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ORIGINAL ARTICLE

Modulatory effects of phytol on the antiemetic property of domperidone, possibly through the D_2 receptor interaction pathway: in vivo and in silico studies

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Abstract

The current study is designed to evaluate the antiemetic effect of the diterpenoid phytol (PHY) using in vivo and in silico studies. For this, emesis was induced in 4-day-old chicks by the oral administration of copper sulfate ($CuSO_4.5H_2O$) at 50 mg/kg. To see the possible antiemetic mechanism of PHY, we used a number of reference drugs such as domperidone (80 mg/kg), ondansetron (24 mg/kg) and hyoscine (100 mg/kg) as positive controls, while the vehicle served as a negative control group. PHY was administered orally at the doses of 50 and 75 mg/kg. Both PHY and reference drugs were given alone or in combined groups to evaluate their synergistic or antagonistic effects on the chicks. Molecular docking of PHY and reference drugs was carried out against $5HT_3$, D_2, D_3, H_1, NK_1, and mAChRs (M_1–M_5) receptors for estimating binding affinity to the receptors. Drug-receptor interactions and active sites of the receptors were observed with the aid of different computational tools. The drug-likeness and pharmacokinetics of all the drugs were predicted through the SwissADME online database. The results suggest that PHY reduces the mean number of retches and increases latency dose-dependently in the birds. In the combination groups, PHY75 showed better antiemetic effects with domperidone and ondansetron. In addition, PHY exhibited the highest binding affinity with the D_2 receptor (6CM4) (− 7.3 kcal/mol). In conclusion, PHY showed an antiemetic activity in chicks, possibly through the D_2 receptor interaction pathway.

Keywords Nausea · Vomiting · Diterpenoid alcohol · Chicks · Molecular docking

Introduction

Emesis, also known as vomiting, is usually a distasteful condition that results in the forcible ejection of stomach objects through the mouth and is distinctly connected with gastrointestinal motor activity. As such, it could be interpreted as the body's reaction to particular medications, disease co-morbidities, and protective mechanisms against food poisoning (Hall and Driscoll 2005). While emesis can perform the operation of emptying noxious substances from the bowel, nausea plays the role of conditioned repercussion to desist from the ingestion of offending elements (Scorza et al. 2007). Emesis can occur for a variety of reasons, including illnesses such as food poisoning, motion sickness, gastroenteritis (diarrhea), intestinal obstruction, head injury, pregnancy, appendicitis, or hangover; or it can be a common side effect of certain diseases such as brain tumors, ionizing radiation overexposure, and elevated intracranial pressure (Grahame-Smith 1986). It is also the most common side effect of cancer chemotherapy and radiation therapy (Shankar et al. 2015).

The mechanisms are quite complex. The vomiting center (VC), known as the central emetic generator, hosted by the fourth ventricle of the brain and an area of that region called

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the chemoreceptor trigger zone (CTZ), mainly plays a crucial role in inducing emesis or nausea (Iqbal and Spencer 2012; MacDougall and Sharma 2021). Besides the CTZ, some other sites such as the GI tract, the higher centers in the cortex, the vestibular system, and the thalamus are also reliable for inducing emesis (Becker 2010). The VC in the reticular construction can be stimulated by either convergent afferent stimuli from the GIT or by the CTZ, and it also synchronizes the activities of smooth muscles and skeletal functions related to emesis (Khan et al. 2014; Navari 2013). Emesis is triggered by the CTZ when various receptors within the CTZ, such as dopamine receptors (D_2, D_3), serotonin receptor (5-HT_3), muscarinic acetylcholine receptors (mAChRs), neurokinin 1 receptor (NK_1) for substance P, histamine (H1), and opioid receptors, detect emetogenic toxins in the blood and CSF and transfer this message to the neighboring nucleus tractus solitarius (NTS) (Hornby 2001; Naylor and Inall 1994). The NTS is the origin of a final extensive pathway by which all the emetic inputs provoke vomiting (Miller and Leslie 1994). During emesis, the stomach muscle relaxes and secretion of HCl is inhibited. A backward extensive contraction of the small intestine appeals to the stomach to provoke retching and vomiting (Lang 1990). At present, a variety of antiemetic drugs are used to treat nausea and vomiting, which can be classified as serotonin antagonists, anti-dopaminergic drugs, antihistamines, anticholinergic drugs, NK_1-receptor inhibitors, corticosteroids, cannabinoids, 5-HT_{1A}, $GABA_B$, and CB_1 receptor agonists (Ahmed et al. 2013).

The search for novel antiemetic medicines derived from natural sources continues to focus mechanism-based methods that involve distinct cellular and molecular targets. Flavonoids, cannabinoids, chalcones, glucosides, hydroxycinnamic acids, diarylheptanoids, lignans, phenylpropanoids, saponins, polysaccharides, and terpenes are some of the bioactive chemicals that fall under this group for searching novel antiemetic drug candidates (Ahmed et al. 2014).

Phytol (PHY) is an organic phytochemical, an acyclic monounsaturated diterpene alcohol in nature, commonly obtainable in particular aromatic plants and having various pharmacological activities (McGinty et al. 2010; Islam et al. 2018). The compound is known to possess antioxidant properties as well as some other medicinal properties (Santos et al. 2013). Recent investigation revealed that PHY is a prominent immunostimulant and assists in activating both innate and acquired immunity (Lim et al. 2006). The compound also has antimicrobial, antidiarrheal (Pejin et al. 2014), antinociceptive (Santos et al. 2013), anti-inflammatory (Islam et al. 2020), antitumor, antifungal, antidiabetic, anticonvulsant, autophagy- and apoptosis-inducing, and hepatoprotective (Islam et al. 2018).

There are a variety of in vivo and in vitro models for evaluating the antiemetic activity of a compound or plant extract. The chick emesis model is one of them (Ahmed et al. 2013). In this model, copper sulfate induces emesis in young chicks (*Gallus gallus domesticus*) when administered orally. The test sample or standard is administered before 30 min of copper sulfate ($CuSO_4$), either orally or peritoneally. The antiemetic activity of the test sample is evaluated by comparing the number of retches with control groups (Akita et al. 1998). On the other hand, computational approaches in drug discovery and development enable rapid screening of a vast compound library and estimation of potential binders via modeling/simulation and visualization techniques. It also helps to predict pharmacokinetics and binding sites, which is indispensable for determination of mechanistic steps and binding in identifying and generating promising drug candidates (Sliwoski et al. 2014; Palermo and De Vivo 2014). Therefore, the objective of this study is to evaluate the antiemetic activity of PHY and to predict the mechanism of action as well as to assess the pharmacokinetic properties of the drugs through computational methods.

