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Original Research Article

Network pharmacology and GEO chip-based elucidation of mechanisms underlying the use of Yi Tieqing for prevention and treatment of postoperative nausea and vomiting

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Abstract

Purpose: *To investigate the mechanism(s) involved in the use of Yi Tieqing for the prevention and treatment of postoperative nausea and vomiting (PONV), using network pharmacology and GEO chip.*
Methods: *The chemical constituents and functional targets of five traditional Chinese medicines in Yi Tieqing were obtained by searching TCMSP database. The PONV disease targets were identified through DisGeNET, GeneCards and DrugBank databases, and differential expression genes of the GEO database chip (GSE7762) were mined. From the intersections of the component targets and disease targets, the core targets of drugs and diseases were obtained. The core targets were investigated in R language using GO-biological process and KEGG enrichment analyses, and their biological activities were verified via molecular docking. Finally, the severity and incidence of PONV in control and treatment groups were determined and compared.*
Results: *A total of 254 bioactive components and 301 related potential targets were obtained from the TCMSP database. There were 2092 related targets in PONV, and 6 intersecting targets were obtained from Venn diagram. The results of GO biological process and KEGG enrichment analysis showed that the incidence of PONV was strongly correlated with the negative regulation of response to wounding and nervous system. Clinical results showed that from 24 – 48 h (T2) after operation, the severity and incidence of PONV in the treatment group were significantly lower than those in the control group ($p < 0.05$).*
Conclusion: *Yi Tieqing alleviates PONV through multi-components, multi-targets, and multi-pathways.*

Keywords: *Network pharmacology, Patch, PONV*

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INTRODUCTION

Postoperative nausea and vomiting (PONV) is a common adverse reaction after surgery. Although short-acting anesthetics, preventive antiemetics, and minimally invasive surgery are frequently used nowadays, PONV still affects about 20 to 40 % of surgical patients, and the

incidence in high-risk patients may be as high as 80 % [1,2]. It causes varying degrees of discomfort and inability to eat and take medicines normally, and in severe cases, it may lead to incisional hernia, wound dehiscence, aspiration pneumonia, acid-base imbalance, and water-electrolyte imbalance, resulting in low surgical effect [3]. In the prevention and treatment of PONV, traditional Chinese medicine therapy offers the advantages of high safety, minimal side effects, and multiple targets [4]. Studies have shown that the application of traditional Chinese medicine acupuncture and transcutaneous acupoint electrical stimulation during the perioperative period significantly reduced the incidence of PONV and promoted postoperative recovery [5].

Yi Tieqing, an in-hospital preparation of Shaanxi University of Traditional Chinese Medicine Affiliated Hospital, has been in use for 11 years. In the prevention and treatment of PONV based on deficiency of *qi* and blood and dissipation of *healthy qi* after operation, *Codonopsis pilosula, Scutellaria baicalensis* and licorice are added to *Zhang Zhongjing Xiao Banxia* decoction. This results in development of acupoint patch comprising a 6:3:3:2:1 ratio of *Codonopsis pilosula, Pinellia ternate, ginger, Scutellaria baicalensis* and *licorice* [6]. In this prescription, *Pinellia ternata* and *ginger* are monarch medicines, and the two are used together to enhance the anti-emetic power of *Yi Tieqing. Codonopsis pilosula*, an auxiliary medicine, *tonifies the temper* by controlling its rise and fall, while *Scutellaria baicalensis*, an adjuvant which relieves heat, is used together with *Pinellia ternata* to disperse *knot*. *Licorice* functions as a harmonic medicine used to moderate coordination compatibility of *Yi Tieqing* [7]. Clinically, *Yi Tieqing* has been effective in reducing the degree and incidence of nausea and vomiting after laparoscopic cholecystectomy and modified radical mastectomy [8,9]. However, the mechanisms underlying these effects are not yet known. Therefore, there is need to study the mechanism involved in the use of *Yi Tieqing* in the prevention and treatment of PONV.

