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Identification of the multiple bioactive derivatives and their endogenous molecular targets that may mediate the laxative effect ofrhubarb in rats

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Keywords: **Rhubarb Laxative Anthraquinones Anthrones C-kit Aquaporin-3**

Abstract— The goal of this study is to use a bioinformatics and in vivo method to figure out how rhubarb (Rheum tanguticum Maxim. ex Balf.) causes rats to go to the toilet more often. Methods: Using high-performance liquid chromatography in conjunction with linear ion-trap quadrupole Orbitrap high-resolution mass spectrometry, substances originating from rhubarb that were found in the colorectum were identified. Using databases and the literature, we identified targets with the potential to have laxative effects. Then, we employed compounds derived from rhubarb in molecular docking modelling. Then, using western blotting, we assessed the expression of potential endogenous target molecules that bind specific rhubarb components in rats that were either given or not given rhubarb for constipation. In the end, the components of compounds that showed promise as bioactive were identified. We found 17 anthraquinones and 21 anthrones in the rat colorectum, which is a good indicator of the plant's medicinal value. Based on G-scoring, three proteins—c-kit, 5-hydroxytryptamine receptor 4 (5-HT4), and aquaporin-3 (AQP3)—may mediate the laxative action. Furthermore, ten components derived from rhubarb—aloe-emodin, emodin, rhein, chrysophanol, physcion, sennoside A, sennoside C, physcionanthrone, aloe-emodinanthrone, and rheinanthrone—were chosen as compounds with high probability of activity due to their strong binding affinity for multiple potential targets. It is possible that the laxative action of rhubarb extract is mediated by the fact that it enhanced the expression of c-kit and 5-HT4 while decreasing the expression of AQP3 in the colon of rats that were constipated. Additionally, we discovered that a single prototype component may undergo metabolism into many active metabolites, and that different molecules from the prototype can be combined to form a single active ingredient. The current research concludes that rhubarb's anthraquinones and anthrones may be metabolised into bioactive chemicals that enhance defecation via c-kit, 5-HT4, and/or AQP3.

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Key words: Al7075 alloy composite, E-glass, MMCs, and short E-glass fibres

Introduction

The Chinese herbal remedy rhubarb, scientifically known as Rheum tanguticum Maxim. ex Balf., is a popular choice for relieving constipation.1, 2 Sennoside A, the main laxative component of rhubarb, is metabolised to rheinanthrone by gut

But recent research has found that rhubarb only contains around 0.08% sennoside A,7,8 which is much too little to explain the plant's strong laxative effects. In addition, we found that rhubarb extract had a much stronger laxative effect than the same amount of sennoside A in our early trial. According to their research, the exact process by which rhubarb produces its laxative effects is still up for debate. Thus, we set out to better understand how rhubarb's laxative action works so that its therapeutic usage may be based on good theoretical grounding.

For clinically-approved laxatives, the two main ways they work are by increasing the rate of intestinal motility and the stimulation of electrolyte and water secretion. One popular medicine, prucalopride, increases colonic motility by stimulating neuronal 5-hydroxytryptamine receptor 4 (5-HT4), which in turn increases the contraction of the muscles lining the intestines.11th to 9th As an example, osmotic laxatives like magnesium sulphate control the expression of aquaporin-3 (AQP3) in colonic epithelial cells, which leads to water buildup in the intestinal lumen and promotes defecation.12, 13 Since these laxatives act on specific molecular targets in the colon, it is vital to identify these targets and the specific rhubarb components involved in order to deduce how rhubarb exerts its laxative effects.

Applying the notion of induced FIT and the lock-and-key principle,14 One efficient method for identifying therapeutic targets and quickly screening possible active molecules is molecular docking modelling. The sole applications for this are the screening of bioactive compounds and potential targets. We wanted to conduct molecular docking analysis utilising active, biotransformed rhubarb derivatives and endogenous molecules that could mediate the laxative action to discover the active compounds in the col-orectum. Our goal was to increase the data's biological relevance. We used the in vivo metabolic understanding of the chemicals in rhubarb to discover the appropriate bioactive derivatives in the prototype by tracing the biotransformation products back to the original plant.15 Establishing suitable quality control procedures and providing a reference for the use of rhubarb derivatives in the clinic would be greatly aided by the identification of bioactive components in rhubarb.

Our goal here was to find endogenous molecular targets that might modulate the actions of rhubarb derivatives that could have cumulative or synergistic effects to stimulate defecation. The first step was to use high-performance liquid chromatography in conjunction with linear ion-trap quadrupole Orbitrap high-resolution mass spectrometry to identify the rhubarb derivatives that were found in the colorectum. The next step was to find possible molecular targets by searching the literature, the Drug Bank Database (DBD), and the Therapeutic Target Database (TTD). As a further step, we ran molecular docking simulations with all of the rhubarb derivatives and all of the possible molecular targets to find binding partners. Next, we performed protein analysis of treated rats' colonic lysates to find out whether the molecular targets that were identified in silico were controlled in vivo. Lastly, the active elements in colorectum were identified by tracing them back to components of rhubarb, utilising knowledge of the metabolism of related chemicals. This study technique has greatly contributed to our understanding of the laxative mechanism of rhubarb by identifying essential components responsible for its therapeutic actions and their prospective molecular targets. Because herbal medicines include several bioactive components with various potential mammalian molecular targets, this technique might be valuable in future mechanistic investigations of these treatments.

Materials and methods

Ethical approval

The study protocols were approved by the Ethics Committee of Beijing University of Chinese Medicine (BUCM-4-2017102601- 1026; Beijing, China).

Chemicals and reagents

Rhubarb was provided by the Modern Research Center of Traditional Chinese Medicine, Peking University (Beijing, China).

Loperamide was purchased from Xian Janssen Pharmaceutical (Xi'an, China). Standard substances, including emodin (lot code: 110756-201512, 98.7%), rhein (lot code: 110757-201607, 99.3%), physcion (lot code: 110758-201616, 99.0%), aloe-emodin (lot code:110795-201710, 98.3%), chrysophanol (lot code: 110796-201721, 99.2%), sennoside A (lot code: 110824-201702, 95.2%), and senno- side B (lot code: 110825-201603, 95.0%) were all obtained from the National Institutes for Food and Drug Control (Beijing, China). Standard substances, including emodin-8-*O*-b-D-glucopyranoside (lot code: 23313-21-5, >95%), aloe-emodin-8-*O*-b-D-glucopyrano- side (lot code: 33037-46-6, >95%), and sennoside C (lot code:37271-16-2,

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>95%) were purchased from Chengdu Biopurify Phytochemicals (Sichuan, China). Mass spectrometry grade acetoni- trile, methanol, and formic acid were obtained from Thermo Fisher Scientific (Fair Lawn, NJ). Ultrapure water was obtained using a Synergy UV water purification system (Millipore, Billerica, MA).

Preparation of references and rhubarb extracts

We weighed and dissolved compounds in suitable volumes of 0.5% sodium carboxymethylcellulose to prepare reference samples(Supplemental Table 1).

The rhubarb herbal medicine (31.0 g) powder was weighed,
300 mL ultra-pure water was added, then the suspension was boiled for 20 min at 100 ˚C. The extracted solution was evaporated to 100 mL.

Rhubarb should be used at a dose of 0.05e0.25 g/kg/day in humans, according to the *Pharmacopoeia of the People's Republic of China* (2015).[16] According to the doseeconversion relationship be- tween human and rat, the dose for rats was 0.31e1.54 g/kg/day. Inour previous study, we showed that the administration of 1.54 g/kg/ day of a rhubarb extract for 3 days exerted an effective laxative in rats.

Rat model of constipation and rhubarb administration

Thirty rats were acclimatized for 7 days, then allocated to threegroups: control, model, and treatment. The control group was administered saline solution, and the model and treatment groups were administered loperamide at a dose of 1.5 mg/kg/day twice daily for 7 days by gavage to induce constipation. During the first 6days, the wet and dry masses of the fecal pellets produced by the rats were measured each day.[17]

After the model of constipation had been successfully estab- lished, the treatment group was fed with rhubarb extract at a dose of 1.54 g/kg/day for 3 days, during which the other groups were administered substances as above. The evacuation index (EI) was used to evaluate the laxative effect of rhubarb.[18] In addition, the feces of rats in the treatment group were collected for analysis. All the rats were sacrificed on the 10th day, their mid-colons were collected, snap-frozen in liquid nitrogen, and stored at 80 ˚C until western blot analysis.