Material and methods

Chemicals and reagents

PHY (3,7,11,15-Tetramethyl-2-hexadecen-1-ol), 97%, mixture of isomers (CAS No. 7541-49-3) was purchased from Sigma-Aldrich (USA), while copper sulfate pentrahydrate ($CuSO_4.5H_2O$) and 1% tween 80 were purchased from Merck (India). Reference drugs, domperidone, ondansetron, and hyoscine butyl bromide were collected from Square Pharma Ltd., Healthcare Pharma Ltd., and Opsonin Pharma Ltd., Bangladesh, respectively.

Animals

Young chickens (*Gallus gallus domesticus*) of either sex, 2 days old, weighing about 55–65 gm (Grade-A) were collected from Nourish Grand Parent Ltd., Rangpur, Bangladesh. All chickens were housed for an additional 2 days prior to starting the experiment in stainless steel cages opened in the upper hood at room temperature with a twelve-hour light and dark cycle and were permitted to take standard food and water ad libitum. After 12 h of fasting, the antiemetic test was carried out. This study was granted by the Department of Pharmacy and acts of the Ethical Committee of Bangabadhu Sheikh Mujibur Rahman Science and Technology University [#BSMRSTU/R2022(1)1].

In vivo study

The study was carried out according to the protocols of Akita et al. (1998) with a slight modification. All the birds

were divided into nine groups, containing five in each. Before being given the treatments, each chick was kept in a large transparent plastic container for 10 min. The two doses of PHY referred as PHY50 (50 mg/kg) and PHY75 (75 mg/kg) were prepared by dissolving them in a 0.9% NaCl solution containing a small amount of (1% tween 80) and administered orally. Domperidone (DPD), ondansetron (ODN) and hyoscine butyl bromide (HYS) were administered orally as positive controls at 80, 24 and 100 mg/kg (b.w.), respectively. Three combined doses of PHY (75 mg/kg) with the positive controls were also administered orally to animals. The vehicle was considered a negative control (NC). After 30 min of treatment, emesis was induced through $CuSO_4.5H_2O$ at a dose of 50 mg/kg (b.w.) by administering it orally to every bird. Then, the latency (first retch after having $CuSO_4.5H_2O$ treatment) and number of retches (within 10 min after having $CuSO_4.5H_2O$ treatment) were recorded carefully. The percentage increase in latency and decrease in retches in comparison with the NC group were calculated according to the following equations:

$$\% \text{ increase in latency} = \frac{A - B}{A} \times 100$$

$$\% \text{ decrease in retches} = \frac{C - D}{C} \times 100$$

where, A = Mean of latency in seconds in NC group, B = Mean of latency in seconds in standard and test groups, C = Mean of retches in NC group, D = Mean of retches in standard and test groups.

Statistical analysis

Values of antiemetic activity are presented as mean ± SEM (standard error of mean). The statistical significance of the difference is calculated by using Graph Pad Prism (version 6.0) considering a 95% confidence interval. *P* values of < 0.05 were considered significant, and $p < 0.0001$ was highly significant.

In silico studies

Selection and preparation of receptors

Based on the literature review, we have targeted 10 receptors responsible for inducing emesis. 3D structures in PDB format of the targeted receptors: $5HT_3$ (PDB ID: 6Y5B) (Gregory and Ettinger 1998), D_2 (PDB ID: 6CM4) (Davis and Walsh 2000), D_3 (PDB ID: 3PBL) (Darmani et al. 1999), H_1 (PDB ID: 7DFL) (Doenicke et al. 2004), M_1 (PDB ID: 6WJC) (Pleuvry et al. 2006), M_2 (PDB ID: 5ZK8) (Pleuvry et al., 2006), M_3 (PDB ID: 4U15) (Pleuvry et al. 2006), M_4 (PDB ID: 7V6A) (Pleuvry et al. 2006), M_5 (PDB ID: 6OL9) (Pleuvry et al. 2006) and NK_1 (PDB ID: 6HLP) (Navari et al. 1999) were collected from the RCSB Protein Data Bank (https://www.rcsb.org/). After collection, the receptors were optimized to avoid docking interference by deleting all unnecessary molecules, e.g., lipids, water molecules, and heteroatoms from the sequence of proteins via the PyMol software package (v2.4.1). Finally, energy minimization and geometry optimization of the receptors were carried out through the SwissPDB Viewer software package by appealing to the GROMOS96 force field and saving the PDB file to perform molecular docking.

Collection and preparation of ligands

3D conformers of aprepitant (Compound CID: 135413536), domperidone (Compound CID: 3151), hyoscine (Compound CID: 3000322), ondansetron (Compound CID: 4595), promethazine (Compound CID: 4927), and phytol (Compound CID: 5280435) were collected in SDF format from the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/). Then, the 3D conformers of the chemical agents were minimized and saved in SDF files and converted into MOL files through the Chem3D 16.0 program package for performing molecular docking and predicting pharmacokinetics, respectively. Finally, all the ligands were optimized utilizing Gaussian view software (v5.0). The two-dimensional images of the chemical agents are displayed in Fig. 1.

Molecular docking and prediction of active site of receptors

Molecular docking was performed by utilizing the PyRx software package to predict the active binding potential of the drugs against the active sites of receptors. For performing docking, the grid box dimensions were set as $76.37 \times 55.95 \times 83.32$ Å along x-, y- and z-axes, respectively, and the calculation was run at 200 steps (Ibrahim et al. 2022). The result of the docking potential is saved in '.csv' format, and the complex of ligand–protein is collected in PDB format for collecting the ligand in PDBQT format. The interactions of ligand-receptors and the receptor's active site were observed under the Discovery Studio Visualizer (v21.1.020298) and PyMol (v2.4.1) program packages, and amino acid residues or receptor (D_2) that interacted with the drug are listed.