The GEO database (Gene Expression Omnibus, GEO) contains a large number of high-throughput experimental biological data and provides gene expression profiles for many diseases. Through bioinformatics analysis, differential genes closely related to diseases may be identified via screening. Network pharmacology describes the complex interactions amongst drugs and biological systems such as targets and diseases; it reveals the material basis of the efficacy of traditional Chinese medicine, and elucidates the molecular mechanisms associated with the therapeutic properties of traditional Chinese medicine compounds [10]. Therefore, in this study, all the components of *Yi Tieqing* were searched and their target genes were screened via network pharmacology methods and GEO chip analysis. A regulatory network of the traditional Chinese medicine compounds was constructed, and the material basis and possible mechanisms underlying their anti-PONV effects were determined.

METHODS

Search for chemical constituents and screening of bioactive components of *Yi Tieqing*

Traditional Chinese Medicine Systems Pharmacology (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) database was used to identify the chemical constituents of *Pinellia ternata*, ginger, *Codonopsis pilosula*, *Scutellaria baicalensis*, and licorice. Then, lipid-water partition coefficient (AlogP) and drug-likeness (DL) were used to predict the bioactive compounds contained in each herbal component of *Yi Tieqing* in this study. The values of DL and AlogP used as criteria for screening were ≥ 0.18 and ≤ 5, respectively [11].

Acquisition of drug and disease target genes

Target genes corresponding to the bioactive components were found in the TCMSP database, and they were imported into the Uniprot (https://www.uniprot.org/) database to standardize them. The DisGeNET (http://www.disgenet.org/), GeneCards (https://www.genecards.org/) and DrugBank (https://www.drugbank.ca/) databases were searched in order to identify the genes related to postoperative vomiting. The target genes related to postoperative vomiting were searched using the key phrase “postoperative nausea and vomiting”. The GEO database (https://www.ncbi.nlm.nih.gov/geo/) was searched to obtain the expression dataset of the GSE7762 chip. Three (3) normal samples and 3 lesion samples were compared and analyzed with the R “limma” package in which the criteria of $p < 0.05$ and logFC > 0.5 were used to screen differential expression genes (DEGs) and draw a DEG volcano map.

Gene mapping

By comparing the bioactive compound targets with the vomiting disease targets, the direct

targets for the prevention and treatment of PONV with the bioactive compounds in *Yi Tieqing* were identified. Venn diagrams of drug bioactive ingredient targets and PONV targets were drawn online using VENNY 2.1 (https://bioinfogp.cnb.csic.es), and the common target genes were screened via mapping. The Venn diagrams helped to intuitively visualize the potential of *Yi Tieqing* to alleviate PONV through multiple gene targets.

Construction of drug bioactive components-targets-PONV disease network

The *bioactive components-potential targets for PONV treatment network* of *Yi Tieqing* was constructed using Cytoscape 3.7.2, and the network analyzer tool built into the software was used to analyze parameters of network characteristics so as to study the relationship amongst the more important components and targets of *Yi Tieqing*.

Biological function and pathway analysis

The GO and KEGG analyses were performed on *Yi Tieqing*-PONV-gene dataset using the "ggplot2", "cluster Profiler" and other packages of R 4.1.2 software ($p < 0.05$). The top 20 enrichment results in GO biological processes and KEGG were presented as bar graphs and bubble plots, respectively.

Molecular docking

The Pdb format for protein structure was downloaded from the Protein Date Bank protein database (https://www.rcsb.org/). The corresponding positive drug-related targets were retrieved from Drugbank, and the sdf file of the ligands was downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) using the LibDock tool from Discovery Studio 4.0. The key target proteins screened with GEO were docked with their corresponding bioactive components of traditional Chinese medicine, and the scores of the results were analyzed.