Animals

Specific pathogen-free male Sprague-Dawley rats weighing 200 ± 20 g were obtained from Beijing Vital River Laboratory An- imal Technology (Beijing, China) and maintained at a constant temperature (23 ± 2 ˚C) and humidity (60 ± 5%).

Identification of rhubarb derivatives in feces

Preparation of samples

Fecal samples were collected over a 24-h period from another 20Sprague-Dawley rats that had been administered solutions of 10 reference substances. These feces were dried in a ventilator, crushed, and then placed in a centrifuge tube. Fecal samples weighing 20 mg were extracted using 200 mL 80% methanol and vortex-mixed for 30 min. Then, the samples were centrifuged at 13 522×*g* at 4 ˚C for 15 min and the supernatant was evaporated to dryness under nitrogen. The residue was reconstituted with 200 mL of 50% methanol, vortex-mixed for 3 min, then centrifuged at 13 522×*g* at 4 ˚C for 10 min.

Chromatography and MS conditions

We used previously optimized chromatographic and MS conditions.[19]

Data processing

The raw mass data generated by the HPLC-LTQ-Orbitrap MS analysis were processed using Qual Browser of Xcalibur 2.1 (Thermo Fisher Scientific, San Jose, CA). Online chemical databases (http://www.chemspider.com/and https://www.ncbi.nlm.nih.gov/) were used to identify the compounds obtained. The identification of potentially novel compounds was accomplished using the Sci- Finder database (https://sso.cas.org/).

Creation of a laxative target database

First, we used the TTD (https://db.idrblab.org/ttd/) to identify molecular targets for the treatment of constipation and the Drug Bank Database (https://www.drugbank.ca/) to summarize the tar- gets of existing laxatives. Then, we searched the literature to further screen for molecular targets with a possible laxative effect.

Homology modeling

The target sequences of 5-HT4 and AQP3 were acquired from Uniprot (http://www.uniprot.org/; Uniprot IDs Q13639 [5-HT4] and Q92482 [AQP3]). Template crystal structures for these target se- quences were identified using BLAST and downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB, http://www.rcsb.org/), using the PDB IDs of 6H7Jfor 5-HT4 and 6F7H for AQP3.[20] MOE v2014.0901 software (Mo- lecular Operating Environment (MOE); Montreal, Canada) was usedfor homology modeling. The QuickPrep module in MOE was used to determine the likely sites of protonation of the proteins and the orientation of hydrogen atoms in pHs up to 7 and temperatures upto 300 K. First, the target sequence was aligned with the template sequence, and 10 independent intermediate models were estab- lished. These models, with differing homologies, were the result of permutational selection of different loop candidates and side-chain rotamers. Then, we selected the intermediate model with the highest generalized born/volume integral score as the final model, which was subjected to further energy minimization using AMBER10: EHT force field (MOE).

Molecular docking between rhubarb derivatives in the colorectumand molecules mediating a laxative effect

The 2D structures of the rhubarb derivatives were drawn in ChemBioDraw 2014 and chemically standardized, which consistedof the addition of hydrogens, ionization at pHs from 5.1 to 9.1, and the formation of stereoisomers and effective single 3D conforma- tions using the LigPrep module in Maestro (version 9.4, Schrödinger).

The 3D structures of the proteins were downloaded from RCSB PDB. In addition, homology modeling was conducted for proteinstructures that were not found in the database. The

3D structure ofc-kit was downloaded from the RCSB PDB (PDB ID: 6GQK),[21] and those of 5-HT4 and AQP3 were obtained by homology modeling.

The Glide module in Schrödinger v2015.09 was used for molecular docking modeling and to predict the binding affinity of ligands for target proteins. The structures of the proteins were manipulated using the "Protein Preparation Wizard" workflow in Maestro. The main operations were exclusion of water molecules, protonation, and optimization via OPLS_2005 force field. The Glide module was used to generate a grid file for each protein receptor, todetermine the location of the active site of the protein, in order to model the binding of ligands and proteins. The grid enclosed a box centered on the binding site with dimensions of 10 10 10 Å. A scaling factor of 0.8 was used for the van der Waals radii of receptor atoms with a partial atomic charge of <0.15.

An extra-precise Glide docking procedure (Glide-XP) was employed to model the docking of the compounds to the binding sites of the proteins and the optimal orientation of the compound, determined using the Glide scoring function (G-score), was recorded. The more negative the G-score is, the stronger is the expected binding affinity of the compound and receptor. Molecular graphics were generated using PyMOL (https://www.pymol.org/).

Western blotting

Protein lysates of colonic tissue were prepared using RIPA Lysis Buffer (Solarbio, Beijing, China) containing protease inhibitors. The protein content of each was quantified using the bicinchoninic acid method (Beyotime, Shanghai, China), then the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10% polyacrylamide gels, and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). The membranes were then blocked with 5% skimmed milk powder (BioRuler, Danbury, CT) in tris-buffered saline for 2 h, and incubated overnight with primary antibodies targeting c-kit (sc-365504, 1:100; Santa Cruz, San Francisco, CA), 5-HT4 (TA323344, 1:200; Origene, Rockville, MD), AQ3 (ab125219, 1:1000; Abcam, Cambridge, UK), or glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:20 000; Proteintech, Chicago, IL) at 4 °C. The membranes were then incubated with a secondary antibody, after three washes. Specific protein bands were detected using Enhanced chemiluminescence solution (Proteintech) and imaged using a ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA).

Statistical analysis

Data are shown as means (SEMs). Student's *t*-test was used to compare data between the two groups. $P < .05$ was accepted as indicating statistical significance. Data analysis was performed using GraphPad Prism 8.0 software (www.graphpad.com).

Results

Laxative effect of rhubarb extract in constipated rats

To confirm the laxative effect of rhubarb, we administered rhubarb extract to Sprague-Dawley rats in which constipation had been induced by loperamide gavage for 7 days. Loperamide is commonly used to increase mucosal contact time, which has the effect of inducing additional absorption of electrolytes and water, thereby reducing fecal mass and the number of bowel movements. Twenty-four-hour fecal water content was monitored for the first 6 days of loperamide administration, and a reduction was identified in the model group from the second day (Fig. 1A), which confirmedthat constipation had been successfully induced. Then, rhubarb

