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Original Article

Molecular docking study and ADMET prediction of the effects of some food alkaloids on thyroxine homeostasis through their interactions with human thyroxine-binding globulin

Naima Maouche[1*], Nesrine Lenchi[1,2]

[1] *Department of Nature and Life Sciences. Faculty of Sciences. University of Algiers, Benyoucef Benkhedda, 2 Rue Didouche Mourad, 16000 Algiers, Algeria*

[2] *Bioinformatics, Applied Microbiology and Biomolecules Laboratory (BMAB), Faculty of Sciences, University of M'Hamed Bougara of Boumerdès. 35000 Boumerdes, Algeria*

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Abstract

Thyroid hormones (THs) play a vital role in several physiological functions of the body. At the circulatory level, thyroxine (T4) has been found to be the predominant form of THs. The distribution of T4 in the blood is mainly carried out by human thyroxine-binding globulin (hTBG). This process can be interfered with by various natural substances present in foods, particularly alkaloids. Some of these alkaloids have been shown to possess a cyclic chemical structure similar to that of T4. It has therefore been hypothesised that this class could potentially compete with T4 transport in the bloodstream. A molecular docking study and ADMET prediction were performed with ten selected food alkaloids. Predicted ADMET analysis revealed that all compounds tested had adequate solubility, high human gastrointestinal absorption and minimal risk of hepatotoxicity and cardiotoxicity. Molecular docking data showed that piperine, nigellidine, capsaicin, nigellicine, and 3-hydroxyquinine had a high affinity for hTBG, with respective binding energies of - 8.1, - 8.0, - 7.7, - 7.2 and - 7.1 kcal/mol. This finding indicates that these alkaloids were successfully positioned in the binding site of hTBG and had the ability to compete with T4 and increase its free level in the bloodstream. Therefore, two suggestions can be withdrawn, depending on the physiological state of the thyroid gland. Overconsumption of these alkaloids may lead to an imbalance in T4 homeostasis in both healthy individuals and hyperthyroid patients. Conversely, this competitive dynamic may offer a therapeutic advantage in the management of hypothyroidism.

Keywords: T4 homeostasis, Food alkaloids, hTBG, Molecular docking, ADMET prediction.

1. Introduction

It is well known that human food contains proteins, lipids, carbohydrates, minerals and vitamins. However, in addition to these compounds, it is possible to find other substances, in particular alkaloids. These substances are secondary metabolites found in many plants. They constitute a very large group of simple or complex natural molecules characterised by the presence of a nitrogen atom, often incorporated in a ring structure. Alkaloids are generally divided into several groups according to their biosynthetic precursor and heterocyclic ring system, including imidazoles, indoles, quinolines, isoquinolines, piperidines, purines, pyrrolidines, pyrrolizidines, quinolizidines and tropanes [1,2].

Several scientific studies have been focused on alkaloids, due to their significant biological activity and therapeutic properties (antioxidant, anti-inflammatory, analgesic and anticancer) [3,4]. Furthermore, other alkaloids have been classified as toxic molecules for human health, such as pyrrolizidine [2]. Some alkaloids can also interfere with several enzymatic systems and transport proteins, including those involved in the thyroid hormone status [5,6].

Thyroid hormones (THs: triiodothyronine or T3 and tetraiodothyronine or thyroxine or T4) are secreted by the thyroid gland. They are derived from tyrosine residues and have a biphenolic structure with iodine substitutions at specific sites on the inner and outer rings. THs play a crucial role in the differentiation, growth, metabolism and physiological function of virtually all tissues in the body [7]. At the circulatory level, T4 has been found to be the predominant form of THs. However, it is imperative that THs are distributed in appropriate amounts from their site of synthesis to their target cells. Furthermore, a minor proportion of the total THs in the blood is in free form (active form) and available to cross target cells. The distribution of THs in the blood is facilitated by transport proteins, including albumin, transthyretin (TTR) and thyroxine binding globulin (TBG). Among these proteins, TBG exhibits the highest affinity for THs and is responsible for transporting the majority of T4 in the blood [8]. Human TBG (hTBG) is a 54-kDa glycoprotein generated by the liver. It consists of a single 395-amino acid chain. hTBG

* Corresponding author.
E-mail address: na.maouche@univ-alger.dz (N. Maouche).
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is encoded by four exons in a single gene, serpin family A (SERPINA7), which is located on the long arm of the X chromosome (Xq21-22) [9]. Its half-life in human blood is around 5 days [8].

In this study, ten dietary alkaloids were selected based on specific criteria. The first criterion was the high global consumption of foods containing these compounds, combined with their recognised medicinal properties. These foods include coffee, tea and dark chocolate, which contain caffeine, theophylline and theobromine, respectively [1]. Furthermore, onions (3-hydroxyquinine and galantamine) and spices such as black pepper and chilli (piperine and capsaicin) are used in the preparation of meals [10-12], as well as nigella seeds and fenugreek (nigellicine, nigellidine and trigonelline), which are frequently employed in numerous recipes [11,13].