Clinical verification

Eighty patients who were scheduled to undergo gynecological surgery in The Affiliated Hospital of Shaanxi University of Chinese Medicine were selected. The patients were in the age range of 40 - 60 years, with body of 50 - 80 kg, height of 155 - 175 cm, and American Society of Anesthesiologists grades I - II. This study was reviewed by the medical ethics committee of the hospital, and patients and their families were informed of the study content and signed informed consent. The patients were randomly divided into control group (n = 40) and treatment group (n = 40). Patients in the control group did not receive any treatment before or after the operation, while those in the treatment group were administered 2 g of *Yi Tieqing* at the *Shenque* point, once daily for 4 h for 3 consecutive days before the operation, and on the day after operation. The incidence of PONV was recorded at 4 time points: 6 h after extubation (T_0), 6 - 12 h after extubation (T_1), 12 - 24 h after extubation (T_2) and 24 - 48 h after extubation (T_3). The incidence of PONV, based on the World Health Organization (WHO) grading standard was categorized into levels viz: level I for no nausea and vomiting, level II for mild nausea without vomiting, level III for obvious nausea and vomiting, but no content vomiting; and level IV for severe nausea and vomiting, with content vomiting. Patients in grades II and above were considered to have PONV.

RESULTS

Collection and screening of bioactive components of *Yi Tieqing*

In this study, a total of 938 compounds were collected from the TCMSP database, comprising 116 compounds from *Pinellia ternate,* 265 compounds from ginger, 134 compounds from *Codonopsis pilosula*, 143 compounds from *Scutellaria baicalensis*, and 280 compounds from licorice samples. Using AlogP ≤ 5 and DL ≥ 0.18 as criteria for screening, 254 of 938 compounds in the 5 traditional Chinese medicines were found to be bioactive components.

Results of drug-disease target screening

A total of 301 targets were obtained from the TCMSP database: 31 targets were found in the DisGeNET database, 1754 targets were found in the GeneCards database, and 43 targets were retrieved from the DrugBank database (official names were given in the Uniprot database). A total of 1772 targets were obtained after merging these target genes and deleting duplicates. Following downloading of the GSE7762 chip and platform data from the GEO database and analyzing 320 significant differential genes, it was found that 149 genes were upregulated, while 171 genes were downregulated. A DEG volcano map was drawn, as shown in Figure 1 A.

Venn diagram of intersection targets

The 301 drug targets obtained from the TCMSP database, and the 1772 disease targets obtained from the DisGeNET, GeneCards, and DrugBank

databases, as well as the 320 DEGs obtained from the GEO database, were imported into VENNY 2.1. Ultimately, 6 drug-disease interaction targets (CDKN1A, ICAM1, G6PC, PLAT, SPP1 and ERBB3) were obtained, as presented in Figure 1 B.

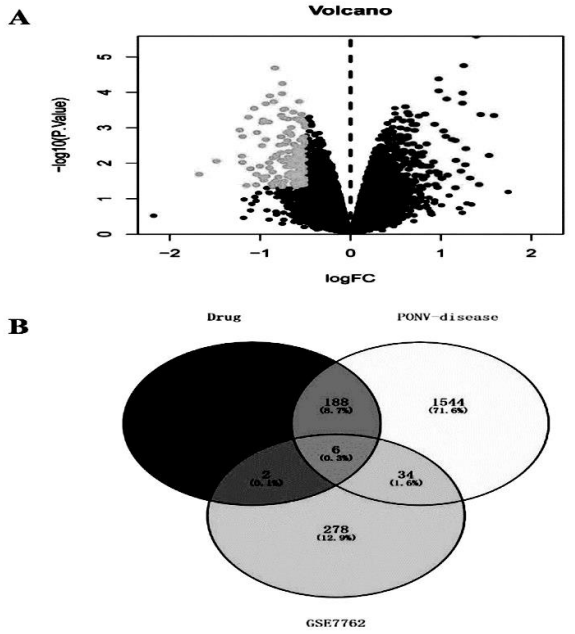

Figure 1 A: Volcano map of differential expression genes between normal group and PONV group; B: Venn diagram of *Yi Tieqing* and PONV disease

Construction of bioactive component-core target network

The "merge" tool of Cytoscape 3.7.2 was used to construct a network diagram of the 8 bioactive components of the drug and 6 PONV targets in *Yi Tieqing* (Figure 2). The network comprised 14 nodes and 15 edges. The 6 key targets of *Yi Tieqing* for the prevention and treatment of PONV corresponded to only 8 components (luteolin, apigenin, vogonine, asaxetine, lutein, quercetin, kaempferol and naringin) from *Codonopsis, Scutellaria baicalensis*, and *Licorice.* Therefore, the Figure shows only the network relationship amongst the 8 components of these 3 drugs and their corresponding targets.