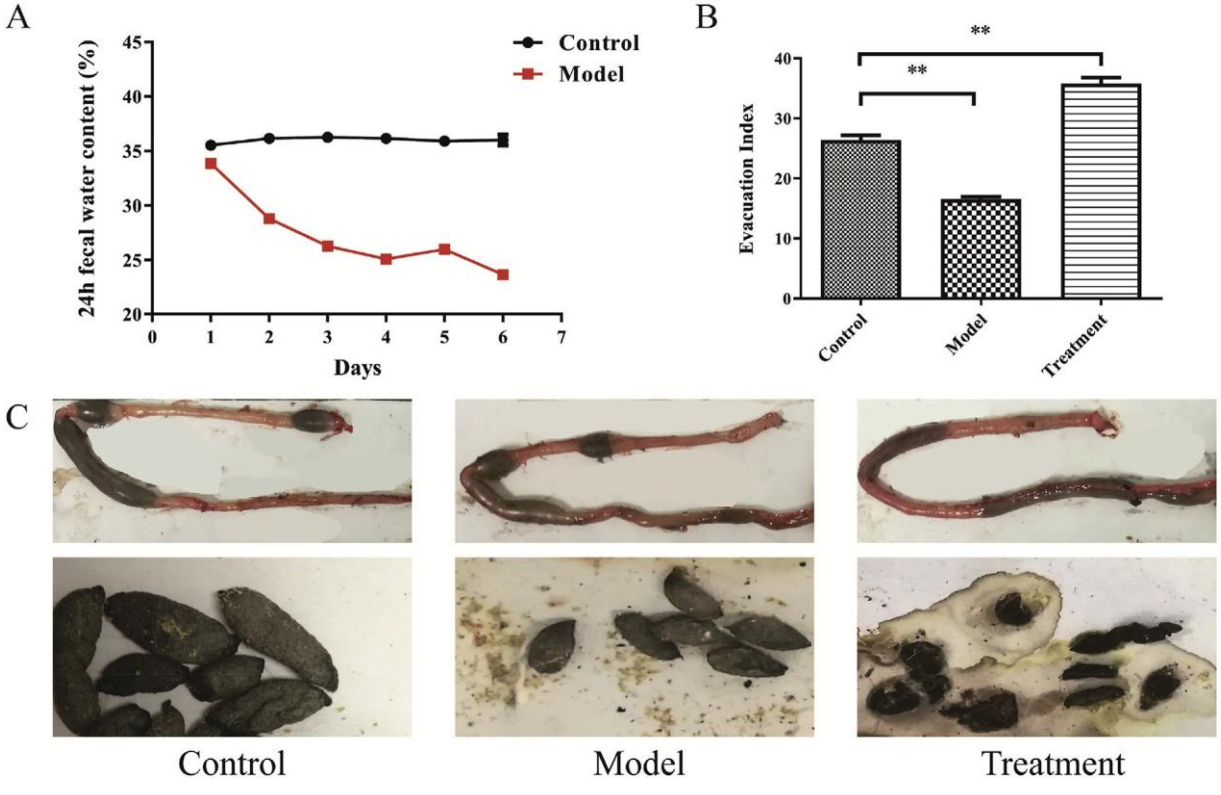

Fig. 1. Laxative effect of rhubarb extract in constipated rats (A) The 24-h fecal water content of the control and model groups (B) The evacuation indices of the three groups (C)

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Thecontents of the colorectum and the shape of the feces.
Note: Data are presented as the mean (SEM), n ¼ 10. **P < .01 vs. the control group.

extract was administered to the treatment group for 3 days. A significant increase in EI occurred in this group (Fig. 1B), which confirmed that rhubarb extract has a laxative effect in constipated rats. We then compared the water content of the colorectal con- tents of the control, model, and treatment groups (Fig. 1C) and found that the rhubarb extract increased this. These results imply that rhubarb may have its laxative effect by increasing the retention of water in the colorectum.

Identification of rhubarb derivatives in rat feces

We used HPLC-LTQ-Orbitrap MS to identify the derivatives of rhubarb in the colorectum that might contribute to its laxative ef- fect. Using the established strategy of characteristic fragment filtration for the detection of rhubarb derivatives,[19,22] a series of anthraquinones and anthrones were identified. The identification process can be illustrated using the example of the metabolite emodin-*O*-glucuronide (Fig. 2). The full ion chromatogram of a fecal sample is shown in Fig. 2A and the extract ion chromatogram for emodin-*O*-glucuronide is shown in Fig. 2B. In negative mode, the diagnostic ion fragments of *m/z* 269.0451 and 225.0552 were detected, which suggested that the skeleton of the target com-pound was the same as that of emodin (Fig. 2C). In addition, the detection of a quasi-molecular ion of *m/z* 445.0765 [M H], which differs from the expected *m/z* 269.0451 for emodin by 176.0814 Da, implied that the compound was emodin-*O*-glucuronide (Fig. 2D). Overall, 38 derivatives of known rhubarb components, including 17 anthraquinones and 21 anthrones, were identified in colorectal samples, including prototypes and various metabolites (Fig. 3), andthe compound identification data are presented in Supplemental Tables 2–4.

Construction of a target protein database

To determine the mechanism of the laxative effect of rhubarb, the molecular targets of clinically-approved laxatives were identi- fied using the TTD, Drug Bank Database, and published literature. As shown in Supplemental Table 5, there are 17 types of molecular target for laxatives. All of these, which include channel proteins and receptors, have been identified to mediate effective treatments for constipation. For instance, magnesium sulfate, which is classified asan osmotic laxative, increases AQP3 expression in the epithelial cells, which has the effect of retaining water in the intestinal lumen and promoting defecation.[23] In addition, linaclotide is a secreta- gogue that activates the guanylate cyclase-C receptor on intestinal epithelial cells to increase fluid secretion into the intestinal lumen.[24,25] Some previous studies have shown that the number of interstitial cells of Cajal (ICCs) is lower in the colons of patients withslow-transport constipation,[26,27] and c-kit is a specific marker of ICCs, which have been shown to be intestinal pacemaker cells.[28] Finally, prucalopride, a highly selective 5-HT4 receptor agonist, directly activates afferent neurons and improves intestinal motility.[29,30] The creation of a molecular target database for laxa- tives provided a tool for us to identify potential targets and bioac- tive derivatives of rhubarb compounds for the treatment of constipation.

Candidate colorectal molecular targets and potentially bioactive compounds

To screen the candidate targets and bioactive rhubarb de- rivatives, molecular docking modeling was performed using 38rhubarb derivatives from the colorectum and 17 endogenous

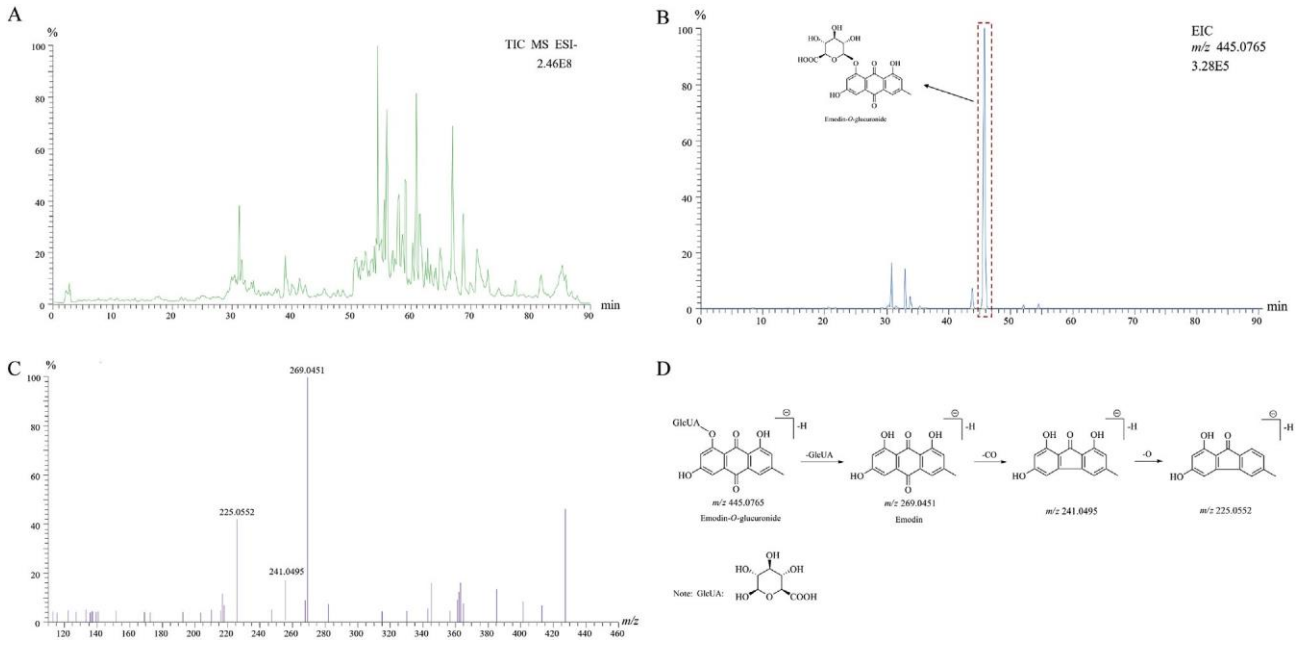


Fig. 2. The identification process for emodin-*O*-glucuronide (A) The total ion chromatogram of a fecal sample (B) The extract ion chromatogram (C) Mass spectrometry (MS)/MSspectrum (D) The identification of an ion with *m/z* 445.0765 in the fecal sample.

