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Cardioprotective Effect of Troxerutin: An *In silico* Study

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ABSTRACT

Background and Objective: Cardiovascular diseases are the leading causes of death worldwide. Therefore, the search for therapeutic alternatives is necessary, and *in silico* studies constitute an important tool for the development of cardioprotective drugs. The aim of this study was predicting the possible target which could explain the cardioprotective effects of the natural chemical marker troxerutin.

Materials and Methods: Compounds with cardioprotective effects were selected using PubMed, ScienceDirect, and SciELO. The Molispiration server was used for oral bioavailability analysis, and Pred-HERG and Pro Tox-II were used to predict cardiac and systemic toxicities. Analyses of biological activities were performed in PASS online and those of molecular targets in Swiss Target Prediction and Superpred. Finally, pharmacophoric analysis and molecular docking were performed. To validate the model employed in molecular docking, redocking was also performed. Troxerutin, a natural flavonoid derivative, is found in the Brazilian Cerrado, extracted from *Sophora japonica* and *Dimorphandra gardneriana*. This molecule had the best scores and was thus selected for further analyses. To define the role of troxerutin as a thrombin antagonist, a pharmacophoric mapping was performed using five thrombin inhibitors with the lowest IC_{50} values.

Results: The results of PASS online corroborated with the cardioprotective effects of troxerutin, which were further confirmed by using thrombin, one of the targets obtained in the prediction studies. This interaction was verified by troxerutin docking with the human thrombin crystallographic structure (PDB ID: 2A2X). Redocking analysis established the parameters of the interaction model. As a result of thrombin antagonist, troxerutin interacted strongly with glycine and serine residues.

Conclusion: Troxerutin is a promising thrombin inhibitor as demonstrated by pharmacophoric model and molecular docking analysis, which account for its use as a medical tool against cardiovascular disease.

INTRODUCTION

Worldwide, cardiovascular diseases (CVD) are the leading cause of death and the total number of deaths from CVD was approximately 31% or 17.7 million in 2015. According to epidemiological data, CVDs kill twice more than cancers, 2.5 times more than accidents and deaths from violence, and 6 times more than infectious causes[1]. The main cause of death from CVDs can be due to either ischemic heart disease or cerebrovascular disease[2]. The other categories of CVDs include rheumatic heart disease, hypertensive heart disease, myocarditis, atrial and flutter fibrillation, aortic aneurysm, peripheral vascular disease and endocarditis[3]. The socioeconomic impact of these pathologies is significant. In Brazil, the cost of hospitalization for CVDs is the highest among the causes of hospitalizations. Total direct spending on hospitalizations and consultations for CVD in 2015 was more than $5 billion[4,5]. With the increase in life

expectancy and consequent aging of the population, there is a tendency towards an increase in the incidence of CVD and, in turn, and its exponential cost.

Thrombin is a serine protease that catalyzes the conversion of fibrinogen to fibrin and regulates the coagulation cascade with the procoagulant effect, acting on hemostasis. It is closely related to thrombogenesis caused by some heart diseases such as arrhythmias, heart failure, and valvulopathies. Thrombin inhibitors are increasingly recognized as therapeutic agents for CVD, preventing acute coronary syndrome, stroke and venous thromboembolism[6].

The search for alternative therapies through *in silico* studies of natural plant products aids in the development of drugs with cardioprotective properties and thus fosters a better therapy for CVD. For instance, acetylsalicylic acid was discovered from natural sources and is currently used to treat this set of diseases. *In silico* approach is based on studies of structure-activity relationship, and also explores the pharmacokinetic aspects of the candidate compounds. In addition, knowledge about the etiology and pathophysiology of diseases helps to optimize the process of discovery and development of new drugs, as it allows the identification of possible therapeutic targets that the substance may act on to stop the pathological process[7].

Troxerutin, known as vitamin P4, is derived from a natural glycosidic and flavonoid extracted from the fruits of the plant species *Sophora japonica* and *Dimorphandra gardneriana*[8].

Drugs from natural sources have nearly 50% FDA approval for commercialization. Supplementation using natural products with cardioprotective action needs to be considered in populations with a high prevalence of CVD and *in silico* screening systems contribute highly to the identification of these compounds[8]. Thus, the main objective of this study was predicting the possible target to natural chemical marker troxerutin which could explain its cardioprotective effects.