Prediction of drug-likeness and pharmacokinetics

Drug-likeness is a qualitative measurement employed in drug design and development to assess how the chemical compound acts like a drug with respect to factors like bioavailability, and it is also related to ADME (Bhadra 2020). Drug-likeness and pharmacokinetics of a chemical agent can

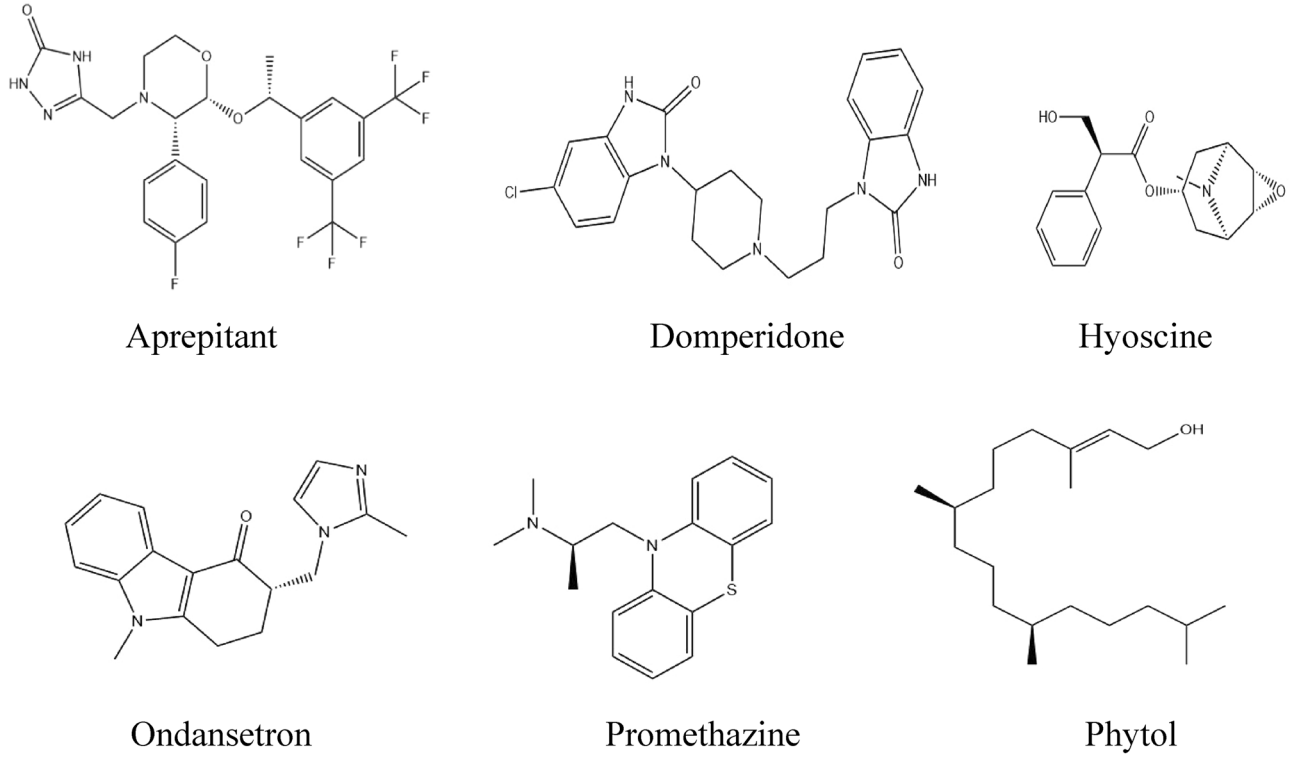

Fig. 1 Structures of phytol and selected standards screened against the receptors

be estimated through various online servers and software. In this study, we described various factors for assessing the selected molecule's physicochemical properties important in drug development with the aid of SwissADME (http://www.swissadme.ch/index.php) (Daina et al. 2017).

Results

In vivo antiemetic activity

The administered doses of PHY remarkably decreased the number of retches and increased the latent period in chicks. The combined drug therapy (standard plus test sample) expressed higher latency, such as first retching was observed in the DPD + PHY-75 group at 146.60 s, on the other hand, at 8.80 s in the NC group (values are mean). The onset of retching in test groups was observed at 25.00 and 61.60 s for PHY-50 and PHY-75, respectively (Fig. 2).

The highest number of retches was observed in the NC group (mean value: 70.60). The number of retches in the test groups reduced gradually with increasing dose, and the values were 12.40 and 7.80 for the PHY-50 and PHY-75 groups, respectively, which presented better antiemetic activity than the NC and HYS groups in the animals. The combined groups expressed a reduced number of retches. The lowest number of retches was observed in the DPD + PHY-75 group (Fig. 3). In comparison with the number of retches and latency of PHY with the number of retches and latency of control groups, PHY provided a mild antiemetic activity in this experiment.

The percentage increase in latency compared to the NC group for the test groups was recorded as 64.80 and 85.71% for the PHY-50 and PHY-75 groups, respectively. The highest percentage increase in latency (94%) was observed in the DPD + PHY-75 group. On the other hand, the highest %decrease in retching in comparison with the NC group was also recorded in the same group. The value of %decrease in retches of test groups compared to the NC group is 82.44% and 88.95% for the PHY-50 and PHY-75 groups, respectively (Table 1). The result demonstrated that PHY provided protective and antiemetic activity against copper sulfate-induced emesis in chicks in a dose-dependent manner.

In silico analysis

Molecular docking study

Molecular docking is carried out to predict the probable binding affinity and interactions between drugs and receptors. In our investigation, aprepitant (APT) was screened against the NK_1 receptor. And the binding energy against