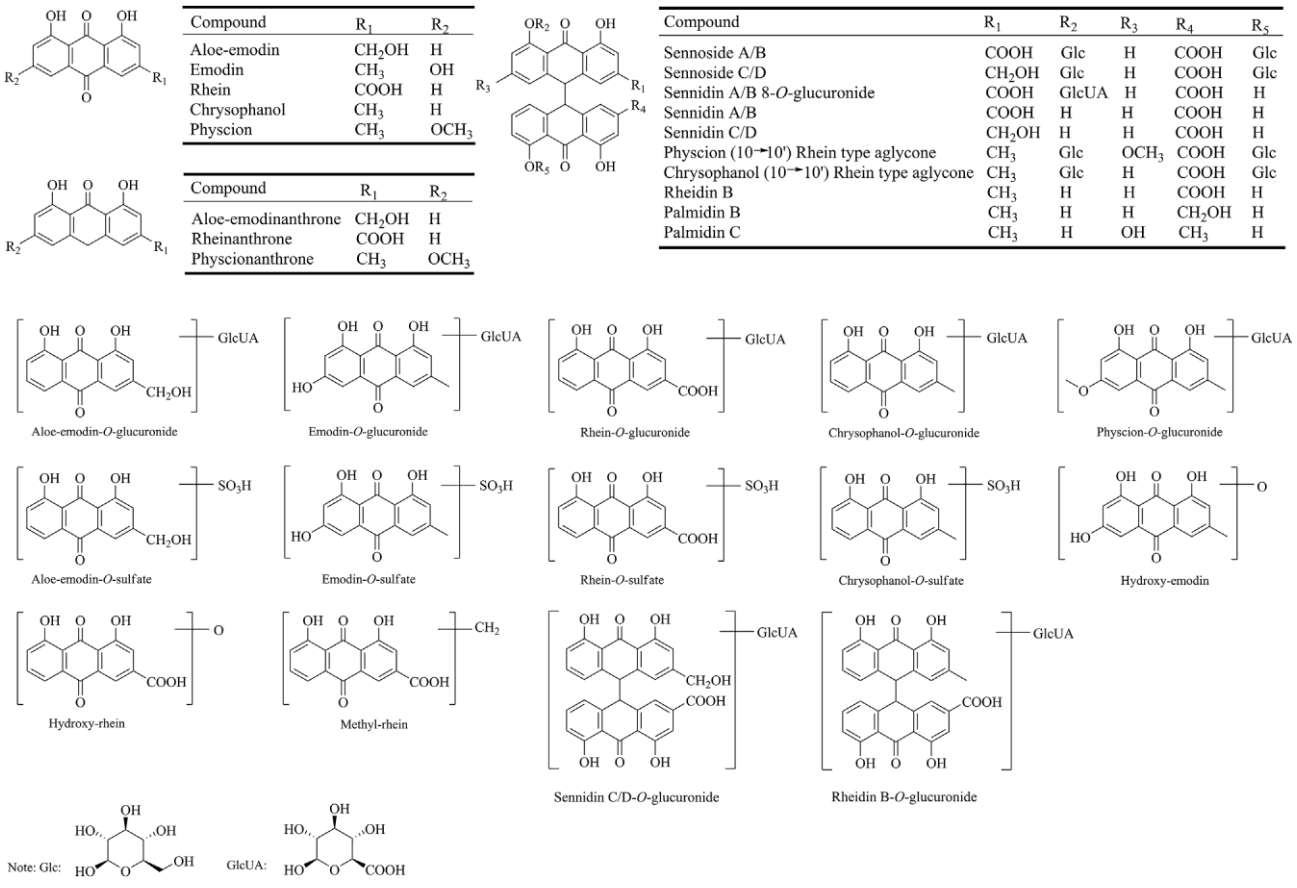
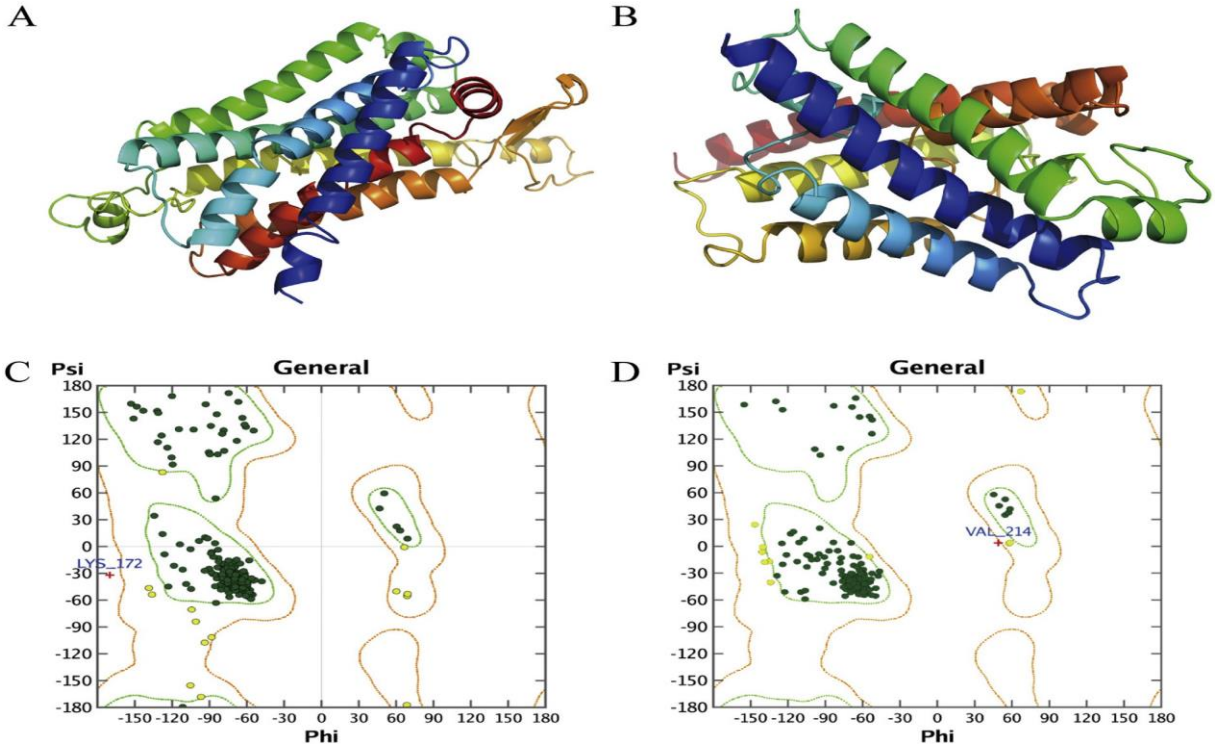


Fig. 3. Structures of the 38 rhubarb derivatives identified in fecal amples.

molecules that mediate laxative effects. First, homology modelingwas conducted to predict the structures of proteins that were not found in the database, such as 5-HT4 and AQP3. The modelsgenerated for 5-HT



4 and AQP3 are depicted in Fig. 4A and B,

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Fig. 4. Structural modeling of molecules mediating laxative effects (A) Homology model of 5-HT4 (B) Homology model of AQP3 (CeD) Ramachandran plot for 5-HT4 and AQP3. Darkgreen dots represent the residues in favored regions, yellow dots represent the residues in permitted regions, and red crosses represent the residues in irrational regions. Abbreviations: 5-HT4: 5-hydroxytryptamine receptor 4; AQP3: aquaporin-3.

respectively. Ramachandran plots for 5-HT4 and AQP3 showed thatmore than 99% of their amino acid residues were in permitted regions, which indicates that the predicted 3D structures were reasonable (Fig. 4C and D). Then, on the basis that the more negative the G-score is, the stronger is the binding affinity between

Table 1
G-scores for the binding of the top-ranked rhubarb derivatives with their potentialmolecular targets.