MATERIALS AND METHODS

Study area and literature search: This study was carried out from January to October 2019 in Catholic Pontifical University of Goiás, Brazil. The flavonoids from medicinal plant species with cardioprotective potential investigated in this article were selected from PubMed, ScienceDirect and SciELO databases. Seven compounds from different species were selected and their molecular structures were confirmed in the Pubchem database (Table 1)

Drug likeness classification: To predict the pharmacokinetics properties from the molecules selected, the Molinspiration web server was used[9]. From properties identified in this server, the investigated compounds could be classified into drug

Table 1: Compounds with cardiovascular potential selected for *in silico* analyses

Compound	Species
Troxerutin	*Sophora japonica* and *Dimorphandra gardneriana*
Catechin Gallet	Grape pomace
Epicatechin Gallet	Grape pomace
Galocatechin Gallet	Grape pomace
Quercetin	Grape pomace
Kaempferol	Grape pomace
Aristolane	*Nardostachys chinensis* Batal

likeness or non-drug likeness. The properties included hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and the presence of pharmacophoric characteristics that influence the behavior of the molecule in a living organism, including bioavailability, transport properties, protein affinity, reactivity, toxicity, stability and others. On this server, Lipinski's rule of five was used. It states that for a compound to have good oral bioavailability (comparable to drugs already used), it must have values for four multiple parameters, such as log P greater than or equal to 5, molecular mass less than or equal to 500 Da, acceptors of hydrogen bond less than or equal to 10 and number of hydrogen bond donors less than or equal to 5[10].

Prediction of toxicity analysis was performed by Pred-HERG[11–13], which predicts cardiac toxicity via hERG inhibition and ProTox-II[14,15], which analyzes molecular similarity to assess toxicity including acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity and immunotoxicity.

***In silico* prediction of biological properties:** To predict biological activities, the PASS webserver was employed[16,17]. From the structure of the molecules, their pharmacological effects, mechanisms of action, toxicity and adverse effects, interaction with enzymes and metabolic transporters, influence on gene expression and other characteristics were explored. Further, the effects that were linked to some cardioprotective activity of these molecules were also analyzed.

Molecular targets selection and docking: After the prediction of biological activities, the molecular targets were studied on two platforms, the Swiss Target Predicition[18] to predict molecular targets using a combination of 2D and 3D similarity measurements and comparison of the query molecule to a library of 280,000 active compounds and then the SuperPred webserver was used[19] for target prediction with 2D and 3D similarity analysis of the compounds.

Pharmacophore perception: Pharmacophoric models were generated using the PharmaGist web server which generates 3D images of pharmacophores from a set of molecules that are known to bind to a common target receptor. The method works for possible spatial features (pharmacophores) and reports the one with the highest score. The selected pharmacophores were identified by multiple flexible alignments of the input molecule ligands, where the flexibility of these molecules was processed and in a deterministic model in the alignment process[20].

In relation to the parameters, a minimum of three to a maximum of six features including hydrogen-bond acceptor, hydrogen bond donor, hydrophobic and ring aromatic features were selected for generating the pharmacophore models. The scoring weight was assigned as 3.0 for aromatic rings, 1.0 for a charge (anion/cation), 1.5 for hydrogen bond (donor/acceptor) and 0.3 for hydrophobic, which represented the default parameters of the webserver.

First, the active molecules against the same target, troxerutin, were selected for pharmacophore perception. From a total of 30 compounds, five molecules with the lowest IC_{50} values were selected in Binding DB[21]. Next, to obtain the fitting pharmacophore models, troxerutin was imputed into the dataset, and the top-scored pharmacophore models were selected.

Molecular docking: To further prepare receptors for docking, the Hermes visualizer in the GOLD Suite 5.7.0 was used[22]. The region of interest used for GOLD docking was defined as all the protein residues within the 10Å radius of the reference ligands that accompanied the downloaded protein complexes. The default values of all other parameters were used, and the complexes were submitted to 10 genetic algorithm runs using the CHEMPLP fitness function.

Statistical analysis: The Piecewise Linear Potential (FPLP) was used to model the steric complementarity between protein and ligand, while for ChemPLP additionally the distance and angle-dependent hydrogen and metal bonding terms from ChemScore are considered[22].

RESULTS

Among the molecules analyzed for cardioprotective activities, classified as drug likeness and with favorable pharmacokinetic profiles, troxerutin (Fig. 1a) was the most promising and presented the highest scores in the employed servers.