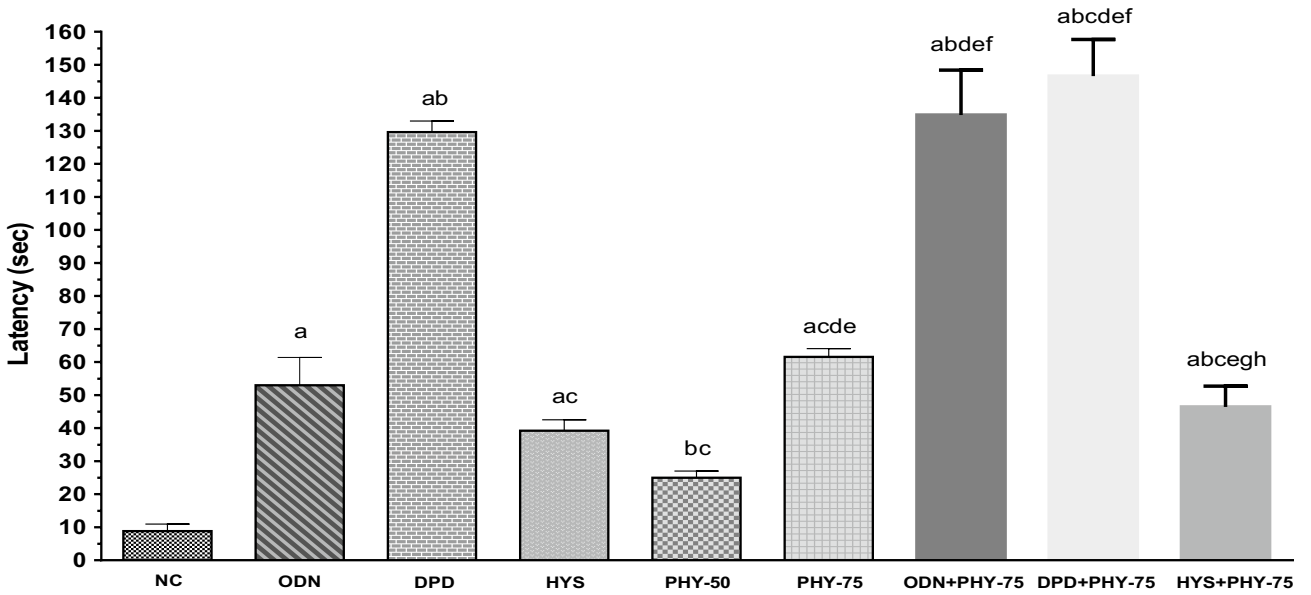

Fig. 2 Latency (sec) of retches observed in test sample, controls and combinations. [Values are mean ± S.E.M. ($n = 5$). [a]compared to the NC (vehicle), [b]compared to the OND (positive control); [c]compared to the DPD; [d]compared to the HYS; [e]compared to the PHY-50; [f]compared to the PHY-75; [g]compared to the ODN + PHY-75;.[h]compared to the DPD + PHY-75; $p < 0.05$ (DPD vs. DPD + PHY-75); $p < 0.01$(HYS vs. PHY-75, PHY-50 vs. HYS + PHY-75); $p < 0.001$(ODN vs. PHY-50); $p < 0.0001$(NC vs. ODN, NC vs. DPD, NC vs. HYS, NC vs. PHY-75, NC vs. ODN + PHY-75, NC vs. DPD + PHY-75, NC vs. HYS + PHY-75, ODN vs. DPD, ODN vs. ODN + PHY-75, ODN vs. DPD + PHY-75, DPD vs. HYS, DPD vs. PHY-50, DPD vs. PHY-75, DPD vs. HYS + PHY-75, HYS vs. ODN + PHY-75, HYS vs. DPD + PHY-75, PHY-50 vs. PHY-75, PHY-50 vs. ODN + PHY-75, PHY-50 vs. DPD + PHY-75, PHY-75 vs. ODN + PHY-75, PHY-75 vs. DPD + PHY-75, ODN + PHY-75 vs. HYS + PHY-75, DPD + PHY-75 vs. HYS + PHY-75)]

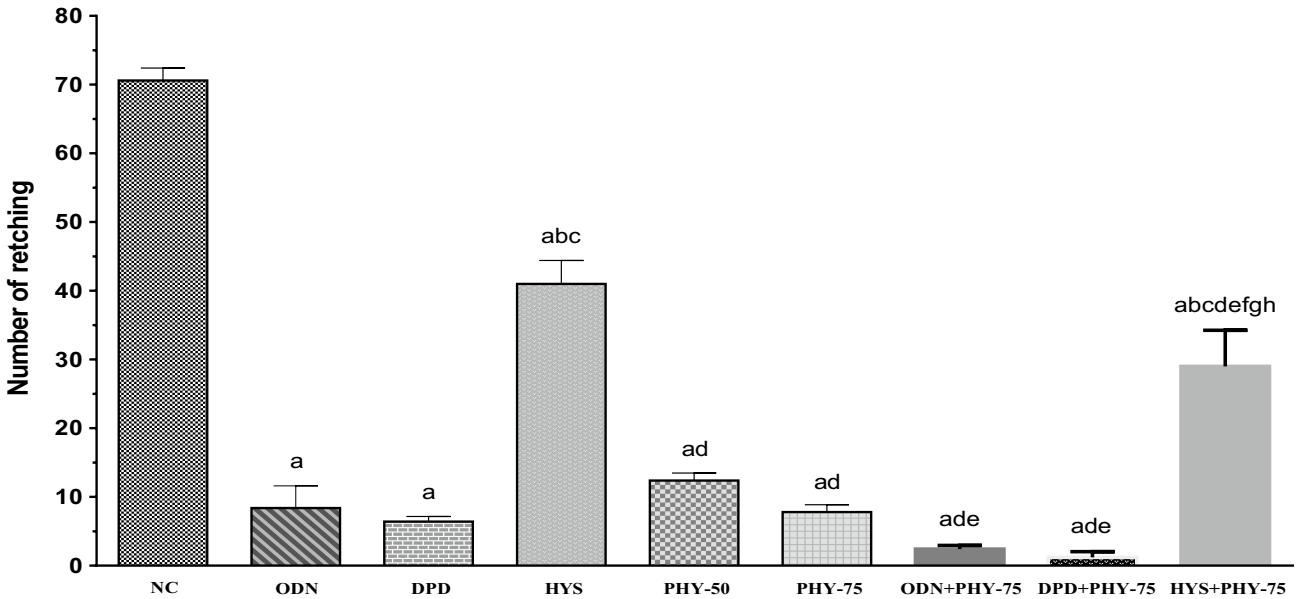

Fig. 3 Number of retches observed in test sample, controls and combinations. [Values are mean ± S.E.M. ($n = 5$). [a]compared to the NC (vehicle), [b]compared to the OND (positive control); [c]compared to the DPD; [d]compared to the HYS; [e]compared to the PHY-50; [f]compared to the PHY-75; [g]compared to the ODN + PHY-75;.[h]compared to the DPD + PHY-75; $p < 0.01$ (PHY-50 vs. ODN + PHY-75); $p < 0.001$ (HYS vs. HYS + PHY-75, PHY-50 vs. DPD + PHY-75); $p < 0.0001$ (NC vs. ODN, NC vs. DPD, NC vs. HYS, NC vs. PHY-50, NC vs. PHY-75, NC vs. ODN + PHY-75, NC vs. DPD + PHY-75, NC vs. HYS + PHY-75, ODN vs. HYS, ODN vs. HYS + PHY-75, DPD vs. HYS, DPD vs. HYS + PHY-75, HYS vs. PHY-50, HYS vs. PHY-75, HYS vs. ODN + PHY-75, HYS vs. DPD + PHY-75, PHY-50 vs. HYS + PHY-75, PHY-75 vs. HYS + PHY-75, ODN + PHY-75 vs. HYS + PHY-75, DPD + PHY-75 vs. HYS + PHY-75)]