No.	Ligand	Receptor (kcal/mol)		
		c-kit	5-HT4	AQP3
1	Aloe-emodin	−8.476	/	−5.289
2	Emodin	−9.378	−5.541	−5.743
3	Rhein	−8.453	−5.963	−5.270
4	Chrysophanol	−8.530	−6.221	−5.514
5	Physcion	−8.275	−5.241	−5.987
6	Sennoside A	−6.854	−8.891	/
7	Sennoside C	−9.019	−8.810	/
8	Physcionanthrone	−9.069	−5.246	−6.809
9	Aloe-emodinanthrone	−9.278	−5.163	−6.165
10	Rheinanthrone	−9.747	−5.914	−5.763

Abbreviations: 5-HT4: 5-hydroxytryptamine receptor 4; AQP3: aquaporin-3.

the compound and receptor, we selected candidate targets and rhubarb derivatives. The G-scores are listed in Table 1, and show that three molecules, c-kit, 5-HT4, and AQP3, represented candi- date targets. Furthermore, 10 rhubarb derivatives (aloe-emodin, emodin, rhein, chrysophanol, physcion, sennoside A, sennoside C, physcionanthrone, aloe-emodinanthrone, and rheinanthrone), were found to have strong binding affinities for the three targets, and were therefore selected as potentially bioactive rhubarb derivatives.

To gain insight into the binding affinity of rhubarb derivatives for the binding sites of the three candidate molecular targets, the ligandereceptor interactions were analyzed *in silico*, and we found that all 10 of the derivatives are likely to bind strongly to c-kit. Multiple oxygen atoms in hydroxyl and carbonyl groups in the basic skeleton of the anthraquinones and anthrones were shown to be located in the binding site and to form hydrogen bonds with the Cys673, Lys623, Glu671, and Leu595 residues of c-kit. The com- pound with the strongest predicted binding affinity for c-kit was rheinanthrone (9.747 kcal/mol). The predicted interaction is shown in Fig. 5A, which involves three hydrogen bonds and one H- p conjugation: the oxygen atoms at the 9- and 3-sites of the carbonyl group, and the oxygen atom at the 1-site of the hydroxyl

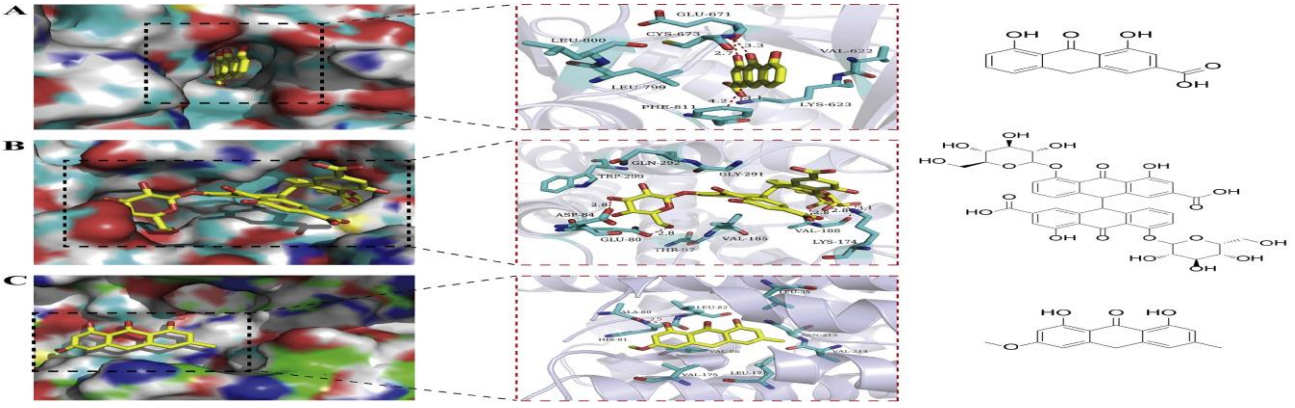

Fig. 5. Interaction models for the three candidate molecular mediators of the laxative effect of rhubarb. The interaction model for rheinanthrone and c-kit (A), sennoside A and 5-hydroxytryptamine receptor 4 (B), and physcionanthrone and aquaporin-3 (C). The ligands are yellow and the surrounding residues in the binding pockets are cyan. The backbone of the receptor is depicted as a light blue ribbon.

group in rheinanthrone formed hydrogen bonds with the Cys673, Lys623, and Glu671 residues of c-kit, respectively. In addition, the carbon atom at the 4-site in rheinanthrone formed an H-p conju- gation with the Phe811 residue of c-kit.

Sennoside A and sennoside C were shown to have the strongest binding affinities with 5-HT4 of the bioactive derivatives. Theywere predicted to bind via hydrogen bonds to the Asp84, Val188, Lys174, Glu80, and Asn279 residues of 5-HT4. The strongest pre- dicted binding affinity for 5-HT4 was that of sennoside A(8.891 kcal/mol). The five hydrogen bonds are shown in Fig. 5B: the oxygen atom at the 8-site of the hydroxyl group in the glucose group of sennoside A formed a hydrogen bond with the Glu80 and Asp84 residues of 5-HT4, separately. In addition, two oxygen atomsof at the 8^{0} site of the hydroxyl group in the glucose group con- nected with the backbone and side-chain of Val188, and the oxygenatom at the 9^{0} site of the carbonyl group of sennoside A interacted with the Lys174 residue of 5-HT4.

However, in contrast to the situation with c-kit and 5-HT4, in which the bioactive compounds interacted with amino acid resi- dues via numerous hydrogen bonds, AQP3 was predicted to have few, weak interactions with the rhubarb derivatives. The strongest binding affinity with AQP3 was shown by physcionanthrone (6.809 kcal/mol): the oxygen atom at the 1-site of the hydroxyl group in physcionanthrone formed a hydrogen bond with the Ala80residue of AQP3 (Fig. 5C). Taken together, the results of the mo- lecular docking modeling showed that various oxygen atoms of basic skeletons and glucose groups of the 10 bioactive compounds derived from rhubarb can form hydrogen bonds with the three molecules that mediate laxative effects.

Effects of rhubarb extract on the expression of c-kit, AQP3, and 5-HT4 in the colons of rats

In order to increase the biological relevance of the molecular docking data, the effects of rhubarb extract on c-kit, 5-HT4, and AQP3 expression were evaluated by western blot analysis. Colonic lysates prepared from rats in the control, model, and treatment groups were prepared and immunoblotted for each of the proteins.As shown in Fig. 6, the expression of c-kit and 5-HT4 was much lower, and that of AQP3 was higher in model rats than in the controlgroup. When constipated rats were treated with rhubarb extract, these changes in expression were all reversed. The increase in c-kitexpression in the colon would tend to maintain the phenotype of ICCs, thereby promoting intestinal motility.[31,32] In addition, the