The second criterion was the similarity of their chemical structures to that of thyroxine, particularly with regard to the number of phenolic rings (ranging from two to three) and the total number of carbon atoms (ranging from seven to twenty). Based on these criteria, the aim of this study is to investigate the high probability of competition between the ten selected alkaloids and thyroxine for binding to the hTBG protein in the bloodstream. This competition has the potential to alter the balance of THs levels in the blood. To verify this theory, an *in silico* molecular docking and ADMET study was conducted.

2. Materials and Methods

2.1. Preparation of protein

The crystal structure of hTBG (PDB ID: 4X30; resolution: 1.55 Å) was obtained and downloaded in the PDB format from the RCSB protein database (http://www.rcsb.org/). Before starting the docking analysis, the hTBG protein was prepared using BIOVIA Discovery Studio 2021 software [14]. During the preparation, water molecules and various bound ligands were removed, hydrogen atoms were added and partial charges were assigned.

2.2. Preparation of ligands

Ten food-derived alkaloid molecules were selected for the comparison (Fig. 1). All ligand (T4 and alkaloid) codes were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). These different structures were downloaded in SDF (Structure data file) format and then converted to PDB format using BIOVIA Discovery Studio 2021 Software.

2.3. Molecular docking

Auto Dock Tool 1.5.7 (ADT) software was used for the second preparation of ligands and protein for docking, by converting them to PDBQT format. Auto grid was used with set dimensions and 0.375 Å spacing. The center of the grid has been adjusted according to the position of the different ligands selected. The docking was carried out first for the hTBG protein and its natural ligand: T4. Secondly, for comparison, the docking was carried out for each ligand tested (caffeine, trigonelline, theobromine, theophylline, 3-hydroxyquinine, galantamine, capsaicin, piperine, nigellicine and nigellidine) with hTBG. The binding energies obtained in kilocalorie per mole (kcal/mol) between each ligand and protein target were determined using the Auto Dock Vina software; the compound with the lowest energy was selected for further analysis [15].

2.4. Docking results visualization

The molecular docking results were visualised using BIOVIA Discovery Studio Visualizer software [14]. This software enabled the identification of the different hydrogen and hydrophobic bonds formed, as well as the amino acids involved in these interactions.

2.5. ADMET prediction

In parallel to molecular docking, it is important to analyse the pharmacokinetic properties of the selected alkaloids, describing the absorption, distribution, metabolism and excretion (ADME). This analysis was performed using the SuissADME (http://www.swissadme.ch/). This procedure allows the relevance of the selected ligands to be assessed for the subsequent development of potential drugs [16]. In addition, the ProTox-III (prediction of toxicity of chemicals) website was used to predict the toxicity of selected alkaloids (https://tox.charite.de/protox3/).

3. Results

3.1. Molecular docking study of T4 with hTBG

Molecular docking is a computational technique used to predict the binding affinity of small molecules or ligands to receptor proteins. However, before starting this process, it is necessary to ensure the quality of molecular docking of the three-dimensional structure of the selected target protein and to confirm its reliability using the RMSD test (*Root Mean Square Deviation*). For hTBG, the prepared pose and the raw pose downloaded from the PDB were found superimposed with an RMSD value of zero (0).

The various molecular docking results from this study are presented in Table 1. Structural analysis between T4 and hTBG showed that a total of eleven amino acid residues were involved in different molecular interactions. T4 had a binding energy of -7.4 kcal/mol. There were four conventional hydrogen bonds with GLN238, LYS270, ASN273 and ARG378, with distances of 2.08 Å, 2.32 Å, 2.11 Å and 2.47 Å, respectively. Furthermore, different alkyl interactions were observed with ALA27, LEU248, LEU269, LYS270, LEU276, LEU376 and ARG381. Pi-alkyl interactions were only located with LEU246, LEU376 and ARG381, respectively with distances of 4.81 Å, 5.20 Å and 4.52 Å (Fig. 2).

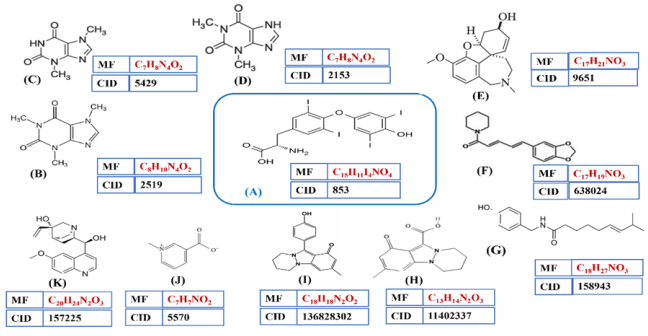

Fig. 1. Chemical structures of Thyroxine and food-alkaloids selected. (A) Thyroxine. (B) Caffeine. (C) Theobromine. (D) Theophylline. (E) Galantamine. (F) Piperine. (G) Capsaicin. (H) Nigellicine. (I) Nigellidine. (J) Trigonelline. (K) 3-Hydroxyquinine. (MF) Molecular Formula. (CID) PubChem Compound Identifier. (Source- PubChem; https://pubchem.ncbi.nlm.nih.gov).

Table 1. Molecular docking results of T4 and selected food alkaloids with hTBG.