Table 1 Percentage increase in latency and decrease in retches in treatment groups

Name of group	% increase in latency	% decrease in retches
NC	–	–
HYS	77.55	41.93
ODN	83.40	88.10
DPD	93.21	90.93
PHY-50	64.80	82.44
PHY-75	85.71	88.95
HYS + PHY-75	81.03	58.92
ODN + PHY-75	93.47	96.60
DPD + PHY-75	94.00	98.30

Values are percentage inspect of NC group (Negative control or vehicle) ($n = 5$); ODN = Ondansetron (Dose 24 mg/kg); DPD = Domperidone (Dose 80 mg/kg); HYS = Hyoscine (Dose 100 mg/kg); PHY-50 = Phytol (Dose 50 mg/kg); PHY-75 = Phytol (Dose 75 mg/kg); ODN + PHY-75 = Ondansetron + Phytol (Dose 24 mg/kg + 75 mg/kg; DPD + PHY-75 = Domperidone + Phytol (Dose 80 mg/kg + 75 mg/kg); HYS + PHY-75 = Hyoscine + Phytol (Dose 100 mg/kg + 75 mg/kg)

NK_1 is − 11.3 and − 6.1 kcal/mol for APT and PHY, respectively. DPD is the antagonist of the dopamine receptors. DPD scored − 9.8 kcal/mol and − 9.7 kcal/mol against the D_2 and D_3 receptors, respectively, whereas PHY scored − 7.3 kcal/mol against D_2, which is the highest score of binding affinity of PHY against an emesis producing receptor. HYS is the mAChRs antagonist. The docking scores against mAChRs such as M_1, M_2, M_3, M_4, and M_5 are − 8.5, − 7.6, − 9.2, − 9, and − 6.3 kcal/mol, respectively. The binding energy of PHY against serotonin receptor ($5HT_3$) is − 5.8 kcal/mol, whereas the standard ODN scored − 8 kcal/mol. The antihistamine PMN scored − 7 kcal/mol against the histaminic H_1 receptor, where PHY exhibited − 5.6 kcal/mol. The binding affinity of all the drugs against the selected receptors is provided in Table 2.

Prediction of drug-likeness and pharmacokinetics

Drug-likeness, which describes the molecular properties of a drug candidate, is an important parameter for developing a chemical compound into a drug and evaluating pharmacokinetics. MW, Log P, HBA, HBD, and MR are the parameters by which drug likeness is assessed. In our findings, the molecular weight of all the drugs was retained under 500 Dalton except APT, the drug also having 12 HBA (Table 3). According to Lipinski's rule of five, except for APT, the values of HBA (≤ 10) and HBD (≤ 5) are within the limit. HYS and ODN are soluble in water, and others are moderately soluble. APT and PHY are slightly absorbable through the GI membrane, and others are highly absorbable. Values of the pharmacokinetic parameters such as BBB permeability, P-gp substrate, CYP2C19 inhibitor, bioavailability score, along with water solubility and GI absorption are also provided in Table 3.

Estimation of non-bond interactions between drug-receptor complexes

PHY forms different types of bonds with the D_2 receptor, such as hydrogen bonds (HB) and hydrophobic bonds (alkyl, pi-alkyl bonds), to provide the best interactions with the D_2 receptor. PHY formed HB with the amino acid residues of Thr119, Val115 and hydrophobic bonds (alkyl and pi-alkyl) with Val91, Leu94, Cys118, Trp100, Phe110, Phe189, Trp386, Phe389, Phe390, Trp413 and Tyr416 of D_2 receptor. On the other hand, DPD formed HB with the amino acid residue of Ser409, an electrostatic bond with Asp114, and hydrophobic bonds (alkyl, pi-alkyl, pi-pi stacked) with Tyr408, Ile184, Val91, Phe189, His393, Tyr408, Trp413, and Leu94 of the D_2 receptor. Besides D2, PHY also interacted with 5HT3 receptors to provide an antiemetic effect by forming HB and hydrophobic bonds (alkyl and pi-alkyl) with the amino acid residues of that receptor. PHY formed HB with Ile93, Val95, and Trp94 amino acid residues, as well as hydrophobic bonds with Leu99, Leu129, Val95, Ile100,

Table 2 Molecular docking scores (kcal/mol) of phytol and selected reference standards

Ligands	Receptors									
	Common Name	$5HT_3$	D_2	D_3	H_1	M_1	M_2	M_3	M_4	M_5	NK_1
	PDB ID	6Y5B	6CM4	3PBL	7DFL	6WJC	5ZK8	4U15	7V6A	6OL9	6HLP
Aprepitant		–	–	–	–	–	–	–	–	–	− 11.3
Domperidone		–	− 9.8	− 9.7	–	–	–	–	–	–	–
Hyoscine		–	–	–	–	− 8.5	− 7.6	− 9.2	− 9	− 6.3	–
Ondansetron		− 8	–	–	–	–	–	–	–	–	–
Promethazine		–	–	–	–	–	–	–	–	–	–
Phytol		− 5.8	− 7.3	− 6	− 5.6	− 5.1	− 6.2	− 6.9	− 5.6	− 6.2	− 6.1

Table 3 Drug-likeness and pharmacokinetic properties of phytol predicted by SwissADME

Parameters	APT	DPD	HYS	ODN	PMN	PHY
MF	$C_{23}H_{21}F_7N_4O_3$	$C_{22}H_{24}C_lN_5O_2$	$C_{17}H_{21}NO_4$	$C_{18}H_{19}N_3O$	$C_{17}H_{20}N_2S$	$C_{20}H_{40}O$
MW	534.43	425.91	303.35	293.36	284.42	296.53
Log P	4.05	3.28	1.19	1.75	3.84	5.25
HBA	12	3	5	2	1	1
HBD	2	2	1	0	0	1
MR	118.82	124.08	83.48	87.39	90.07	98.94
Solubility (water)	Moderately soluble	Moderately soluble	Soluble	Soluble	Moderately soluble	Moderately soluble
GI absorption	Low	High	High	High	High	Low
BBB permeant	No	Yes	No	Yes	Yes	No
P-gp substrate	Yes	Yes	No	Yes	No	Yes
CYP2C19 int	No	Yes	No	Yes	No	No
BIO Score	0.55	0.55	0.55	0.55	0.55	0.55