As presented in Fig. 1, the flavonoids possess some known characteristics, such as antioxidant, anti-inflammatory, antineoplastic, antithrombotic and antifibrinolytic. By predicting the biological activity of this molecule in the online PASS server, the effects that corroborated the hypothesis that troxerutin has a cardioprotective activity, such as hemostatic effect, vasoprotection, free radical scavenging, antioxidant, antithrombotic, vasodilator, platelet adhesion inhibitor, therapy for peripheral vascular disease and other descriptions that support the applicability of this species was confirmed.

The aglyconated part of troxerutin is absorbable and thus does not present any cardiotoxicity. Evaluation of troxerutin on Pro-Tox II, revealed its toxicity at level 5, with 6 being the lowest. The average similarity was 82.08% and the prediction accuracy was 70.97%. The analysis further revealed inactive hepatotoxicity, cytotoxicity, mutagenicity, carcinogenotoxicity and other toxicities with a propensity of 61% to 97%.

Troxerutin fitted with required features of the pharmacophore model of thrombin inhibitors, and a greater part of the molecules was aligned (Fig. 1b). The three features shared between troxerutin and the thrombin inhibitors with the lowest IC_{50} values indicate that troxerutin may present this inhibitor activity, which was corroborated by molecular docking analysis.

Redocking showed that the values of the Root Mean Standard Deviation (RMSD), used to measure the distance between the 5 best poses and the crystallized structure, were less than 1 (Fig. 2), confirming the docking analysis. Thus, the model obtained here was robust to be used in the docking as can be seen in Fig. 3a-b, probably the most stable pose of the interaction between troxerutin and the target selected (thrombin). Troxerutin showed strong thrombin antagonist activity through interaction with glycine (GL255 and GLY257) and serine (SER232 and SER253) residues.

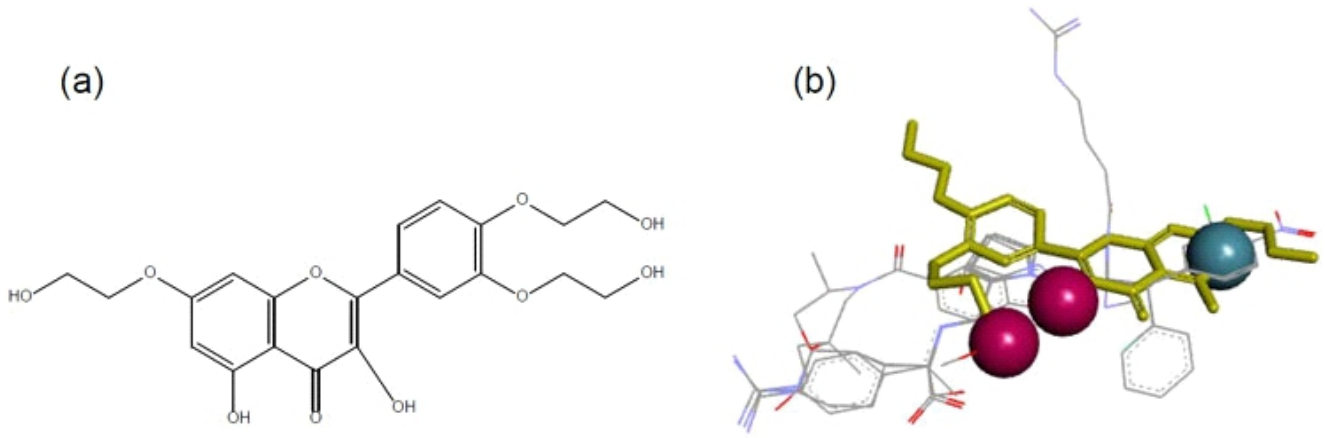

Fig 1: (a)Structure of the troxerutin, (b)Pharmacophore features are color-coded in red for hydrogen-bond acceptors, blue for aromatic rings