Results of enrichment analysis

The biological processes and pathways of the major enrichments of the 6 key targets were analyzed using R language. It was found that *Yi Tieqing* mitigated PONV through negative regulation of response to wounding and response to nutrient levels. The KEGG pathway was enriched to 5 PONV-related signaling pathways (Table 1). The results show that the bioactive components of *Yi Tieqing* prevented PONV through the PI3K-Akt and other signaling pathways. Biological process analysis of the bioactive components of *Yi Tieqing* was visualized, as shown in Figure 3 (A & B). A bubble chart depicting the KEGG pathway analysis of *Yi Tieqing* is presented in Figure 4.

Molecular docking analysis

Discovery Studio 4.5 software was used to conduct molecular docking for the 6 key target proteins screened with GEO and their corresponding compounds. At the same time, the positive drugs for the corresponding target proteins were used for comparison. Since the three-dimensional G6PC target protein was not found in the PDB protein database structure, the key proteins CDKNIA, PLAT, SPP1, ERBB3 and ICAM1 were subjected to molecular docking. The comparison of the binding activity scores between targets and compounds and positive drugs are shown in Table 2 and Table 3, while the interactions diagram between components and targets are shown in Figure 5. The results are expressed in terms of LibDock scores. The higher the LibDock score, the better the affinity.

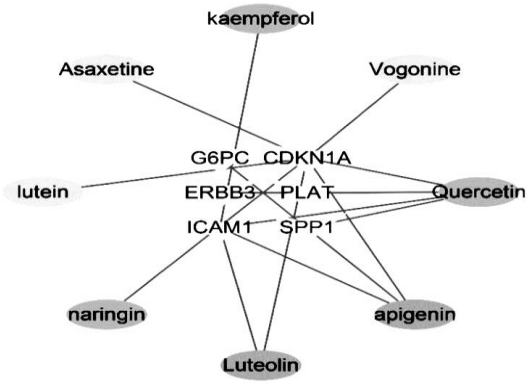

Figure 2: Network diagram of *Yi Tieqing* bioactive components-PONV key targets interactions

Table 1: Analysis of information on PONV-related signal pathway

KEGGID	Channel name	Target	Quantity
hsa04151	PI3K-Akt signaling pathway	CDKN1A/SPP1/G6PC/ERBB3	4
hsa04012	ErbB signaling pathway	CDKN1A/ERBB3	2
hsa05215	Prostate cancer	CDKN1A/PLAT	2
hsa04068	FoxO signaling pathway	CDKN1A/G6PC	2
hsa04371	Apelin signaling pathway	PLAT/SPP1	2
hsa05418	Fluid shear stress and atherosclerosis	PLAT/ICAM1	2