MF Molecular formula, *MW* Molecular weight (g/mol), *LogP* Log $P_{o/w}$ (MLOGP), *HBA* Hydrogen bond acceptor, *HBD* Hydrogen bond donor, *MR* Molar refractivity, *APT* Aprepitant, *PMN* Promethazine, *CYP2C19 int* CYP2C19 inhibitor, *BIO Score* Bioavailability Score

Pro110, Pro113 (alkyl), Phe103, and Phe153 (pi-alkyl). In contrast, reference drug ODN interacted with the respective receptor by forming HB with Ile256, Phe254, Thr257 amino acid residues and hydrophobic bonds (pi-sigma, pi-alkyl, alkyl) with Ile256, Leu260 amino acid residues. The drug-receptor interactions (PHY, DPD with D_2 and PHY, ODN with $5HT_3$) and active sites of the receptors (D_2 and $5HT_3$) are presented in Fig. 4 and Table 4.

Discussion

Ingestion of toxic $CuSO_4$ potentially provides a specific vagal emetic stimulus because it is an oxidizing agent and corrosive to the mucous membranes of GIT (Horn et al. 2014). Emesis is persuaded by peripheral functions through the excitation of visceral afferent nerve fibers of the GIT by way of transmitting the stimuli to the vomiting center (Hossein et al. 2005; Bowman and Rand 1980). It has also been confirmed that the peripheral serotonin receptors (5-HT_3, 5-HT_4) (Fukui et al. 1993, 1994), NK_1 receptor (Ariumi et al. 2000) and H_1-histamine receptors (Katzung 2007) are engaged in emesis. And some other types of receptors, such as dopamine-type 2 (D_2) (Becker 2010), muscarinic acetylcholine receptors (M_1, M_2, M_3, M_4) within the CTZ are also stimulated at their own receptor sites and induce emesis (Kudlak and Tadi 2021; Hornby 2001). Our selected standard drug (DPD) functioned as a peripherally selective antagonist of dopamine receptors, especially D_2 and D_3 receptors, and ensured redemption by antagonizing or inhibiting the activity of the receptors at CTZ in the brain (Jacoby 2018). In our investigation, the DPD ingested group exhibited 6.40 (mean) retches in chicks and the mean of retches in the NC group was 70.60. On the other hand, $5HT_3$ receptors play a role in inducing emesis by transforming information in the GTI, and in the enteric nervous system they regulate peristalsis and bowel motility (Galligan 2002). And the $5HT_3$ antagonists such as ODN block the function of the receptor and provide relief from vomiting.

In this experiment, ODN and HYS (mAChRs antagonist) also reduced the number of retches in the chick group compared to the vehicle group. On the basis of experimental results, it can be hypothesized that PHY exerts a protective effect against toxicity by reducing or preventing nerve stimuli that are liable to induce emesis. Because both the groups of PHY remarkably diminished the number of retches in comparison with the NC group, the value is near to the value of the standard groups. But the potency of DPD is greater than the others in this experiment, and it is related to the D_2 receptor.

In pharmacology, when the combined effect of two or more medications is greater than the effects observed in the drug administered alone, it is called a synergistic effect, and this term is called synergism (Garcia-Fuente et al. 2018). The combined drug therapy in this study exhibited a lower number of retches and an elevated latency period in chicks, resulting in a synergistic effect. The study says that antiemetic drug therapy delayed nausea or vomiting against the emesis stimuli created by cancer chemotherapy or acute toxicity (Perwitasari et al. 2011). In our investigation, the latency of retching in seconds of the test groups was higher than the NC group, and the highest latency (sec) was observed in the combined group (DPD + THY-75).

Copper sulfate does not follow the vagal nerve stimulation; in a study, it was observed that vagotomy (cutting the end of the vagus nerve in GIT) could not stop emesis (Wang and Borison 1951; Niijima et al. 1987), so there could be

