular Modeling and Design Laboratory.
2.4 TOXICOLOGICAL STUDY IN HUMAN SILICO

The human in silico toxicology study for flavone analogs was conducted to predict toxicity by the AMES Test (T: toxic; NT: non-toxic), carcinogenicity (C: carcinogenic; NC: non-carcinogenic) and the Acute Oral Toxicity of analogs to classify them into categories. The study was also carried out with the help of the Chinese admetSAR® platform (http://lmmd.ecust.edu.cn/admetsar2/) (YANG, et al., 2018).

3 RESULTS AND DISCUSSION

3.1 MOLECULAR MODELING

Molecular stability and biological activity of the ligand (analog) is closely correlated with intermolecular interactions between the ligand (analog molecule) and the biomacromolecule (biological receptor = biological target). This study is known as SBDD (Structure Based Drug Design), in which the free energy of the intermolecular ligand-receptor interactions in the active gap (active binding sulcus) of the biological receptor is determined by physicochemical parameters (MORRIS and LIM-WILBY, 2008). Energy minimization and optimization of the molecular geometry of the chemical structure consist of the process in which the atomic coordinates of the binder molecule will change in order to reduce the steric energy of the molecular chemical structure that corresponds to its local minimum (SANT'ANNA, 2009).

After performing the Molecular Modeling step (2D Design, 3D Design, Energy Minimization and Geometric Optimization) for the chemical structure of each analog, the optimized chemical structures were saved in MDL molfiles (.mol) and the steric energies (E1, E2 and E3) were tabulated. Table 2 represents the two-dimensional (2D) chemical structure of the test analogs and the respective energies of the local minima E1, E2 and E3 in Kcal/mol of each chemical structure after the above steps.
Table 2: Chemical Structure in 2D of the analogs and values of the steric energies E1, E2 and E2.

<table>
<thead>
<tr>
<th>Analog</th>
<th>Two-Dimensional Drawing (2D)</th>
<th>E1 - 3D (kcal/mol)</th>
<th>E2 - PM3 (kcal/mol)</th>
<th>E3 - DFT (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td><img src="image" alt="Apigenin Structure" /></td>
<td>53.6392</td>
<td>50.1223</td>
<td>35.5465</td>
</tr>
<tr>
<td>Chrysin</td>
<td><img src="image" alt="Chrysin Structure" /></td>
<td>49.6687</td>
<td>47.3861</td>
<td>34.3402</td>
</tr>
<tr>
<td>Luteoline</td>
<td><img src="image" alt="Luteoline Structure" /></td>
<td>59.4799</td>
<td>52.7262</td>
<td>41.3988</td>
</tr>
</tbody>
</table>

Source: ChemSketch® Freeware version 2021, Arguslab® Freeware version 4.0 and MMPplus® version 8.0

E1: Steeric Energy (3D Drawing); E2: Energy after Energy Minimization and energy minimization after Geometric Optimization.

The analysis in table 2 clearly shows that the hybrid method used (Energy Minimization: PM3 + Geometric Optimization: DFT) considerably reduced the steric energy of all flavone analogs.

3.2 PHARMACOKINETIC STUDY IN SILICO HUMAN FOR ORAL BIOAVAILABILITY

After performing molecular modeling, some molecular properties were determined with the aid of the Chemoinformatics Database: Molinspiration Cheminformatics® (https://www.molinspiration.com), with the aim of performing the Pharmacokinetic Study in silico human to evaluate the bioavailability of analogs when administered orally. The study revealed whether the analog will obey Lipinski’s Rule (Rule of Five): the molecule will show a high level of human intestinal absorption, plasma solubility and tissue liquids, and permeability by biomembranes.

Lipinski's rule indicates whether or not a given analog will possess promising characteristics to be a drug. The determined molecular properties also evaluate the capacity of the compounds to be promising pharmaceutical industrial synthesis drugs,
since failure to do so as a rule means enormous losses of time and money. Currently, researchers from several companies such as Pfizer, GSK, Boehringer Ingelheim, AstraZeneca, Bayer and Lilly, among others, use this type of Virtual Screening to evaluate molecular properties in order to increase the probability of industrial organic synthesis.