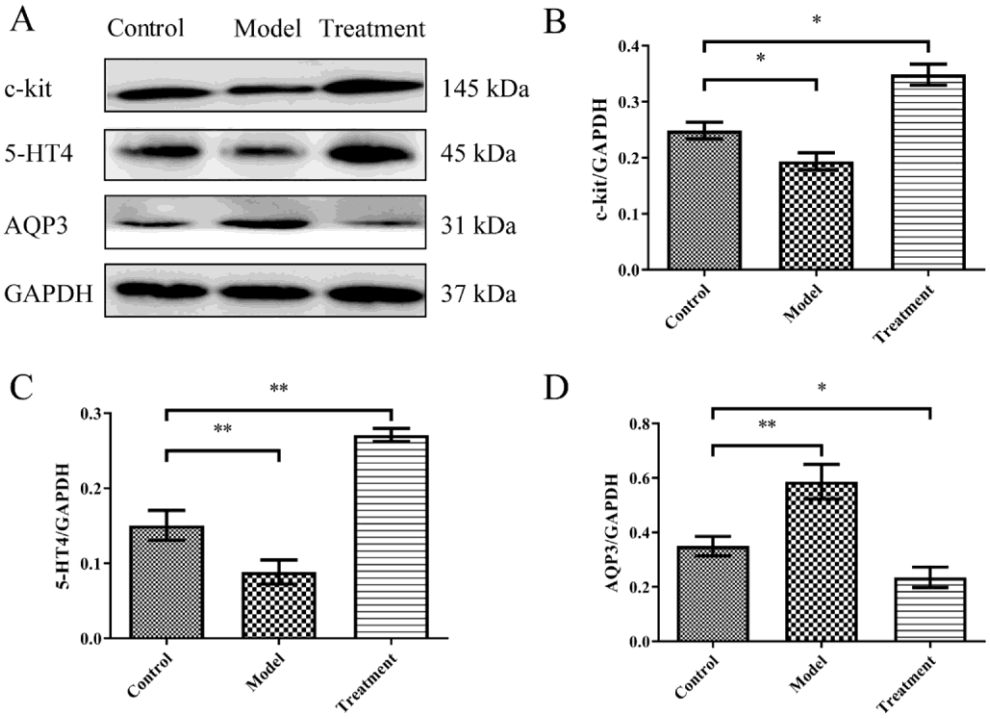

Fig. 6. The effects of rhubarb extract on the expression of c-kit, 5-HT4, and AQP3 in the colons of rats (A) c-kit, 5-HT4, and AQP3 protein expression in the colon, measured usingwestern blotting (BeD): c-kit, 5-HT4, and AQP3 protein expression, normalized to that of GAPDH, respectively.
Note: 5-HT4: 5-hydroxytryptamine receptor 4; AQP3: aquaporin-3; GAPDH: glyceraldehyde 3-phosphate dehydrogenase. Data are expressed as mean (SEM). n ¼ 3. *P < .05,
**P < .01 *vs*. the control group.

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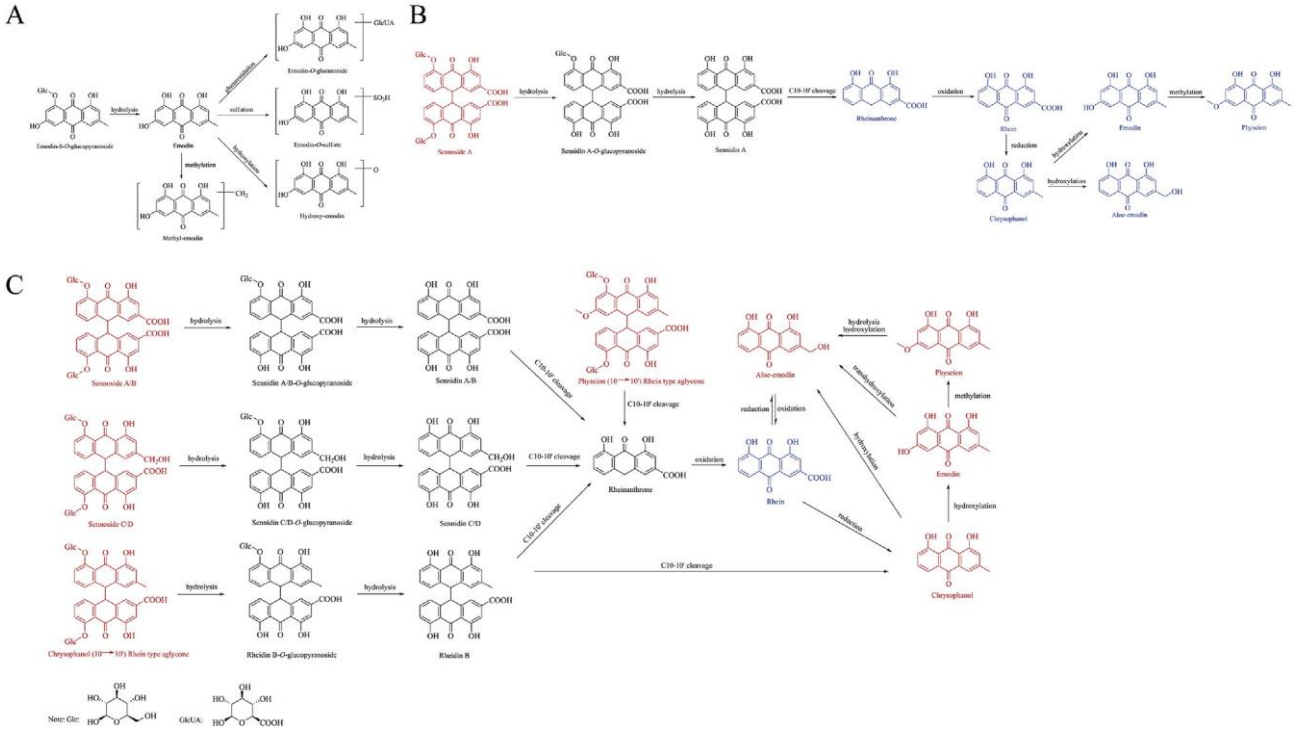

Fig. 7. Potential metabolic pathways for the anthraquinones and anthrones (AeC) Biotransformation of emodin, sennoside A, and rhein as a product of the metabolism of various prototype compounds. The prototype components are shown in red and the active metabolites are shown in blue.

increase in 5-HT4 expression in the colon would be expected to increase intestinal transport.[11,33,34] Finally, and as expected, lower expression of AQP3 was shown in the colon of treated rats, which would be expected to inhibit water absorption, which would also have a laxative effect.[35–37] Thus, rhubarb extract regulates the expression of the identified potential mediators of a laxative effect.

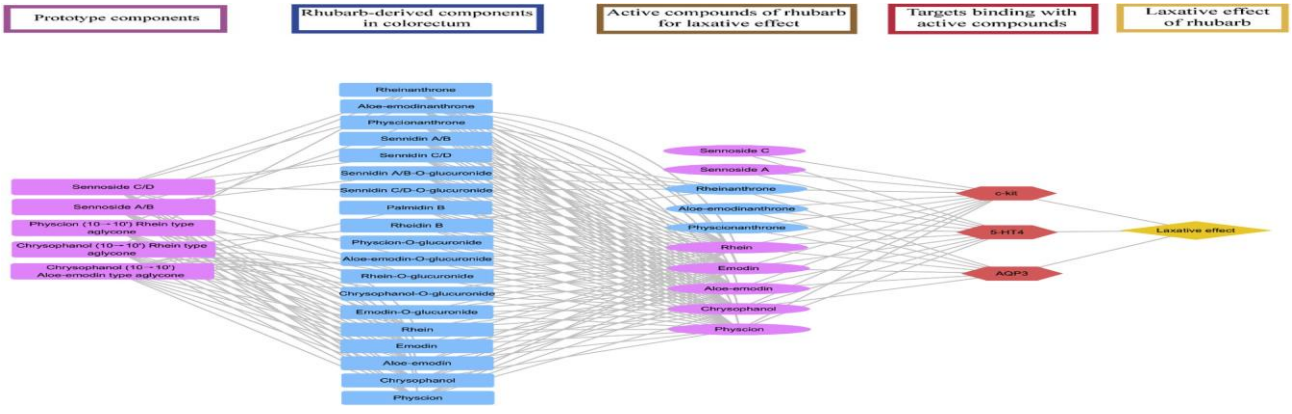

Fig. 8. Network describing the prototype components of rhubarb, their colonic derivatives, and the endogenous molecular targets potentially mediating a laxative effect. Theprototype components are shown in pink and the rhubarb-derived components present in the colorectum are shown in blue.

Tracing of the candidate bioactive derivatives back to the components of rhubarb

To trace the biotransformation products to its prototype, we explored the *in vivo* metabolic transformation of the compounds in rhubarb. We studied the metabolism of 10 representative com- pounds, including both anthraquinones (emodin, rhein, physcion, aloe-emodin, chrysophanol, emodin-8-*O*-b-D-glucopyranoside, aloe-emodin-8-*O*-b-D-glucopyranoside) and anthrones (sennoside A, sennoside B, and sennoside C). As shown in Fig. 7A,anthraquinone-type components are metabolized to glycosides *in vivo*, and the principal metabolic pathways involved are hy- droxylation, methylation, glucuronidation, and sulfonation. For anthrone-type compounds (Fig. 7B), the glucose group is typically lost as a result of hydrolysis. In addition, it was predicted that anthrone-type compounds would principally undergo C10eC10'bond cleavage *in vivo* and be further metabolized to anthraquinone-type metabolites. Overall, similar metabolic pat- terns were predicted for the 10 representative compounds. We found that a prototype component, for instance sennoside A(Fig. 7B), may be metabolized to form several active metabolites (rheinanthrone, emodin, chrysophanol, aloe-emodin, rhein, and physcion), and a single bioactive molecule, such as rhein (Fig. 7C), can also be generated from various prototype compounds (senno- side A, sennoside B, sennoside C, physcion [10 ◢ 10^0] rhein type aglycone, chrysophanol [10 ◢ 10^0] rhein type aglycone, aloe- emodin, emodin, physcion, and chrysophanol).