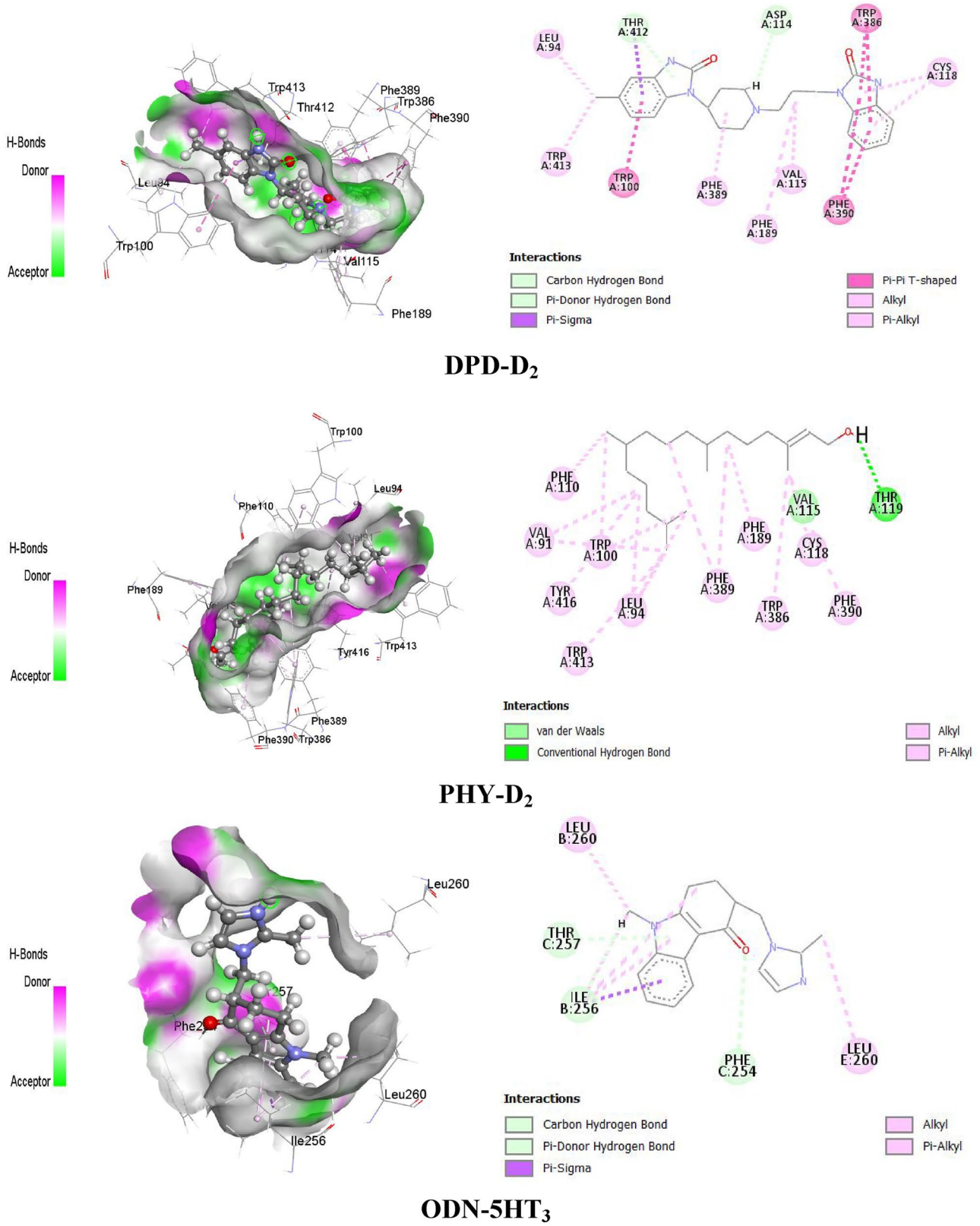

Fig. 4 Active sites and drug-receptor interactions among drugs (PHY and DPD) and receptors (D_2 and $5HT_3$)

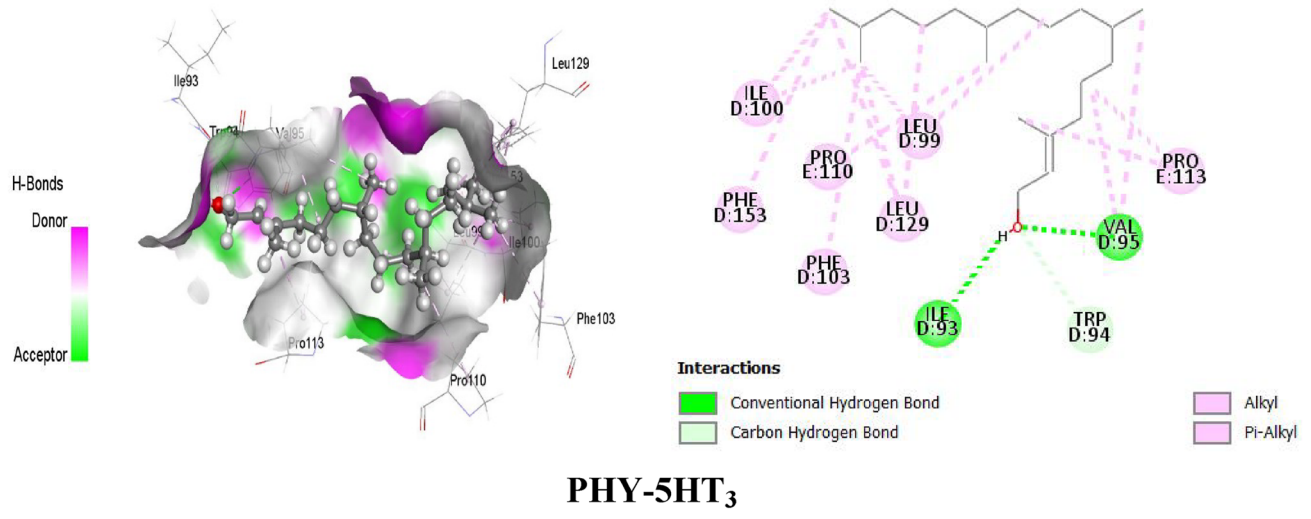

Fig. 4 (continued)

Table 4 Amino acid residues of non-bond interactions between the ligands and receptors

Drug-receptor complexes	Non-bond interactions		
	Amino acid residues	Bond type	
		HB	Others
DPD-D_2	Ser409	CHB	–
	Asp114:	–	Pi-anion
	Tyr408	–	Pi-Pi Stacked
	Ile184, Val91, Leu94	–	Alkyl
	Phe189, His393, Tyr408, Trp41, Leu94	–	Pi-alkyl
PHY-D_2	Thr119	CHB	–
	Val115	CaHB	–
	Val91, Leu94, Cys118	–	Alkyl
	Trp100, Phe110, He189, Trp386, Phe389, Phe390, Trp413, Tyr416	–	Pi-alkyl
ODN-$5HT_3$	Ile256, Phe254	CaHB	–
	Thr257	Pi-DHB	–
	Ile256	–	Pi-sigma
	Ile256, Leu260,	–	Alkyl
	Ile256	–	Pi-alkyl
PHY-$5HT_3$	Ile93, Val95	CHB	–
	Trp94	CaHB	–
	Leu99, Leu129, Val95, Pro113, Ile100, Leu129, Pro110,	–	Alky
	Phe103, Phe153	–	Pi-alkyl

HB Hydrogen bond, *CHB* Conventional hydrogen bond, *CaHB* Carbon hydrogen bond, *Pi-DHB* Pi-donor hydrogen bond

involvement of chemoreceptor signaling like the PHY follows in Fig. 5.