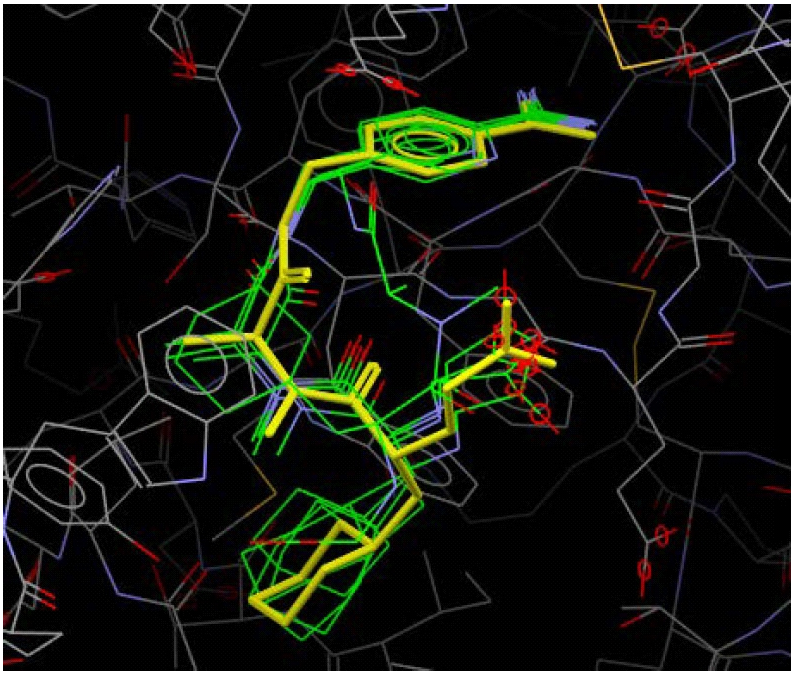

Fig. 2: Redocking image for ligand ID NA09 on thrombin protein (2A2X). The image is a result of the 5 best poses NA09: 2-((R) -1- (((S) -1- ((6-CARBAMIMIDOYLPYRIDIN -3- YL) METHYLAMINO) -1- OXOPROPAN -2- YL) (METHYL)AMINO) -3- CYCLOHEXYL -1- OXOPROPAN -2- YLAMINO) ACETIC ACID (C_{22} H_{34} N_6 O_4)

DISCUSSION

This study showed that thrombin can be considered a potential target to the natural chemical marker troxerutin, which could explain its cardioprotective effects and this effect of the plant species that this substance is present. Besides, there is a few works in literature regarding *in silico* studies with troxerutin, however its binding with DNA, corroborated by *in vitro* assays was performed[23].

Furthermore, there is relevant evidence about the cardioprotective effects of the troxerutin, such as anti-thrombotic, anti-fibrinolytic and rheological activities. Anti-arrhythmogenic and anti-inflammatory effects was also showed by Zhang *et al*[24] and Najafi *et al*[25]. These facts suggested the importance of elucidation of the probably mechanism of the troxerutin in cardioprotective effect.

To explore the key ligand-enzyme intermolecular interactions between troxerutin and human thrombin, molecular docking simulations were performed using a previously reported crystal structure of thrombin (PDB ID: 2A2X)[26]. Then, molecular docking was carried out to illustrate the binding mode of the selected flavonoid and the target. As presented in Fig. 3a, troxerutin could be docked in the catalytic site of human thrombin, indicating that this compound could serve as competitive inhibitor of thrombin, explaining its cardioprotective effects[25].

The key interactions between troxerutin and thrombin were analyzed. Fig. 3b shows that in the catalytic site of human thrombin, troxerutin could strongly interact with two glycine (GLY255 and GLY257), and two serine (SER232 and SER253) residues by hydrogen bonding. In addition, two cysteines interact *via* sulfur-TT (CYS228 and CYS258), and the anion-TT (GLU229) interactions contribute to establishing the docking and stabilize the ligand in the catalytic site. In the protein structure obtained from PDB (2A2X), the crystalized inhibitor also presents the hydrogens bond acceptors, and displays an important role of the enzyme active site[27].

Perhaps the cardioprotective effects of the species *Sophora japonica* and *Dimorphandra gardneriana* could be justified by the presence of flavonoid compounds,

including troxerutin, which probably contributes to beneficial properties of the extracts of this plants[8-10]. Thus, this study proposed a potential mechanism that would explain the effects of these species and others that present troxerutin in its composition, besides future assays employing this chemical marker could be corroborated by the data raised in this work. The standardization of the extracts could be employing the chemical marker troxerutin to control the several processes to obtainment of liquid and dried extracts.