Aiming to analyze compliance with the Lipinski Rule of flavone analogs for the analysis of oral bioavailability and also as possible pharmaceutical industry's 'synthesis' drugs, the results obtained were compared with the physico-chemical parameters standardized according to Lipinski's Rule. Table 3 shows the descriptor values (miLog P = Log P: octanol-water partition coefficient; PM: Molecular Weight; SDLH: Hydrogen Bond Donor Sites; SALH: Hydrogen Bond Acceptor Sites; TPSA: Polar Surface Topology Area; NLR: Number of Rotatable Links and VM: Molecular Volume). The Number of Rotatable Links (NLR) and Molecular Volume (VM) are considered molecular descriptors in extension. The extension of Lipinski's rule, known as Veber's rule, indicates that molecular flexibility, as measured by rotatable bonds, can influence oral bioavailability considerably, i.e., the greater the molecule's flexibility, the lower the probability of the molecule being bioactive when administered orally (VEBER et al., 2002). Table 3 shows the values of molecular descriptors obtained with the help of the Molinspiration Cheminformatics® database (https://www.molinspiration.com).

<table>
<thead>
<tr>
<th>Analogs</th>
<th>miLogP</th>
<th>PM</th>
<th>SDLH</th>
<th>SALH</th>
<th>TPSA</th>
<th>Violations*</th>
<th>NLR*</th>
<th>VM***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>2.46</td>
<td>270.24</td>
<td>3.5</td>
<td>90.89</td>
<td>0</td>
<td>1.224</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysin</td>
<td>2.94</td>
<td>254.24</td>
<td>2.4</td>
<td>70.67</td>
<td>0</td>
<td>1.216.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteoline</td>
<td>1.97</td>
<td>286.24</td>
<td>4.6</td>
<td>111.12</td>
<td>0</td>
<td>1.232.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Molinspiration Cheminformatics®
* Violations: Violations of Lipinski's Rule; ** NLR: Number of Rotatable Links; *** VM: Volume Molecular.

Analysis of table 3 showed that the results are favorable for all flavone analogs, since the three derivatives did not violate Lipinski's Rule, indicating that they have a good prediction as to the oral bioavailability profile. For a better understanding of the Lipinski Rule criteria for the oral bioavailability profile, we have:

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1) Octanol-water partition coefficient \( \text{Log P (miLogP)} \leq 5 \): molecular descriptor of hydrophilic/hydrophobic nature. This molecular property is related to the intestinal absorption of the ligand, its bioavailability when administered orally, the hydrophobic/hydrophilic ligand-receptor interactions and also the metabolizing process of the molecules. All the analogs under study had values lower than five (5).

2) Molecular Weight (PM) \( \leq 500 \text{ Da (Dalton)} \): all analogs had molecular weight values lower than 500 Da.

3) Hydrogen Link Donor Sites (SDLH) \( \leq 5 \): all analogs had values less than five (5).

4) Hydrogen Link Acceptor Sites (SALH) \( \leq 10 \). All analogs presented values less than ten (10).

5) Polar Surface Topological Area TPSA \( \leq 140 \text{ Å}^2 \): Descriptor characterized as the sum of polar surfaces of atoms bound to hydrogen in the molecule (usually fluorine, oxygen and nitrogen). All study analogs had TPSA values less than 140 \( \text{Å}^2 \).

3.3 PHARMACOKINETIC STUDY IN HUMAN SILICO (ADME IN SILICO)

After conducting the human in silico PK Study for prediction of oral bioavailability, the human in silico PK study was conducted to predict the following molecular descriptors for the three analogs: Human Intestinal Absorption (HIA), Haematoencephalic Barrier Permeability (BBB), Caco-2 epithelial cell permeability, P-glycoprotein inhibition, and Cell distribution of analogs in the human body.

When a given drug is administered orally, its molecules will be transported via the bloodstream and distributed to the receiving targets. An orally administered drug will have an effect on the central nervous system (CNS) if it can penetrate barriers that include intestinal wall tissues, intestinal capillary walls, and the blood-brain barrier.