Ligands interacting with hTBG	Binding energy (kcal/mol)	Residues of hydrogen bonding	Residues of hydrophobic interaction
T4	-7.4	GLN238, LYS270, ASN273, ARG378	ALA27, LEU246, LEU248, LEU269, LYS270, LEU276, LEU376, ARG381
Caffeine	-6.0	LYS270	LEU246, LEU269, LEU376, ARG381
Theobromine	-6.2	SER23, SER24, LYS270.	ALA27, LEU269, LYS270, ARG381.
Theophylline	-6.1	SER23, SER24, LYS270.	ALA27, LEU269, ARG381.
Trigonelline	-5.3	SER23, SER24, SER266, ASN273.	LYS270, LEU269, LEU376, ARG381.
3-Hydroxyquinine	-7.1	LEU269, ASN273, ARG378.	TYR225, LEU246, LEU248, TRP272, LEU276, LEU376.ARG378.
Galantamine	-5.6	ASN273, ARG378, ARG381.	LEU269, ARG378, ARG381.
Piperine	-8.1	GLN238, LYS270, ARG378.	TYR225, LEU246, LEU269, LEU276, LEU376, ARG381.
Capsaicin	-7.7	SER23, SER24, LYS270, ARG381.	ALA27, LEU246, LEU248, LEU269, LEU276, LEU376, ARG378, ARG381.
Nigellicine	-7.2	LYS270, ASN273, GLU377.	ALA27, LEU246, LEU269, LEU376, ARG378, ARG381, ILE383.
Nigellidine	-8.0	ARG381.	LEU246, LEU248, LEU269, LEU376, ARG378.

3.2. Molecular docking of caffeine and trigonelline with hTBG

Caffeine belongs to the group of purine alkaloids, which is mainly found in coffee. The structural analysis of caffeine with hTBG revealed that a total of five amino acid residues were involved in different molecular interactions. The binding energy was -6.0 kcal/mol. The caffeine ligand formed a single conventional hydrogen bonding interaction with Lys270 at a distance of 1.82 Å and three alkyl interactions with LEU246, LEU376 and ARG381. Moreover, four pi-alkyl interactions were observed with LEU269 (two bonds), LEU376 and ARG381 (Fig.3).

Concerning trigonelline, which is the most alkaloid present in Fenugreek. Structural analysis exposed that trigonelline was bound to eight amino acid residues of the hTBG and had a binding energy of -5.3 kcal/mol. Trigonelline formed a single conventional hydrogen bond with SER24 with distance of 2.78 Å. There were also four carbon-hydrogen bonds with SER23, SER24, SER266 and ASN273, with distances of 2.95 Å, 2.51 Å, 2.67 Å and 3.73 Å, respectively. Trigonelline showed an attractive charge with LYS270 and three alkyl interactions with LEU269, LEU376 and ARG381 (Fig. 3). The results obtained for these two food alkaloids showed that caffeine had the most negative binding energy value. However, their binding affinity seems to be lower than that of the native molecule. About the hTBG binding site, precisely the similarity of the amino acid residues involved, caffeine and trigonelline were found to share five amino acid residues in common with T4 (45.5%).

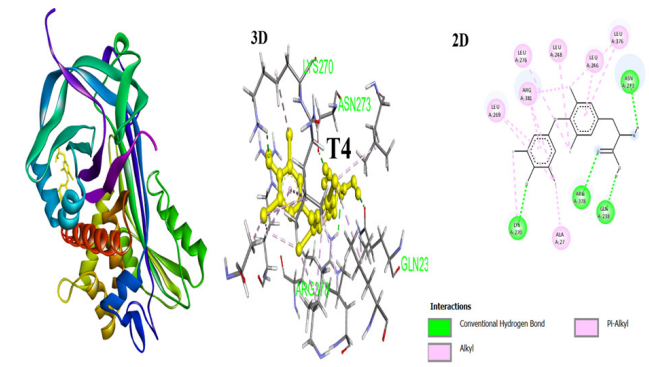

Fig. 2. Crystal structure of hTBG complexed with T4 and molecular interactions between them. H-bonds are indicated by green dashed lines, Alkyl and Pi-alkyl interactions are represented by pink dashed lines.

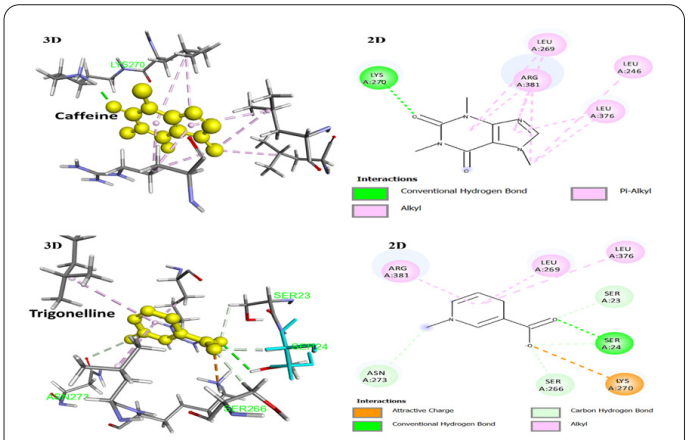

Fig. 3. Molecular interactions of caffeine and trigonelline with hTBG. H-bonds observed between protein and ligand are indicated by green dashed lines, Alkyl and Pi-alkyl interactions are represented by pink dashed lines, and attractive charge interaction is represented by orange dashed line.

