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Anti-Obesity Properties of Black Pepper *(Piper nigrum)*: Completing Puzzle using Computational Analysis

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ABSTRACT. *Pepper (Piper nigrum) is one of the most common spices found in almost every food. Current knowledge informed that pepper regulates physiological activity in obesity. However, the exact mechanism is still poorly understood. This study determined the potential of piperine and piperidine as major compounds in pepper as GHSR-Ghrelin inhibitors due to over-activity of Ghrelin as appetite hormone in obesity. Molecular docking was performed to simulate the binding pattern of piperine and piperidine as GHSR-Ghrelin antagonist. The result showed that piperidine has a lower potential as GHSR-Ghrelin antagonist than piperine based on binding energy calculation and amino acid interaction. Further, piperine binding to GHSR could shift the Ghrelin binding site to the GHSR. In conclusion, piperine may act as an inhibitor of GHSR-Ghrelin interaction to prevent appetite behavior resulting in bodyweight loss in obesity.*

Keywords: *Ghrelin, GHSR, obesity, piperidine, piperine*

INTRODUCTION

Obesity included in a serious health concern worldwide. with the number of prevalence was increasing year by year, obesity becomes one of dangerous health risk among modern civilizations. Obesity have bigger risk to develop another health problem, particularly related to cardiovascular diseases (CVD) [1]. Moreover, obese patients also have high susceptibility to COVID-19 and increase the probability to death [2]. Interestingly, countries with mid-to-high income have more obesity cases compared to the underdeveloped countries globally [3]. On the other hand, people with high annual income are also more susceptible to obesity, suggesting a socio-economic status also played a role in developing this metabolic disease [4, 5].

Since obesity is included as a metabolic disease, controlling food consumption has a significant role in regulating obesity status and development [6]. Thus, physiological regulation related to appetite also become a promising site to counteract obesity development [7]. One appetite hormone, known as Ghrelin, has a significant role in obesity treatment [8, 9]. Ghrelin is overproduction in obese conditions compared to the normal [10], suggesting a physiological target to decrease the obesity prevalence among civilizations [8, 11]. Hence, this study focused in Ghrelin as a target to prevent the obesity progression.

Controlling bodyweight using food regulation has a key role in suppressing obesity status [12, 13]. A familiar and frequently used food spices, Pepper (Piper nigrum), known to has some physiological benefit to improve human health [14]. Among two pepper types, black pepper is the most used spices in numerous foods due to its strong taste. The strong taste of black pepper is due to the presence of piperin and piperidine as alkaloid [15]. Those compounds also have numerous biological activity, such as antimicrobial action, immunomodulatory, antioxidant, antitumor, antimetastatic, antidepressant and many other activities [16–18], suggesting a promising role as Ghrelin inhibitor for obesity treatment. Therefore, this study aimed to investigate possible mechanism of piperine and piperidine as Ghrelin inhibitor to suppress the development of obesity.

RESEARCH METHODS

Data Mining of Compound and Protein Structures

Piperine (CID: 638024) and piperidine (CID: 8082) were retrieved from PubChem database and used as the ligands. The protein were retrieved from RCSB PDB with PDB ID 6KO5 and 6H3E for Growth Hormone Secretagogue Receptor (GHSR) as receptor for Ghrelin and Ghrelin itself, respectively. Protein's structure was prepared using Biovia Discovery Studio version 16 (Dassault Systemes BIOVIA, 2015) to remove attached ligands and water molecule. Ligands were prepared using OpenBabel integrated in PyRx 8.0 [19, 20] to minimize the energy prior to docking process.

Molecular Docking and Analysis

Protein-ligands docking was simulated using AutoDock Vina in PyRx software with maximum grid setting as performed in the previous study [21], while protein-protein interactions were docked by the HDOCK server [22]. Structure visualization and interacting residues then analyzed using Biovia Discovery Studio. Binding energy from docking process and amino acid residue interaction were used as consideration to assess the inhibitory activity of piperine and piperidine.