Initially, the prediction of flavone analogs for human intestinal absorption (HIA) was evaluated. The molecular property HIA is related to the dissolution and dissociation of the compounds and also to intestinal permeability, factors considered limiting as regards the absorption rate of the active ingredients when administered orally in the form of their presentation (POLLI et al., 1996). Human intestinal absorption (HIA) indicates the sum of the intestinal absorption rate with the bioavailability of the unchanged fraction.
of the analog reaching the systemic circulation. It is important to note that when administered orally, plasma concentration will always be less than 100% as a function of loss in the intestinal absorption process and elimination of liver biotransformation-related compound. Therefore, the molecular descriptor HIA represents the percentage of the dose that was administered orally and that reaches the hepatic portal system (WANG et al., 2015).

Subsequently, a prediction was made regarding the molecular parameter BBB (blood-brain barrier), a molecular property related to permeability via endothelial cells and indicating the restriction of the compound to the passage from the blood stream to the Central Nervous System (CNS) (SHARMA et al., 2016), (DOLABELA et al., 2018) and (KANAZAWA, 2018).

The molecular property P-glycoprotein was also evaluated as it is a selective descriptor for the entry of xenobiotics into tissues. P-glycoprotein (present in epithelial cells) has a primary function in excretion and consequently reduces the absolute bioavailability of several analogs (AMIN, 2013) and (ASHORAJ et al., 2003). P-glycoprotein in the endothelium in the capillaries of the cerebral vessels acts as a "defense mechanism" with the ability to return to the blood xenobiotic chemicals that could eventually cross the blood-brain barrier (GOLAN et al., 2017).

Table 4 represents the evaluation of the pharmacokinetic profile in silico (ADME in silico) of the following parameters: Human Intestinal Absorption (HIA), Permeability by the Blood Brain Barrier (BBB), Permeability by Caco-2 epithelial cells, Inhibition of P-glycoprotein and Cell distribution of analogs in the body, qualitatively (Q = positive/negative) and quantitatively (P = probability) of the three flavone analogs.

Table 4: Pharmacokinetic profile assessment in silico human

<table>
<thead>
<tr>
<th>Analogs</th>
<th>BBB</th>
<th>HIA</th>
<th>Distribution</th>
<th>P-glycoprotein inhibitor</th>
<th>Caco-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q</td>
<td>P</td>
<td>Q</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Apigenin</td>
<td>negative 77.5%</td>
<td>positive</td>
<td>96.7%</td>
<td>mitochondria</td>
<td>66.5%</td>
</tr>
<tr>
<td>Chrysin</td>
<td>negative 77.5%</td>
<td>positive</td>
<td>96.7%</td>
<td>mitochondria</td>
<td>66.5%</td>
</tr>
<tr>
<td>Luteoline</td>
<td>negative 77.5%</td>
<td>positive</td>
<td>90.7%</td>
<td>mitochondria</td>
<td>58.9%</td>
</tr>
</tbody>
</table>

Source: admetSAR®
The analysis in table 4 shows that all the analogs under study were positive for HIA, with high HIA rates with probabilistic values between 90.7% (luteoline) and 96.7% (apigenin and chrysin), i.e. much higher than 70%, and are then classified as analogs with high intestinal absorption rate with the following order of HIA for the analogs under study: apigenin, chrysin = luteoline, (MATOS, 2017). The evaluation of the molecular descriptor HIA takes into consideration as a reference the following values of intestinal absorption: 0 to 20% low absorption rate, 20 to 70% moderate absorption rate and 70 to 100% high absorption rate (YAKAIAH et al., 2015).

It is important to note that the HIA parameter results are correlated with permeability by Caco-2 endothelial cells (derived from human colon adenocarcinoma), since for all flavone analogs they are positive for both descriptors. Over the years, Caco-2 cells have been used in tests to help identify molecules that have therapeutic potential for absorption and pharmacological permeability analyzes.

It is also noted that the three flavone analogs evaluated cannot cross the blood brain barrier, as indicated by the molecular descriptor BBB which is correlated with the molecular property P-glycoprotein, since all analogs do not have inhibitory capacity (I) of P-glycoprotein. As regards the distribution of flavone derivatives, all presented as cell target the mitochondria, the organelle responsible for cellular respiration.