3.3. Molecular docking of theobromine and theophylline with hTBG

Theobromine and theophylline belong to the group of purine alkaloids, which are mainly found in chocolate, tea, and soft drinks. The result of the molecular docking of theobromine with hTBG showed that a total of six amino acid residues were involved in several molecular interactions. Theobromine exhibited a binding energy of - 6.2 kcal/mol. It formed three conventional hydrogen bonds with SER23, SER24 and LYS270, with interaction distances ranging from 2.24 Å, 2.47 Å and 2.05 Å, respectively. In addition, theobromine formed pi-alkyl interactions with ALA27, LEU269, LYS270 and ARG381. One pi-sigma interaction was found with ARG381 with a dis-

tance of 2.68 Å. (Fig. 4).

About theophylline, the molecular docking study revealed the involvement of six amino acid residues in various molecular interactions. Theophylline showed a binding energy of -6.1 kcal/mol. It formed three conventional hydrogen bonds with SER23, SER24 and LYS270, with interaction distances of 2.15Å, 2.77Å and 2.28 Å, respectively. Theophylline also formed two carbon-hydrogen bonds with SER23 and SER24, with interaction distances of 3.05 Å and 3.03 Å. Furthermore, theophylline established several pi-alkyl interactions with ALA27, LEU269 and ARG381 (Fig. 4). The results obtained for these two food alkaloids showed that theobromine had the most negative binding energy value. In contrast, their binding affinity seems to be lower than that of T4. About the hTBG binding site, precisely the similarity of the amino acid residues involved, theobromine and theophylline were found to share four amino acid residues in common with T4 (36.4%).

3.4. Molecular docking of 3-hydroxyquinine and galantamine with hTBG

3-Hydroxyquinine and galantamine are alkaloids found in onion (*Allium cepa* L.). The result of molecular docking of 3-Hydroxyquinine with hTBG showed that a total of nine amino acid residues were involved in several molecular interactions. 3-Hydroxyquinine exhibited a binding energy of -7.1 kcal/mol. It formed a single conventional hydrogen bond with ARG378, with interaction distance of 2.47 Å. There were also two carbon-hydrogen bonds with LEU269 and ASN273, with distances of 3.39 Å and 3.62 Å, respectively. In addition, this alkaloid formed two alkyl interactions with LEU248 and LEU276. Five pi-alkyl interactions were found with TYR225, LEU246, TRP272, LEU376 and ARG378 (Fig. 5).

About galantamine, the result of molecular docking showed that a total of four amino acid residues were involved in several molecular interactions. Galantamine showed a binding energy of 5.6 kcal/mol. It formed a single conventional hydrogen bond with ARG381, with interaction distance ranging from 1.84 Å. There were also three carbon-hydrogen bonds with ASN273, ARG378 and ARG381, with distances of 3.49 Å, 3.59Å and 3.05 Å, respectively. Moreover, galantamine formed two alkyl interactions with LEU269 and ARG381 and two pi-alkyl interactions with ARG378 and ARG381 (Fig. 5). The results obtained for these food alkaloids showed that 3-hydroxyquinine had the most negative binding energy value. Nevertheless, their binding affinity appears to be lower than that of T4. With regard to the hTBG binding site, specifically the similarity of amino acid residues involved, it was found that 3-hydroxyquinine shared seven amino acid residues in common with T4 (63.6%), and galantamine shared four amino acid residues in common with T4 (36.4%).

3.5. Molecular docking of capsaicin and piperine with hTBG

Capsaicin is an alkaloid (capsaicinoid) found in the Capsicum family and is the main ingredient responsible for the hot, pungent taste of chilli peppers. Structural analysis revealed that capsaicin was bound to eleven amino acid residues of the hTBG and had a binding energy of -7.7 kcal/mol. The capsaicin ligand formed four conventional hydrogen bonds with SER23, SER24 (two bonds) and LYS270 with interaction distances ranging from 1.98 Å, 2.70 Å, 2.53 Å and 2.16 Å, respectively. There was also a single carbon-hydrogen bond with ARG381, with distance of 2.25 Å. Various alkyl interactions were also observed with LEU246, LEU248, LEU269, LEU276, LEU376 and ARG378. Two pi-alkyl interactions were also detected with ALA27 and ARG381 (Fig. 6).

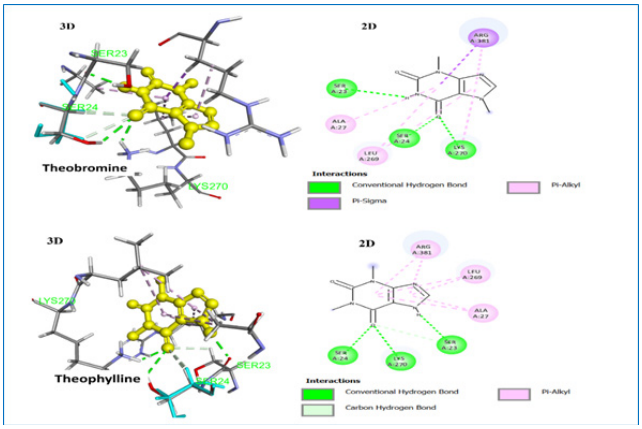

Fig. 4. Molecular interactions of theobromine and theophylline with hTBG. H-bonds are indicated by green dashed lines, Pi-alkyl interactions are represented by pink dashed lines and Pi-sigma by purple dashed lines.