Molecular docking is a computational technique for exploring a suitable ligand that fits the receptor's binding site both geometrically and energetically (Kumar Bhardwaj et al. 2022; Sing et al. 2022a). Computational studies have recently opened up a new avenue for screening, designing, and developing drug candidates. It also reduces total evaluation time and animal and laboratory costs (Kumar et al. 2022; Sing et al. 2022b). The level of interaction

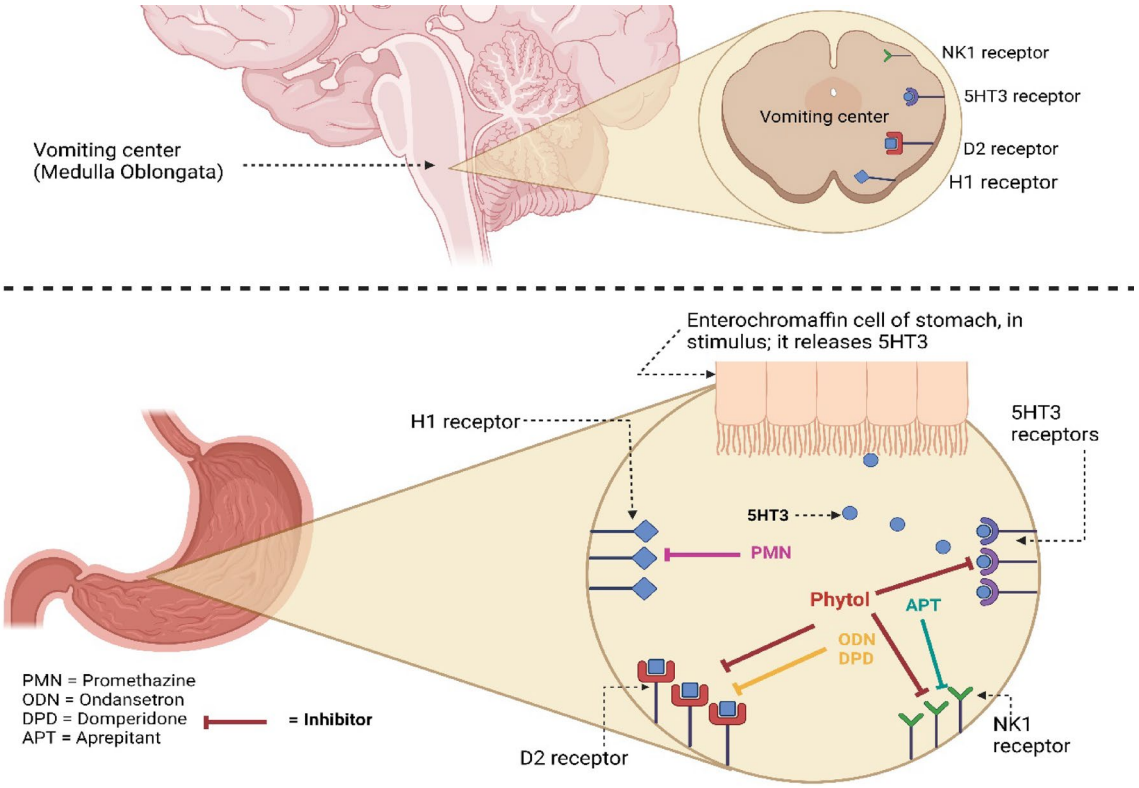

Fig. 5 Proposed anti-emetic mechanism of the test sample and reference drugs [This figure represents possible anti-emetic mechanisms of PHY, ODN, PMN, DPD and APT based on the binding affinity of these ligands with the H_1, $5HT_3$, NK_1, and D_2 receptors. Here, PHY act as inhibitor of $5HT_3$, NK_1 and D_2 receptors, whether ODN and DPD inhibited D_2 receptor, APT blocked NK_1 receptor and PMN acted on H_1 receptor. Antagonizing of these stomach receptors leads to no stimulation of the vomiting center (medulla oblongata), which results in no GIT contraction, no muscle contraction, and finally no emesis]

between ligand and receptor is estimated through binding affinity (Azam and Abbasi 2013). In this experiment, PHY expressed more elevated binding interactions with the D_2 receptor (PDB ID: 6CM4) than the other receptors responsible for inducing emesis. The binding energy of PHY required for interacting with D_2 is − 7.3 kcal/mol, where the standard DPD expressed the value of − 9.8 kcal/mol. As a result, its our view that PHY is more potent for dopaminergic receptors than the other receptors liable for emesis as the docking scores of PHY for D_2 receptor are higher than the other receptors as well as in vivo combined therapy with DPD demonstrated more activity than other combination.

Drug-likeness is an important parameter in the case of drug discovery and development, and it predicts qualitatively the possibility of a chemical compound becoming an oral medication with respect to bioavailability. It is estimated through the physicochemical properties of the drug, indicating drug nature related to pharmacokinetics (Daina et al. 2017). Lipinski's rule of five is widely used to predict drug-likeness and pharmacokinetics. According to Lipinski's rule of five, a drug candidate should have a molecular weight of not more than 500 g/mol, not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, and lipophilicity ($Log P_{o/w}$) within 5, and the acceptable range of violation of the rule is 0–1 (Lipinski 2004). According to Lipinski's rule, all the ligands are within the limits of becoming drugs though except PHY all are established drugs. According to our in silico investigation, the absorption properties of PHY are lower through GI than the standard except for APT, but it can be overcome through parental administration. The visualization of drug-receptor interaction estimates that the binding sites of PHY and DPD are more closely related to D_2 than the interaction of PHY and ODN with $5HT_3$ because some of the amino acid residues of D_2 are identical to those that form hydrophobic bonds with the drugs, such as Val91, Leu94, Phe189, and Trp413. Phe110, Phe390, Met117, Phe164, Cys118, Phe189, Trp386, Val190, and His394 residues of the D_2 receptor form a mostly hydrophobic pocket for dopamine (Kalani et al. 2004). Whereas, we found that PHY interacts with Cys118, Phr110 and Phe390 residues of D_2 which formed the same hydrophobic pocket for dopamine. Therefore, we predict that Cys118, Phe110 and Phe390 are the key residues involved in the antagonizing activity of PHY against D_2.

So, the result of this investigation revealed that the antiemetic activity of PHY against copper sulfate-induced emesis is due to its D_2 receptor antagonizing capacity, and the response is dose-dependent.

Conclusion

The results of this investigation demonstrate that PHY has significant antiemetic activity and the compound protects against $CuSO_4.5H_2O$-induced retching in chicks, perhaps by peripheral action. The molecular docking study confirmed that PHY has a higher affinity for dopamine receptors, especially D_2, than the other receptors liable for inducing emesis. The compound also has synergistic effects when combined with the established antiemetic drugs targeting different receptors. Based on the in silico ADMET analysis, it was also thought that the compound has good pharmacokinetics and drug-like properties. Taken together, PHY reduced $CuSO_4.5H_2O$-induced emesis in chicks in combination with DPD, suggesting its antiemetic potential, possibly through interacting with the D_2 receptor. PHY may be one of the plant-derived antiemetic agents. More research is needed to determine the optimal dose and exact mechanism in emesis caused by other causes.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval This article is according with to the international, national and institutional rules considering biodiversity rights.

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