The hepatic metabolization or biotransformation stage that occurs in liver cells (hepatocytes) is a biochemical process that promotes the structural modification of chemical compounds through biochemical reactions resulting in products called metabolites. The liver is the main organ of the human body responsible for the biotransformation of chemical substances and the components resulting from the process are: the reagents (drug or xenobiotic), the product (metabolite) and the catalyst of the biochemical reaction (hepatic enzymes). Hepatocytes have an enzyme apparatus responsible for metabolism found in the membranes of the smooth endoplasmic reticulum (REL) and the rough endoplasmic reticulum (RER). REL enzymes, also called microsomal enzymes, are called cytochrome P450 complex enzymes.

The first-pass effect (hepatic metabolism), along with excretion, are processes responsible for drug elimination. Hydrophilic drugs are readily excreted in urine due to high plasma solubility and poor reabsorption in renal tubules. Chemical compounds with high hydrophobicity (lipophilicity) undergo metabolism to be converted to hydrophilic metabolites and consequently excreted in the kidney via urine.
Cytochrome P450 enzymes responsible for biotransformation of chemicals catalyze many oxidative hydroxylation and hydrolysis reactions by metabolizing a variety of lipophylic compounds. The cytochrome P450 complex enzyme superfamily is subdivided into families (e.g. CYP1), subfamilies (e.g. CYP3A) and finally isoenzymes (e.g. CYP1A2). Enzymes of the cytochrome P450 (CYP450) oxidative complex are abundant in the liver, but are also found in the kidneys, intestine, lung, and brain, enabling the metabolism of approximately 90% of drugs. (DE MONTELLANO, 2010), (SOUSA, 2012), (MATOS, 2017), (SILVADO et al., 2018) and (DOLABELA et al., 2018).

The human in silico ADME study on hepatic metabolism for the inhibitory prediction of cytochrome P450 (CYP450) isoenzymes will indicate inhibitory capacity (I) and likelihood of inhibition (P). Table 5 represents evaluation of the pharmacokinetic profile in silico for prediction of flavone analogs for inhibition and interaction with hepatic cytochrome P450 complex isoenzymes (CYP450).

<table>
<thead>
<tr>
<th>Analogs</th>
<th>CYP450 1A2</th>
<th>CYP450 2C9</th>
<th>CYP450 2D6</th>
<th>CYP450 2C19</th>
<th>CYP450 3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>yes</td>
<td>92.2%</td>
<td>yes</td>
<td>77.4%</td>
<td>not</td>
</tr>
<tr>
<td>Chrysin</td>
<td>yes</td>
<td>92.2%</td>
<td>yes</td>
<td>77.4%</td>
<td>not</td>
</tr>
<tr>
<td>Luteoline</td>
<td>yes</td>
<td>91.1%</td>
<td>not</td>
<td>58.2%</td>
<td>not</td>
</tr>
</tbody>
</table>

Source: admetSAR®

The analysis in table 5 reveals information on inhibitory prediction of cytochrome P450 complex enzymes. Flavone analogs are noted to inhibit hepatic isoenzymes (CYP450 1A2, CYP450 2C9, CYP450 2C19, CYP450 3A4), with CYP4502D6 being the only isoenzyme not inhibited by the analogs. Therefore, analogs can affect hepatic metabolism, leading to adverse effects impacting the hepatic biotransformation of other chemical compounds, formation of toxic (xenobiotic) metabolites, and possible changes in the genetic formation of other enzymes. (DEVLIN, 2002), (GALLI and FEIJOO, 2002).
3.4 TOXICOLOGICAL STUDY IN HUMAN SILICO

The human in silico toxicology study for flavone analogs was conducted to predict toxicity according to the Ames test (T: toxic; NT: non-toxic), carcinogenicity (C: carcinogenic; NC: non-carcinogenic) and the Acute Oral Toxicity of derivatives in categories (I, II, III and IV).