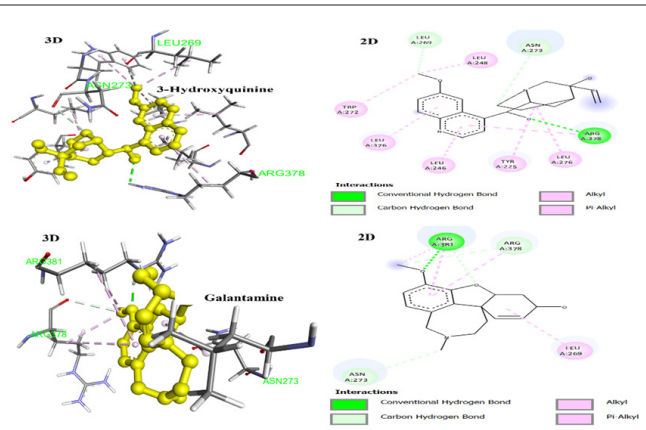

Fig. 5. Molecular interactions of 3-hydroxyquinine and galantamine with hTBG. H-bonds are indicated by green dashed lines, Alkyl and Pi-alkyl interactions are represented by pink dashed lines.

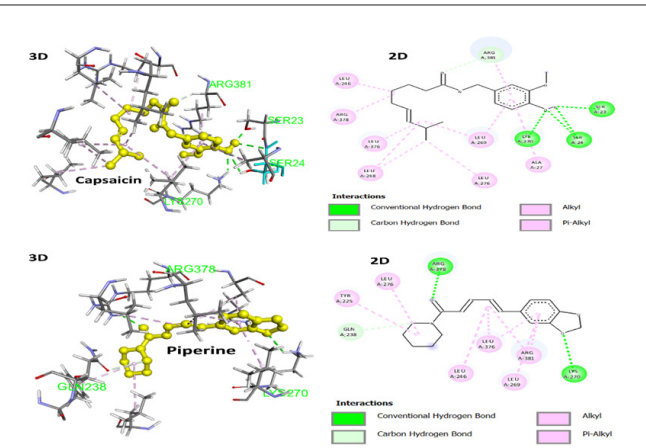

Fig. 6. Molecular interactions of capsaicin and piperine with hTBG. H-bonds are indicated by green dashed lines, Alkyl and Pi-alkyl interactions are represented by pink dashed lines.

Piperine belongs to piperidine alkaloid group and is found in the fruit of *Piper nigrum* (black pepper). The results of the molecular docking revealed that a total of nine amino acid residues were involved in different molecular interactions. Piperine exhibited a binding energy of -8.1 kcal/mol. It formed two conventional hydrogen bonds with LYS270 and ARG378, at distances of 2.66 Å and 2.46 Å, respectively. Additionally, Piperine established a single carbon hydrogen bond with GLN238 at a distance of 3.35 Å. There were also various alkyl interactions with TYR225, LEU246, LEU276, LEU376 and ARG381. Three pi-alkyl interactions were detected with LEU269, LEU376 and ARG381 (Fig. 6). Comparing with the data obtained with T4, the results showed that piperine and capsaicin had a high negative binding value. In terms of the hTBG binding site and the similarity of the amino acid residues involved, capsaicin was found to be the most similar, sharing nine amino acid residues with T4 (82%). Piperine was found to have eight amino acid residues in common with T4, placing it in the second position with 73% similarity.

3.6. Molecular docking of nigellicine and nigellidine with hTBG

Nigellicine and nigellidine are indazole-type alkaloids obtained from the seeds of *Nigella sativa*. The results of the molecular docking between nigellicine and hTBG showed that a total of ten amino acid residues were involved in different molecular interactions. Nigellicine showed a binding energy of -7.2 kcal/mol. It formed a single conventional hydrogen bond with LYS270 with distance of 2.09 Å. There were also two carbon-hydrogen bonds with ASN273 and GLU377, with distances of 3.38 Å and 3.64 Å. Nigellicine formed alkyl interactions with LEU246, LEU269, LEU376, ARG378, ARG381 and ILE383. Three pi-alkyl interactions were visualised with ALA27, LEU269 and ARG381 (Fig. 7).

Regarding nigellidine, structural analysis showed that this molecule interacted with six amino acid residues of hTBG and exhibited a binding energy of -8.0 kcal/mol. It formed a conventional hydrogen bond with ARG381 at a distance of 2.14 Å, as well as a carbon-hydrogen bond with the same residue at a distance of 2.83 Å. Additionally, various alkyl interactions were observed with LEU246, LEU248, LEU269, and ARG378. Five pi-alkyl interactions were identified with LEU246, LEU269, LEU376, ARG378, and ARG381 (Fig. 7).