RESULTS AND DISCUSSIONS

Piperidine has higher binding energy than piperine (Table 1). Also, the binding of the ligands to the GHSR showed lower binding energy than piperine or piperidine binding to Ghrelin (Table 1). This result suggests that piperine or piperidine acted more efficiently as GHSR inhibitor rather than Ghrelin. Interaction of piperidine-Ghrelin involved two residues, GLU:8 and PHE:4 with hydrogen and hydrophobic bonds (Figure 3). Previous research, revealed that GLU:8 may be a low percentage of the α-helix region [32], and PHE:4 is an active core neither displaces ghrelin from its receptor nor stimulates growth hormone release [33]. According to binding energy calculation and amino acid interaction, piperine provided a potential to be an inhibitor of GHSR-Ghrelin interaction. Therefore, piperine was directed into further analysis related to its inhibitory mechanism.

Table 1. The binding energy of analyzed ligands interacted with targeted proteins

Macromolecule	Binding energy (kcal/mol)	
	Piperidine	Piperine
Ghrellin	-2.5	-5.7
GHSR	-4.3	-8.2

Amino acid interaction revealed that piperine has more interaction residue with the ligand (GHSR) compared to the Ghrelin (Figure 1). This data was in line with the binding energy measurement which piperine-GHSR complex has less energy necessity to form a complex compared to piperine-Ghrelin (Table 1). Interaction of piperine-Ghrelin involved only two residues in active site of Ghrelin-GHSR complex, i.e. GLN:10 and GLU:17 with hydrogen bond and van der waals interaction, respectively. Conversely, piperine could interact with GHSR through several amino acid residues using several chemistry interactions such as hydrogen bond, electrostatics, and van der waals (Figure 1). Hydrogen bond serves an important function in protein folding, as the facilitators of the protein bond with ligan, and the enzymatic catalysis [23]. However, electrostatics and other chemistry interaction also play role in protein-ligand structure stability [24, 25]. So that, interaction of piperine with GHSR was preferable to achieve inhibitory mechanism.

To understand the influence of piperine binding to GHSR-Ghrelin interaction, protein-protein docking was performed using piperine-bounded GHSR (GHSR+piperine) with Ghrelin as well as GHSR with piperine-bounded Ghrelin (Ghrelin+piperine) (Figure 2). Structural visualization showed that piperine binding to GHSR was more effective to alter binding motif of GHSR-Ghrelin (Figure 2C) compared to the binding of piperine with Ghrelin (Figure 2B). Analysis of amino acid interaction also strengthen the inhibitory mechanism of piperine to the GHSR-Ghrelin signaling with different amino acid involvement of (Ghrelin+piperine)-GHSR compared to the Ghrelin-(piperine+GHSR) complex (Table 2). This result emphasized the antagonism properties of piperine through the binding into GHSR to prevent GHSR-Ghrelin complex formation and inhibit appetite behavior, reduce the intake of glucose and lipid in the body, so that weight loss minimize obesity.

Development of GHSR antagonist gains an insight to reduce bodyweight. Previous study revealed that GHSR antagonist succeed to reduce food intake and bodyweight [26, 27]. Several bioactive compounds extracted from natural sources also have beneficial impact in reducing bodyweight, particularly related to obesity [27, 28]. Black pepper-contained diet could suppress the appetite [29]. Besides, piperine as major alkaloid in black pepper also induces lipid-lowering effect and body weigh shrinkage [30, 31]. With current result using computational analysis, anti-obesity mechanism of piperine has assembled successfully.