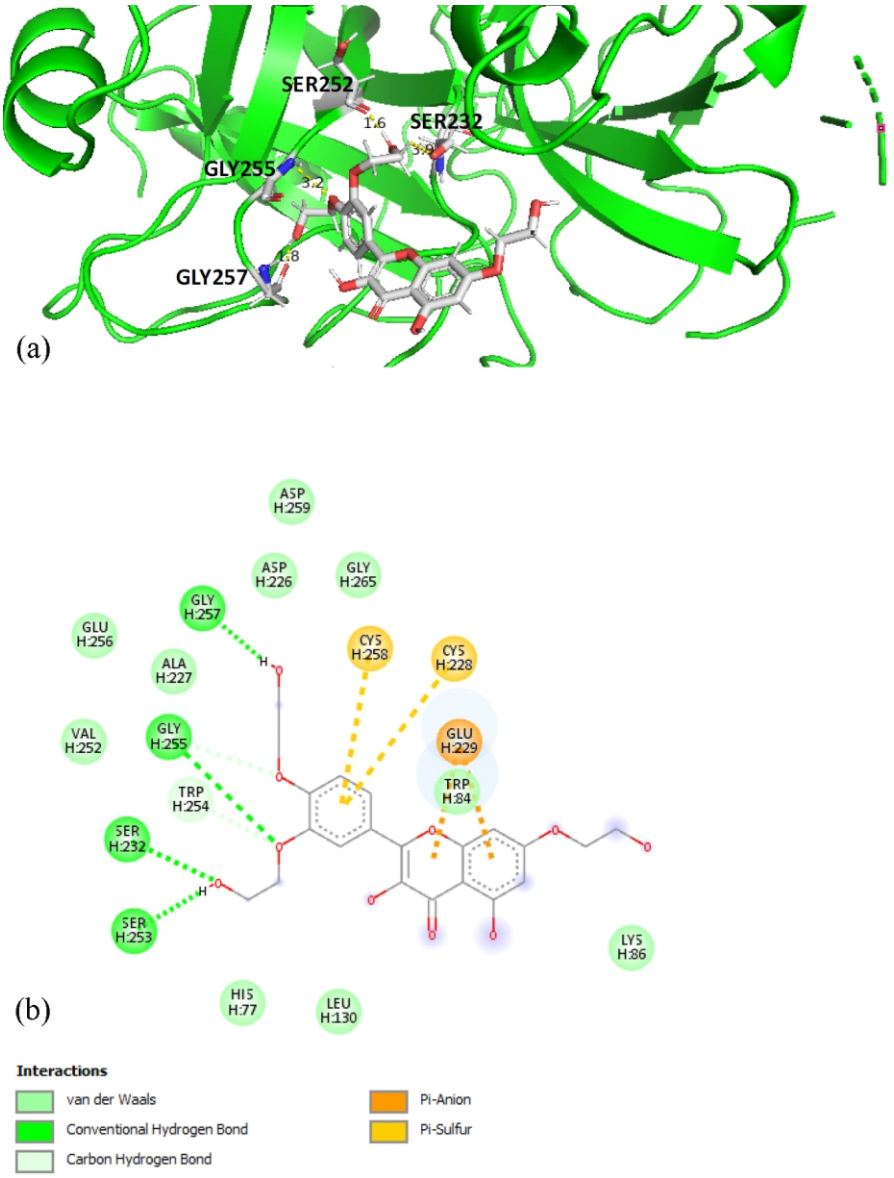

Fig 3: Docked pose 1 of troxerutin within thrombin active site. This figure was generated using Pymol 1.1r1 software (a), 2D interaction diagram of troxerutin pose 1 within thrombin. This figure was generated using Discovery Studio 3.5 Visualizer (b)

CONCLUSION

CVDs have increased at an alarming rate in recent years. This has led to an increasing interest in the cardiopreventive effects of medicinal plants. The pharmacophoric approach in this study demonstrated that troxerutin is a promising thrombin inhibitor because it shares chemical features with different thrombin inhibitors. Thus, some medicinal plants such as *S. japonica* and *D. gardneriana*, which are rich in troxerutin, protect against CVDs; further studies of other plants that possess troxerutin must be encouraged to explore its cardioprotective activity. *In vitro* and *in vivo* assays using

thrombin showed that it can be a starting point for the development of novel therapeutic options with thrombin-inhibitory and CVD-preventing activities. Finally, with *in silico* studies, the use of animals in experimental studies could decrease significantly since they would only be performed to confirm the researcher's hypothesis.

SIGNIFICANCE STATEMENT

This study discover the model to interaction between troxerutin and the possible target thrombin, that can be beneficial for rise future studies *in vitro* and *in vivo*, highlighting this natural compound as a promisor substance with therapeutically apply.

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