Compared with the data obtained with T4, the results showed that nigellidine had the most negative binding energy value, followed by nigellecine. Concerning the hTBG binding site, specifically the similarity of the amino acid residues involved, nigellicine was found to have the highest similarity, with eight amino acid residues in common with T4 (73%), identical to piperine. Nigellidine was found to have six amino acid residues in common with T4 (54.5%).

3.7. ADMET prediction

SwissADME is a cheminformatics tool that predicts the pharmacokinetic properties of a compound by using SMILES strings as input. The pharmacokinetic properties of the ten selected dietary alkaloids are presented in Tables 2 and 3, respectively. In this study, we concentrated mainly on solubility and gastrointestinal absorption to verify whether these compounds enter the bloodstream at high concentrations. With regard to solubility data, SwissADME used three different approaches based on the *in-silico* methods ESOL, Ali and SILICOS-IT.; The results

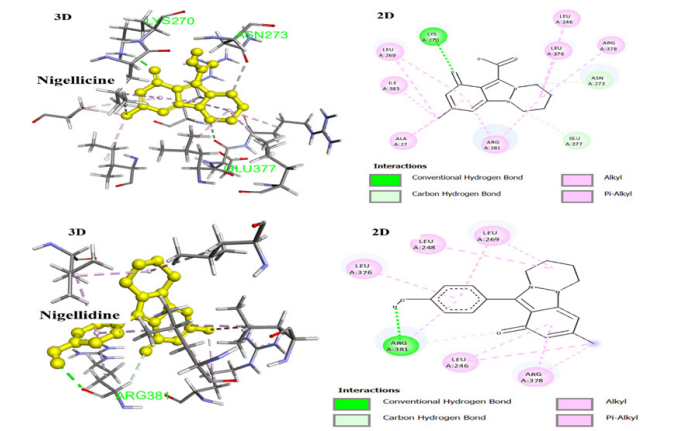

Fig. 7. Molecular interactions of nigellicine and nigellidine with hTBG. H-bonds are indicated by green dashed lines, Alkyl and Pi-alkyl interactions are represented by pink dashed lines.

Table 2. The ADME properties of caffeine, trigonelline, theobromine, theophylline, and 3-hydroxyquinine.

Characteristic	Caffeine	Trigonelline	Theobromine	Theophylline	3-hydroxyquinine
Molecular weight (g/mol)	194.19 g/mol	137.14 g/mol	180.16 g/mol	180.16 g/mol	340.42 g/mol
Log *S* (ESOL)	-1.48	-1.39	-0.98	-1.46	-3.12
Log *S* (Ali)	-0.78	-1.00	-0.27	-1.06	-2.80
Log *S* (SILICOS-IT)	-0.67	-0.94	-1.10	-1.10	-3.94
Gastro intestinal absorption	High	High	High	High	High
Blood-brain-barrier permeant	No	No	No	No	Yes
Glycoprotein P substrate	No	No	No	No	Yes
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	Yes
CYP3A4 inhibitor	No	No	No	No	No
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Bioavailability Score	0.55	0.55	0.55	0.55	0.55

Table 3. The ADME properties of galantamine, capsaicin, piperine, nigellicine, and nigellidine.

Characteristic	Galantamine	Capsaicin	Piperine	Nigellicine	Nigellidine
Molecular weight (g/mol)	287.35 g/mol	305.41 g/mol	285.34 g/mol	246.26 g/mol	294.35 g/mol
Log *S* (ESOL)	-2.93	-3.53	-3.74	-2.55	-3.95
Log *S* (Ali)	-2.34	-4.50	-3.96	-2.34	-3.58
Log *S* (SILICOS-IT)	-2.96	-4.87	-3.00	- 2.04	-4.60
Gastro intestinal absorption	High	High	High	High	High
Blood-brain-barrier permeant	Yes	Yes	Yes	Yes	Yes
GlycoproteinP substrate	Yes	No	No	No	Yes
CYP1A2 inhibitor	No	Yes	Yes	No	Yes
CYP2C19 inhibitor	No	No	Yes	No	No
CYP2C9 inhibitor	No	No	Yes	No	No
CYP2D6 inhibitor	Yes	Yes	No	No	Yes
CYP3A4 inhibitor	No	Yes	No	No	No
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Bioavailability Score	0.55	0.55	0.55	0.85	0.55

Table 4. Toxicity prediction of the ten selected food alkaloids.

Compound name	LD50 (mg/kg)	Cardiotoxicity	Hepatotoxicity	Neurotoxicity	Mutagenicity
Caffeine	127	Inactive (0.98)	Inactive (0.97)	Active (0.96)	Inactive (0.94)
Trigonelline	3720	Inactive (0.75)	Inactive (0.60)	Active (0.76)	Inactive (0.94)
Theobromine	837	Inactive (0.98)	Inactive (0.92)	Active (0.96)	Inactive (0.95)
Theophylline	127	Inactive (0.98)	Inactive (0.92)	Active (0.96)	Inactive (0.95)
3-hydroxyquinine	263	Inactive (0.81)	Inactive (0.92)	Active (0.64)	Inactive (0.80)
Galantamine	85	Inactive (0.82)	Inactive (0.93)	Active (0.78)	Inactive (0.76)
Capsaicin	47	Inactive (0.81)	Inactive (0.88)	Active (0.83)	Active (0.51)
Piperine	330	Inactive (0.77)	Inactive (0.91)	Active (0.66)	Inactive (0.96)
Nigellicine	1300	Inactive (0.75)	Inactive (0.60)	Active (0.59)	Inactive (0.59)
Nigellidine	1000	Inactive (0.78)	Inactive (0.64)	Active (0.70)	Inactive (0.58)

revealed that all the analysed compounds were soluble, but caffeine and theobromine had the highest solubility. In addition, all selected compounds showed high human gastrointestinal absorption. 3-hydroxyquinine, galantamine and nigellidine were the only P-glycoprotein substrates.