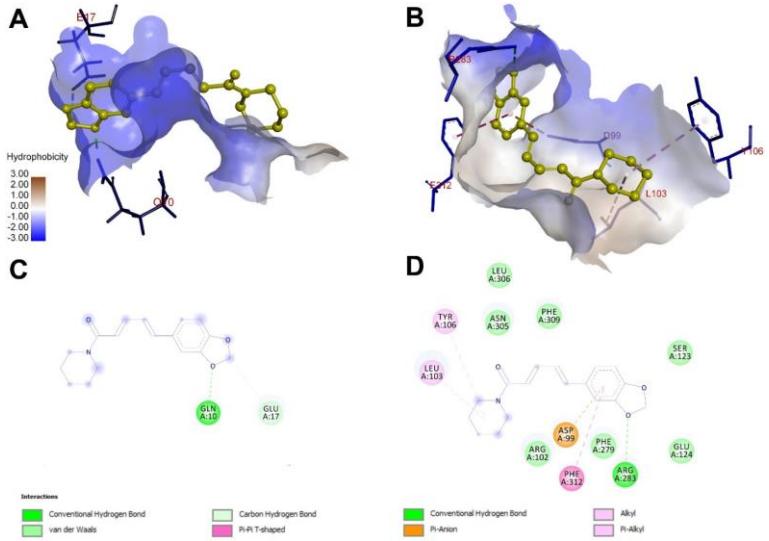

Figure 1. The binding pose of piperine compound with GHSR and Ghrelin, (A,C) interactions sites, hydrophobicity level & 2D view of Ghrelin-piperine complex, (B,D) interactions sites, hydrophobicity level & 2D view of GHSR-piperine complex

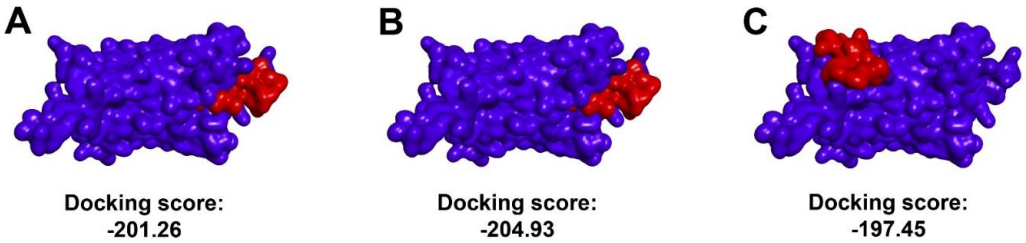

Figure 2. Structural orientation of ligand protein complex. Ghrelin-GHSR (A), [Ghrelin + piperine]-GHSR (B), Ghrelin-[piperine + GHSR] (C).

Table 2. Interacting residues in Ghrelin and GHSR with piperine and piperidine compound

Complex	Interacted residue		Distance	Category	Types
	From	To			
GHSR-Piperine	A:GLY1:N	A:GLU124:OE2	3.30	Hydrogen Bond;Electrostatic	Salt Bridge
	A:ARG102:NH2	A:SER2:OG	3.02	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG283:NH2	A:SER2:O	2.29	Hydrogen Bond	Conventional Hydrogen Bond
	A:PHE286:N	A:SER6:O	3.09	Hydrogen Bond	Conventional Hydrogen Bond
	A:SER289:OG	A:HIS9:O	2.85	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLY1:N	A:THR127:OG1	3.05	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG11:NE	A:TYR106:OH	2.67	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG11:NH2	A:TYR106	3.89	Hydrogen Bond;Electrostatic	Pi-CationDonor Hydrogen Bond
	A:PHE286	A:PHE4	5.76	Hydrophobic	Pi-Pi T-shaped
	A:ARG199	A:ARG11	4.02	Hydrophobic	Alkyl
	A:PRO7	A:LEU285	4.16	Hydrophobic	Alkyl
	A:PHE279	A:LEU5	5.49	Hydrophobic	Pi-Alkyl
[Ghrellin+Piperine]-GHSR	A:GLY1:N	A:GLU124:OE2	3.35	Electrostatic	Attractive Charge
	A:ARG102:NH2	A:SER2:OG	3.27	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG283:NH2	A:SER2:O	2.29	Hydrogen Bond	Conventional Hydrogen Bond
	A:SER289:OG	A:HIS9:O	2.78	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLY1:N	A:THR127:OG1	2.91	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG11:NE	A:TYR106:OH	2.60	Hydrogen Bond	Conventional Hydrogen Bond
	A:SER2:CA	A:ASP99:OD2	3.74	Hydrogen Bond	Carbon Hydrogen Bond
	A:ARG11:NH2	A:TYR106	3.76	Electrostatic	Pi-Cation