Finally, using this information regarding the likely metabolic pathways involved, the active compounds identified in colorectal contents were traced back to the prototype components of rhubarb. As shown in Fig. 8, anthraquinone derivatives, anthrone derivatives, and their glucosides in rhubarb can all be metabolized to generate the 10 potentially bioactive compounds identified, which have the potential to provide three complementary molecular mechanisms for the laxative effect.

Discussion

In the present study, we have used an MS-based approach and molecular modeling to determine the mechanisms involved in the laxative effect of rhubarb. We have shown that anthraquinone and anthrone derivatives in rhubarb can be metabolized in the rat in- testine to generate various bioactive compounds, which are all capable of regulating molecular targets in the colon that mediate the laxative effects of known drug substances (Fig. 9). Thus, de- rivatives of rhubarb may have additive or synergistic effects to promote defecation.

This strategy represents a valid approach to ensure quality control and to provide a reference for the rational medicinal use of rhubarb in the clinic. Because of the complexity of potential me- dicinal compoundetarget interactions, the identification of inter- action domains is still in its infancy. However, high-affinity ligandereceptor interactions can be validated using surface plas- mon resonance studies,[38,39] and his method may be used to

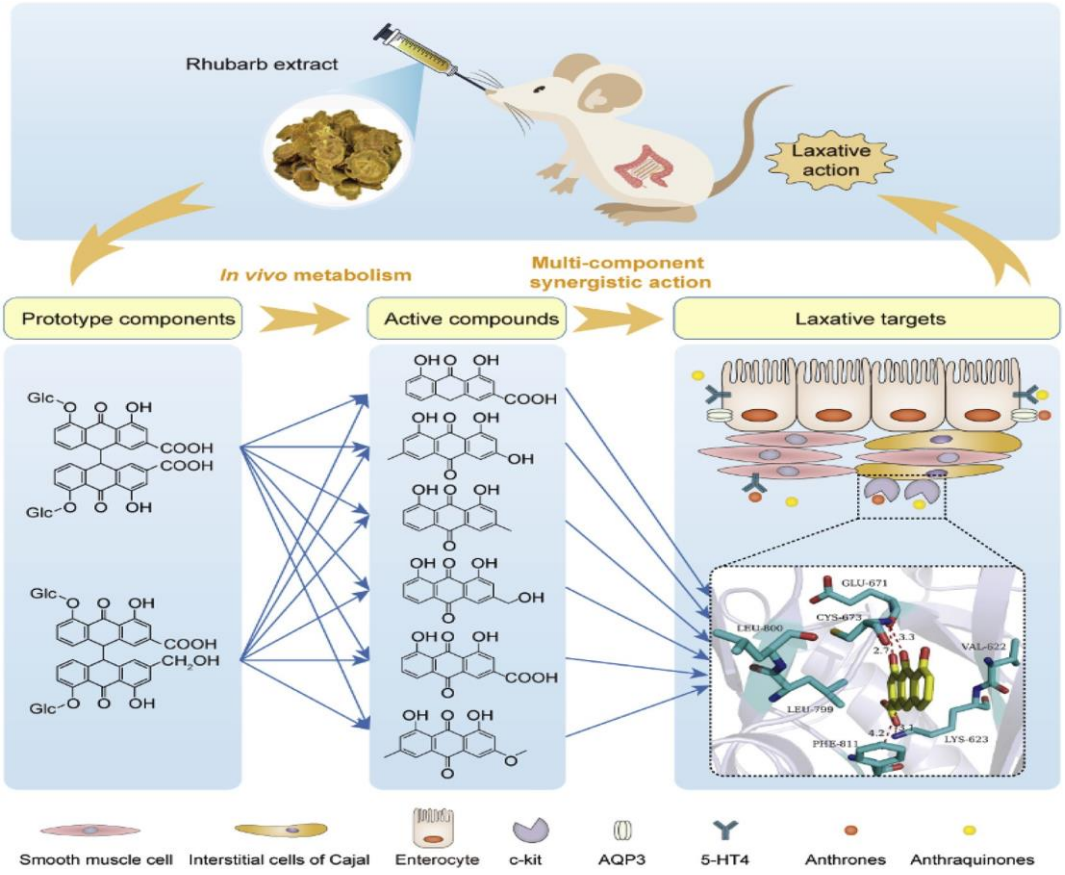

Fig. 9. Summary of the study findings.
Abbreviations: 5-HT4: 5-hydroxytryptamine receptor 4; AQP3: aquaporin-3.

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further characterize the molecular mechanisms involved in thelaxative effect of rhubarb and to identify lead compounds for further investigation.

Most herbal medicines contain numerous components that may be bioactive or metabolized to form bioactive derivatives, such as flavonoids, alkaloids, and organic acids. Rhubarb is a common Chinese medicine that also contains several components of this type, including anthraquinone derivatives, anthrone de- rivatives, glucosides, tannins, and organic acids. In the present study, rhubarb was used as an example to illustrate an approach tothe determination of the mechanisms of a medicinal effect of a plant product. In it, we identified bioactive compounds, deter- mined their origin in the plant, and identified potential binding partners that are known mediators of the medicinal effect. This strategy can provide insights into the mechanisms whereby multiple components of herbal medicines may have additive or synergistic effects, exerted via multiple molecular targets, to achieve a therapeutic endpoint.

Conclusion

By binding to c-kit, 5-HT4, and/or AQP3, the current research showed that bioactive chemicals with a laxative action might be generated by metabolism of anthraquinones and anthrones in rhubarb. It was indicated using molecular docking modelling that five anthraquinones (aloe-emodin, emodin, rhein, chrysophanol, and physcion) and five anthrones (sennoside A, sennoside C, physcionanthrone, aloe-emodinanthrone, and rheinanthrone) interact with c-kit, 5-HT4, and AQP3. Importantly, we have shown that rhubarb extract, which is believed to mediate the laxative action of the plant, raised the expression of c-kit and 5-HT4, while reducing that of AQP3 in the rat colon. Additionally, we discovered that a single proto-type component in rhubarb, like sennoside A, can be metabolised into multiple active metabolites (rheinanthrone, emodin, chrysophanol, aloe-emodin, rhein, and physcion), as well as one active ingredient,

compounds including physcion, emodin, chrysophanol, aloe-emodin, sennoside C, and sennoside A are potentially potential building blocks for compounds like rhein. Our results suggest that rhubarb's laxative action could be due to the interaction of several derivative chemicals with various molecular targets in the colon, which might lead to cumulative or synergistic effects.

References

1. 1. Huang Q, Lu GD, Shen HM, Chung MC, Ong CN. Rhaquinones from rhubarb and their anti-cancer effects. Medical Research Review. 2007;27(5):609–630. Presented by Matsuda, Morikawa, Toguchida, Park, Yoshikawa, and Harima. New anthraquinone glucoside compounds and structural criteria for stilbene activity: anti-oxidant components derived from rhubarb. The published article is from 2001 and is titled "Bioorg Med Chem." it covers pages 41–50.