With regard to metabolism, the data focused on cytochrome P450 (CYP) enzyme, which play an important role in the biotransformation of drugs. The main CYP isoforms are CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The results showed that, with the exception of capsaicin, all the compounds selected were not inhibitors of the CYP3A4 enzyme. Similarly, with the exception of piperine, these compounds were not found to be inhibitors of the CYP2C9 and CYP2C19 enzymes. We also noted that capsaicin, piperine and nigellidine inhibited CYP1A2. 3-hydroxyquinine, galantamine, capsaicin and nigellidine inhibited CYP2D6.

About toxicity, the data obtained are presented in Table 4. This study focused on cardiotoxicity, hepatotoxicity, neurotoxicity and mutagenicity. The toxicological assessment showed that all compounds tested had a minimal risk of cardiotoxicity and hepatotoxicity, with probability scores ranging from 0.75 to 0.98 and 0.60 to 0.97, respectively. However, they all had potential neurotoxic effects with scores ranging from 0.59 to 0.96. With regard to mutagenicity data, none of the tested compounds were mutagenic, except for capsaicin.

4. Discussion

In addition to the most common thyroid disorders, such as hyperthyroidism and hypothyroidism, there are a number of exogenous factors, such as pollutants and certain dietary substances, which are implicated in disturbing the balance of THs in the blood and altering thyroid function [17, 18]. This study therefore, focused on dietary alkaloids and their ability, after intestinal absorption, to bind to hTBG, the main carrier protein for THs in the bloodstream. Transthyretin and albumin also transport THs, but with low affinity.

Molecular docking of the native hormone (T4) with its target protein, hTBG, revealed a strong affinity, with a delta G of -7.4 kcal/mol. The amino acid residues involved in binding were similar to those identified by Sheikh (2020) in his study of the binding of certain alkylphenol and bisphenol A compounds to the hTBG protein [19].

The results of the present study showed that the ten selected dietary alkaloids all bind to the hTBG ligand's binding pocket. In terms of binding energy value, piperine had the most negative value, followed by nigellidine, capsaicin, nigellicine and 3-hydroxyquinine. In contrast, the binding affinity of caffeine, theobromine, theophylline, galantamine and trigonelline was lower than that of the native ligand. Regarding the hTBG binding site, specifically the similarity of the amino acid residues involved, capsaicin was found to had the highest similarity, with nine amino acid residues in common with T4. Piperine and

nigellicine were found to had eight amino acid residues in common with T4, placing them in second position. 3-hydroxyquinine, on the other hand, exhibited seven amino acid residues in common with T4, thereby occupying the third position. Furthermore, when compared with T4, the amino acid residues of the target protein that were most implicated in these different linkages were, respectively, LEU269, ARG381 and LYS270.

One of the reasons for the increased binding potential of piperine, nigellidine, capsaicin, nigellicine and 3-hydroxyquinine compared to the other alkaloids tested may be due to the structural similarity of these molecules to T4. The native hormone has a biphenolic structure consisting of two rings containing a total of fifteen carbons, and the same was observed for these five alkaloids, which have one, two or three rings and contain between thirteen and twenty carbons. It can be hypothesized that this similarity may facilitate binding to the same sites on the hTBG protein. This would result in stronger interactions and greater competition, leading to an imbalance in T4 homeostasis.

However, the issue of competition for binding with THs transport proteins has already been addressed in previous *in vivo* and *in vitro* studies, which had explored the binding of many widely used drugs, such as diclofenac, ibuprofen, furosemide, tamoxifen and salicylate [20, 21]. Consulting the chemical structures of these drugs revealed that many of them share a few characteristics with THs, such as aromatic structures and hydrophobicity.

Few studies have investigated the effects of the selected alkaloids on thyroid function. An earlier experimental study in Swiss albino mice showed that administration of an ethanolic extract of *Piper nigrum* induced an increase in TH levels [22]. Another study on male Wistar rats, conducted by Vijayakumar and Nalini, also reported that piperine supplementation significantly decreased thyroid-stimulating hormone (TSH) and increased T4 and T3 levels [23].

In the case of capsaicin, there are no studies on the relationship of this alkaloid with THs transport proteins in the blood. However, capsaicin has been extensively studied as a potential anti-cancer agent in several types of cancer, both *in vivo* and *in vitro*; a study of differentiated thyroid cancer revealed that treatment of patients with capsaicin produced encouraging results. This is due to the fact that this alkaloid restores the uptake of radioactive iodine by the sodium-iodine symporter, thereby bypassing the standard TSH-TSHR pathway [24].