Complex	Interacted residue		Distance	Category	Types
	From	To			
Ghrellin-[GHSR+Piperine]	A:PHE286	A:PHE4	5.97	Hydrophobic	Pi-Pi T-shaped
	A:ARG237:NH2	A:GLU17:OE2	4.79	Electrostatic	Attractive Charge
	A:ARG159:NH1	A:ASP3:O	3.13	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG159:NH1	A:PHE4:O	3.19	Hydrogen Bond	Conventional Hydrogen Bond
	A:HIS9:CD2	A:TYR142:OH	2.71	Hydrogen Bond	Carbon Hydrogen Bond
	A:TYR142:OH	A:HIS9	3.24	Hydrogen Bond	Pi-Donor Hydrogen Bond
	A:HIS9	A:TYR142	5.21	Hydrophobic	Pi-Pi T-shaped
	A:VAL153	A:LEU5	5.45	Hydrophobic	Alkyl
	A:CYS227	A:ARG11	4.64	Hydrophobic	Alkyl
	A:ARG15	A:LEU234	4.20	Hydrophobic	Alkyl
	A:HIS9	A:LEU234	5.19	Hydrophobic	Pi-Alkyl
GHSR-Piperidine	N:UNK1:C	A:VAL68	5.12	Hydrophobic	Alkyl
	A:TYR330	N:UNK1:C	5.13	Hydrophobic	Alkyl
Ghrelin-Piperidine	N:UNK:1:H	A:GLU:8:O	2.18	Hydrogen Bond	Conventional Hydrogen bond
	A:PHE4	N:UNK1:C	4.82	Hydrophobic	Pi-Alkyl

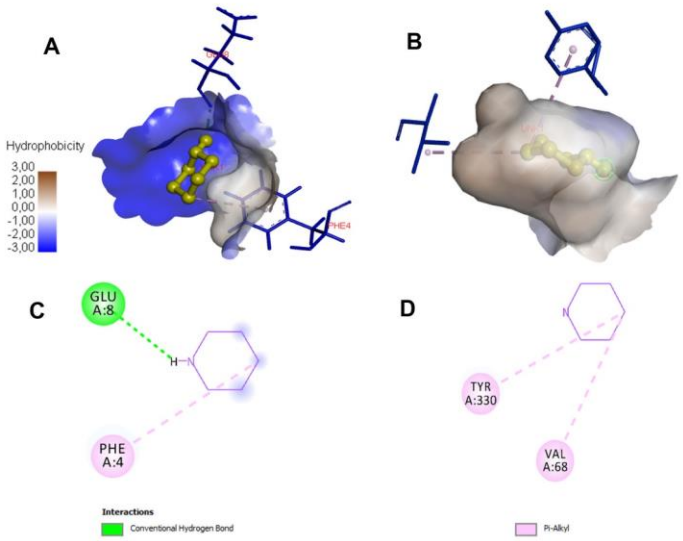

Figure 3. The binding pose of piperidine compound with GHSR and Ghrelin, (A,C) interactions sites, hydrophobicity level & 2D view of Ghrelin-piperidine complex, (B,D) interactions sites, hydrophobicity level & 2D view of GHSR-piperidine complex

CONCLUSION

Anti-obesity of piperine may achieve through inhibition of GHSR as the receptor of appetite hormone Ghrelin. Accordingly, piperine has a good potential to be a GHSR antagonist for anti-obesity treatment.

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