3. Leng-Peschlow E. Sennosides and similar substances induce rats to have a larger intestinal transit time that is accelerated. The article was published in the Journal of Pharmaceutical Pharmacy in 1986 and ran from page 369 to 373.
The effects of sennosides on canine colonic motility: proof of a prostaglandin mediator (Staumont G, Fioramonti J, Frexinos J, Bueno L., 2004). Endocrinology. 1988;29(9):1180e1187.
Sakai, Yamauchi, and Kuwano (2019). 5. Activation of sennoside A metabolism in mice.
Botanical Medicine, 1979, 37(4): 370–378.
6. Vand Hoestenberghe A, Geboes K, Nijs G, Lemli J, Eyssen H, De Witte P. In germ-free rats, the effects of rhein and rhein anthrone on the transfer of intestinal fluid and large intestine transit. Article published in 1992 in the European Journal of Pharmacology, volume 212, pages 121–123.
The authors of this work are Liu YH, Huang ZH, Dong L, Pei WX, Sun Y, and Gao XY. Rhei Radix et Rhizoma's 14 constituents were all measured simultaneously using a high-performance liquid chromatography system. [Chinese] Zhongguo Zhongyao Zazhi. 2017;42(23): 4514e4519.
Ito K, Yamasaki K, Tagami T, Sawabe Y, Takatori S. eight. Quick and easy determination of sennoside A and sennoside B in unprocessed pharmaceuticals and

pharmaceutical byproducts using solid-phase extraction and HPLC. Proceedings of the National Academy of Sciences. 2010;64(2):126–132.
This is the ninth work by Ma, Qi, Xu, Dong, and Yang. How emodin causes smooth muscle cells in the colon to contract: the signal channels involved. Journal of the World Gastrointestinal and Hepatology, 2004;10(10):1476–1479.
10. Huang YH, Liu MT, Gershon MD, Wang JW, Hen R. The enteric nervous system of adult mice: 5-HT4 receptor-mediated neuroprotection and neurogenesis. Journal of Neuroscience. 2009;29(31):9683–9699.
North RA, Galligan JJ, and Tonini M. 11. Circulatory cholinergic transmission and propeller-induced ileal motility in guinea pigs as a result of cisapride. "Gastroenterology" (Volume 96, Issue 1, 1989): 1257–1264.
Section 12: Ikarashi, Kon, and Sugiyama. A novel target for the treatment of diarrhoea and constipation: aquaporins in the colon. Due to elevated PGE2 release from macrophages, aquaporin-3 expression in the colon is reduced (Int J Mol Sci. 2016;17(7):E1172). U.S. Journal of Physiology Journal of Gastrointestinal and Hepatobiliary Physiology. 2011;301(5):G887eG895.
14. Kohland DE. Use of enzyme specificity theory in protein synthesis. The publication is "Proc Natl Acad Sci U S A. 1958;44(2):98e104."
14. Yang XW. A method for discovering the active and beneficial components of Chinese medicinal herbs by studying their metabolism and intra-body distribution. “Zhongguo Zhongyao Zazhi” [Chinese] 2007; 32(5): 365–370.
16. The Commission for the Chinese Pharmacopoeia. Chapter 1 of the People's Republic of China Pharmacopoeia was published in 2015 by China Medical Science Press in Beijing, China. The ISBN for this volume is 2015:391.This work is by Zhang YG, Ge TR, Xiang P, and others. Pharmacological treatment of loperamide-induced constipation in Sprague-Dawley rats and its underlying mechanism using the SLIGRL-NH2 protease receptor 2 agonist. Article 18. Qin Y, Wang JB, Kong WJ, et al. (2018) in Drug Development and Therapeutics, Volume 12, pages 2403–2411. The possible mechanism of rhubarb's diarrhoeogenic and antidiarrheal dual actions. In a 2011 article published in the Journal of Ethnopharmacology, the authors Xu Y, Zhang L, Wang Q, Luo G, and Gao XY discussed... Case study: rhubarb, a natural plant-derived component in rats, and an integrated approach based on characteristic fragment filter and multivariate statistical analysis in multi-stage mass spectrometry chromatograms for large-scale detection and identification. In a 2019 article published in the Journal of Pharmaceutical and Biomedical Anals, Gotfryd K, Mo'sca AF, Missel JW, and colleagues (20) delve into the topic. An AQP10 pH gate controls the flux of adipose glycerol in humans. Kettle JG, Anjum R, Barry E, et al. published their findings in the journal Nature Communications in 2018 with the DOI: 9.1379. The development of AZD3229, a powerful inhibitor of pan-KIT mutants, has improved the prognosis for gastrointestinal stromal tumours. Article published in the Journal of Medical Chemistry in 2018 with the DOI: 8797e8810. Chapter 22.A group including Wang, Lu, Zhang, and others... Using high-performance liquid chromatography (HPLC) in conjunction with linear ion trap-Orbitrap mass spectrometry to quickly detect and identify components in rhubarb by the use of distinctive fragment filtering. Volume 40, Issue 14, Pages 2854–2862, Journal of Separation Science, 2017.Hirano K, Okahira M, Kubota M, Iguchi K, Usui S. Effects of magnesium ion on aquaporin 3 gene expression. "Camilleri M, Ford AC, Mawe GM, et al. Chronic constipation." European Journal of Pharmacology. 2008;588(1):26–32. Scientific Reviews: Drug Primers. 2017;3:17095. 25.An study and evaluation of the efficacy and safety of guanylate cyclase-C agonists for the treatment of chronic idiopathic constipation and irritable bowel syndrome with constipation was conducted by Shah ED, Kim HM, and Schoenfeld P. American

Chinese Traditional Medicine Journal

ISSN : 2693 6356
2024 | Vol 7 | Issue 1

Journal of Gastroenterology. 2018;113(3):329-338. 26. Wedel T, Spiegler J, Soellner S, et al. Megacolon and slow-transit constipation are associated with changes in the interstitial cells of Cajal and enteric nerves. Gastroenterology. 2002;123(5):1459–1467. 27This is according to Yin JQ, Liang YC, Wang DL, and colleagues. In loperamide-induced constipated mice, naringenin increases the expression levels of c-Kit, SCF, and aquaporin 3, leading to laxative effects. Scientific Reports in Molecular Medicine. 2018;41(2): 649-658. 28.G. Bassotti, V. Villanacci, CA Maurer, and others. Enteric neuronal death and glial cell function in the neuropathogenesis of persistent delayed transit constipation. Ref.: Gut. 2006;55(1):41–46. 29.In individuals who are not satisfied with laxatives, prucalopride (Resolor) is used to treat severe persistent constipation (Tack et al., 2019). "Gut" (2009, 58(3): 357–365). 30.The authors of the study include Yu Y, Chen JH, Li H, and others. Regulation of rat colonic motility by 5-HT3 and 5-HT4 receptors. Journal of Neuro-Gastroenterology and Molecular Physiology. 2015;27(7):914–928. 31. Mostafa RM, Moustafa YM, Hamdy H. The Cajal interstitial cells, a master in both health and illness. In a 2010 article published in the World Journal of Gastroenterology, the authors Parthasarathy G., Chen J., Chia N., O'Connor HM., Gaskins HR., and Bharucha AE. Neuro Gastroenterol Motil. 2017;29(10):1e10. 33. Assessing faecal microbiota in chronic constipation: a reproducible research method.John Galligan and Pan H. Enteric neurons inhibit and facilitate rapid synaptic transmission via 5-HT1A and 5-HT4 receptors. In the article "G230eG238" published in the American Journal of Physiology in 1994, the authors Hoffman, Tyler, MacEachern, and colleagues were cited. Propulsive motility is accelerated and visceral hypersensitivity is inhibited when 5-HT(4) receptors in the intestinal mucosa are activated. Laforenza U. Water channel proteins in the gastrointestinal tract. Gastroenterology. 2012;142(4):844e854. e4. (Kon R, Ikarashi N, Hayakawa A, et al.) published in Mol Aspect Med in 2012, volume 33, issue 6, pages 642–650. An increase in serotonin release leads to an upregulation of aquaporin-3 in the colon, which in turn causes morphine-induced constipation. Citation: Toxicol Sci. 2015;145(2):337e347. 37.Kon R, Nagoya C, Ikarashi N, et al. Rheinanthrone, a sennoside A metabolite, is responsible for the laxative effects of rhubarb extract by activating macrophages and reducing aquaporin-3 expression in the colon. Citation: 38. Zong CL, Huang RR, Condac E, et al. J Ethnopharmacol. 2014;152(1):190e200. Discovering Robo1's heparan sulphate ligand needs by an integrated approach. Journal of the American Chemical Society, 2016;138(39): 13059–13067. 39. Zhang Y, Wang ZH, Ma XY, et al. Glycyrrhetinic acid blocks RAS-effector protein interactions by binding to the conserved P-loop region. The article "Acta Pharm Sin B. 2019;9(2):294" addresses this

2. topic.