Regarding nigellicine and nigellidine, a study conducted on patients with Hashimoto's thyroiditis revealed that eight weeks of treatment with *Nigella sativa* powder led to a reduction in serum TSH and anti-thyroid peroxidase antibodies (anti-TPO), as well as an increase in serum T3 [25]. Another study showed that the daily oral administration of an ethanolic *Nigella sativa* extract for fourteen days significantly increased the serum concentration of T4 in normal albino Wistar rats [26]. The rise in serum T4 could be explained by the competition of certain bioactive compounds found in Nigella seeds, such as nigellicine and nigellidine, for binding to hTBG and other transport proteins, including TTR and albumin.

On the other hand, a cross-sectional study revealed an inverse relationship between excessive onion consumption and the prevalence of subclinical hypothyroidism in women. The authors of the research also suggested that this reduction could be linked to onion constituents [27]. Onions are known for their high alkaloid content, including 3-hydroxyquinine and galantamine.

Of all the purine alkaloids, caffeine has been the subject of the most extensive study. Much of this research has focused on the effect of caffeine on plasma level of TSH. However, a study by Zhao et al. (2023) on humans showed that drinking 2–4 cups of coffee daily reduced serum TSH levels [28]. With regard to theobromine and theophylline, there was very little research on the relationship with the function of the thyroid axis. The same applies to trigonelline, contained in fenugreek. Apart from one experiment was carried out on hyperthyroid rats that were given an oral dose of fenugreek extract, which showed a protective effect against hyperthyroidism [29].

From the data discussed on the various selected food alkaloids, it appears that these molecules are used for therapeutic purposes. For this reason, it is important to conduct an ADMET analysis to obtain further information on pharmacokinetics and toxicity risks. The *in silico* ADMET prediction study of the ten alkaloids revealed a favourable pharmacokinetic profile, characterised by adequate solubility and high gastrointestinal absorption. However, previous studies showed that most alkaloids, including caffeine and piperine, were rapidly and easily absorbed by the body after ingestion [30, 31]. Additionally, 3-hydroxyquinine, galantamine, and nigellidine were the only P-glycoprotein (P-gp) substrates, which is a biological barrier that removes toxins and xenobiotics from cells [32]. Regarding metabolism, apart from capsaicin, all the other tested alkaloids were not CYP3A4 inhibitors. It is well known that the CYP3A4 isoenzyme is the principal metaboliser of approximately 75% of all drugs metabolised by CYP [33].

The organ-predicted toxicities indicated that the ten alkaloids posed a minimal risk of cardiotoxicity and hepatotoxicity, suggesting that they were inactive in these zones. However, they exhibited potential neurotoxicity, highlighting the need for further research into their effects on the nervous system. In addition, data revealed that capsaicin is the only alkaloid that is mutagenic. Therefore, from a dietary perspective, its consumption should be limited compared to other alkaloids.

Finally, it is important to underline that when the hTBG protein binding site is occupied by these various food alkaloids, they effectively take the place of T4. As a result, the latter will be present in excess in the bloodstream in its free form. From an endocrine regulation perspective, in healthy individuals, the increase in free T4 in the blood exerts an inhibitory effect on both the hypothalamus and the pituitary gland, reducing the release of TRH and TSH, respectively [34]. On the other hand, excess free T4 in the bloodstream accelerates its penetration into target tissues such as liver, brain, kidney and heart and its deiodination to T3, the active form. In fact, it should be noted that a slight variation in T3 levels in these target tissues can lead to changes in their physiological functions [35]. In general, the data obtained suggests that certain dietary recommendations should be adopted depending on the physiological state of the thyroid gland.

In view of the predicted results of this study, further *in vivo* and *in vitro* experiments would be interesting in order to provide a more detailed explanation of the effects of these alkaloids on the transport of THs and thyroid func-

tion. To develop a comprehensive understanding of these alkaloids, it is also crucial to examine other targets, such as the key proteins responsible for thyroid hormone synthesis inside the thyroid gland.

This study suggests that all food alkaloids tested were successfully positioned at the hTBG binding site and were able to compete with T4, and increase its free level in the blood. Predicted ADMET analysis indicated that all compounds tested had adequate solubility, high human gastrointestinal absorption and minimal risk of cardiotoxicity and hepatotoxicity. However, there was a potential for neurotoxicity, although these predictions are based on computer modelling and need further confirmation. Overall, these different data allowed us to deduce two suggestions, depending on the physiological state of the thyroid gland. Excessive consumption of these alkaloids may lead to an imbalance in T4 homeostasis, both in healthy individuals and in hyperthyroid patients. Conversely, this competitive dynamic may offer a therapeutic advantage in the treatment of hypothyroidism. Finally, more profound computational and experimental studies will be required to confirm these various proposals.

Abbreviations

THs (Thyroid Hormones), T4 (Thyroxine), hTBG (human Thyroxine Binding-Globulin), ADMET (Absorption-Distribution- Metabolism-Excretion and Toxicity).

Author's contribution

NM: conceptualization, writing, data curation, formal analysis, methodology, review, and editing. NL: data curation, review.

Conflict of interest

The authors declare no conflict of interest.

Consent for publications

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