The AMES test has important relevance as it represents the ability to detect mutations in the genetic material involved in the synthesis of the amino acid histidine (KAUFFMANN et al.¹; 2020). The AMES test is a bacterial assay that assesses the mutagenicity of chemical compounds using the strain Salmonella typhimurium (TA100 and TA1535) (MIRANDA et al., 2021).

Assessment of Acute Oral Toxicity of Analogs according to the US Environmental Protection Agency (EPA_https://www.epa.gov) classifies chemicals according to LD50 (median lethal dose) into the following categories: 1) Category I covers LD50 value less than or equal to 50 mg/kg; 2) Category II LD50 value greater than 50 mg/kg and less than 500 mg/kg; 3) Category III LD50 value greater than 500 mg/kg and less than 5,000 mg/kg; 4) Category IV LD50 value greater than 5,000 mg/kg. Compounds classified as Category I and Category II are the most toxic, with Category I being highly toxic and Category II being moderately toxic. Table 6 provides relevant information regarding the assessment of the toxicological profile in human silico.

Table 6: Assessment of the Toxicological Profile in human silico.

<table>
<thead>
<tr>
<th>Analog</th>
<th>AMES test</th>
<th>Carcinogenic</th>
<th>Acute Oral Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*Q **P</td>
<td>*Q **P</td>
<td>***C **P</td>
</tr>
<tr>
<td>Apigenin</td>
<td>NT</td>
<td>83.00% CN</td>
<td>95.00% II.</td>
</tr>
<tr>
<td>Chrysine</td>
<td>NT</td>
<td>86.00% CN</td>
<td>95.00% III.</td>
</tr>
<tr>
<td>Luteoline</td>
<td>NT</td>
<td>51.00% CN</td>
<td>100.0% III.</td>
</tr>
</tbody>
</table>

Source: admetSAR®
* Q: Qualitative; **P: Probability *** C: Category.

The analysis in table 6 shows promising data for all flavone analogs that have been shown to be non-toxic for AMES testing and also because they are not carcinogenic. For Acute Oral Toxicity, the analogs apigenin and chrysin fall into category III, i.e. they are of low toxicity, while the analog luteoline is of moderate toxicity as it falls into category II.
4 CONCLUSION

The present study made it possible, through in silico methodologies, to predict oral bioavailability, pharmacokinetic parameters and toxicological descriptors. Molecular Modeling performed for flavone analogs indicated that the hybrid method used (Energy Minimization: PM3 + Geometric Optimization: DFT) considerably reduced the steric energy for the chemical structure of all analogs. The Virtual Screening for predicting the oral bioavailability profile revealed that the analogs did not violate Lipinski's rule. The ADME study in human silico for prediction of pharmacokinetic parameters showed that all derivatives exhibit high intestinal absorption rate (HIA), do not cross the blood brain barrier (BBB), are permeable by Caco-2 cells, and lack inhibitory capacity of P-glycoprotein. The ADME study in silico human to evaluate the inhibitory prediction of hepatic cytochrome P450 complex enzymes (CYP450) indicated that all flavone analogs inhibit hepatic isozymes (CYP 4501A2, CYP4502C9, CYP4502C19, CYP4503A4), with only the CYP4502D6 isoenzyme not inhibited by the analogs. Flavone analogs can be said to cause adverse effects, hepatotoxicity and consequently may hinder the excretion of xenobiotic compounds and also lipophylic compounds from the organism. The human in silico toxicology study showed that all analogs are non-toxic for the AMES test, non-carcinogenic and acute oral toxicity apigenin and chrysin are non-toxic, while luteoline is moderately toxic. Further studies of flavone analogs for in silico pharmacodynamic prediction, study of the quantitative relationship between chemical structure-biological activity (QSAR) and structure-based drug design (SBDD) studies such as molecular docking and molecular dynamics are of paramount importance in order to elucidate the intermolecular ligand-receptor interactions.
REFERENCES


FIALHO, S. N. Leishmanicidal activity of synthetic derivatives of cinnamic acid against Leishmania amazonensis in vitro. Porto Velho, RO, Brazil, TCC (Graduation) - Bachelor of Biological Sciences Course, 2017.