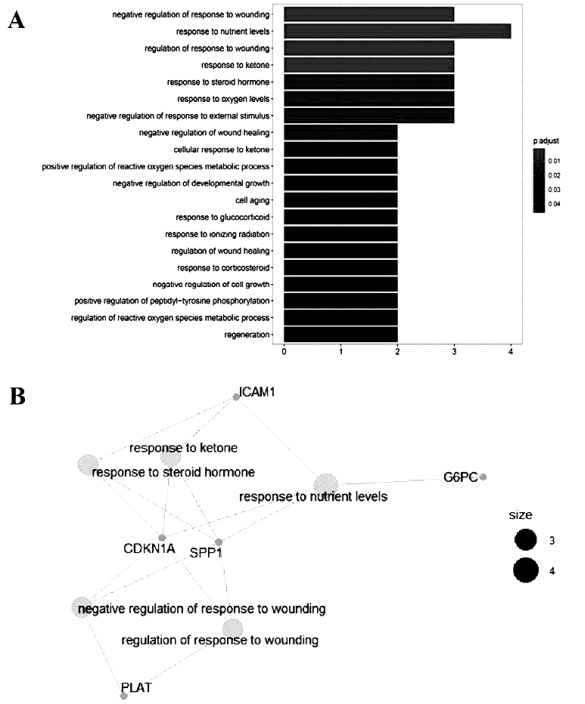

Figure 3: Biological process analysis diagram for *Yi Tieqing* in the prevention and treatment of PONV. A: Biological process histogram; B: biological process target pathway diagram

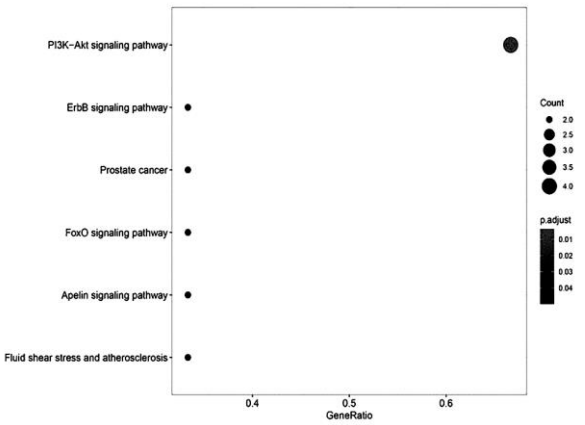

Figure 4: Bubble chart for KEGG pathway analysis of *Yi Tieqng* for preventing and treating PONV

Clinical outcomes

There was no significant difference in the general profile of the two groups ($p > 0.05$), indicating comparability (Table 4). There were significant decreases in PONV in the two groups at the postoperative T_2 period ($p < 0.05$; Table 5).

DISCUSSION

Nausea and vomiting are defensive reflex activities of the body due to ingestion of toxic substances which affect the vomiting center. The mechanism underlying nausea and vomiting involves electrical activity of brain neurons and

Table 2: Score of binding activity between core targets and corresponding compounds

Key target	Small molecule ligand	LibDock score
CDKN1A	Naringin	134.678
CDKN1A	Quercetin	90.0397
CDKN1A	Acacetin	89.4702
CDKN1A	Luteolin	85.6737
CDKN1A	Apigenin	84.9414
CDKN1A	Chrysin	78.8468
CDKN1A	Wogonin	77.6719
PLAT	Quercetin	74.1938
SPP1	Quercetin	112.526
ERBB3	Quercetin	101.488
ICAM1	Quercetin	108.588
ICAM1	Apigenin	108.177
ICAM1	Luteolin	96.1653
ICAM1	Kaempferol	95.5213

Table 3: Positive control docking scores

Key target	Positive drug	LibDock score
CDKN1A	Staurosporine	102.686
PLAT	Dexibuprofen	78.2872
SPP1	N-[(2R)-4-Diazonio-3-oxoniumylidene-1-phenylbutan-2-yl]-1-phenylmethoxymethanimidate	129.137
ERBB3	Tucatinib	115.959
ICAM1	Simvastatin	106.484

the action of neuroactive substances. The activation pathway is a complex nerve-related network, and its mechanism is still being investigated [12]. Studies have revealed that stimulation by serotonin, substance P, dopamine and other neurotransmitters are important mechanisms that underlie PONV. In this study, based on the combined use of network pharmacology and GEO chip for screening 6 core targets of *Yi Tieqing* used for preventing and treating PONV, it was found that *Yi Tieqing* regulated the nervous system. Then, the effect of *Yi Tieqing* was clinically verified. Results of enrichment analysis showed that the biological processes associated with the targets involved negative regulation of injury response, response to nutritional levels, ketone response, response to steroid hormones, and response to oxygen levels.

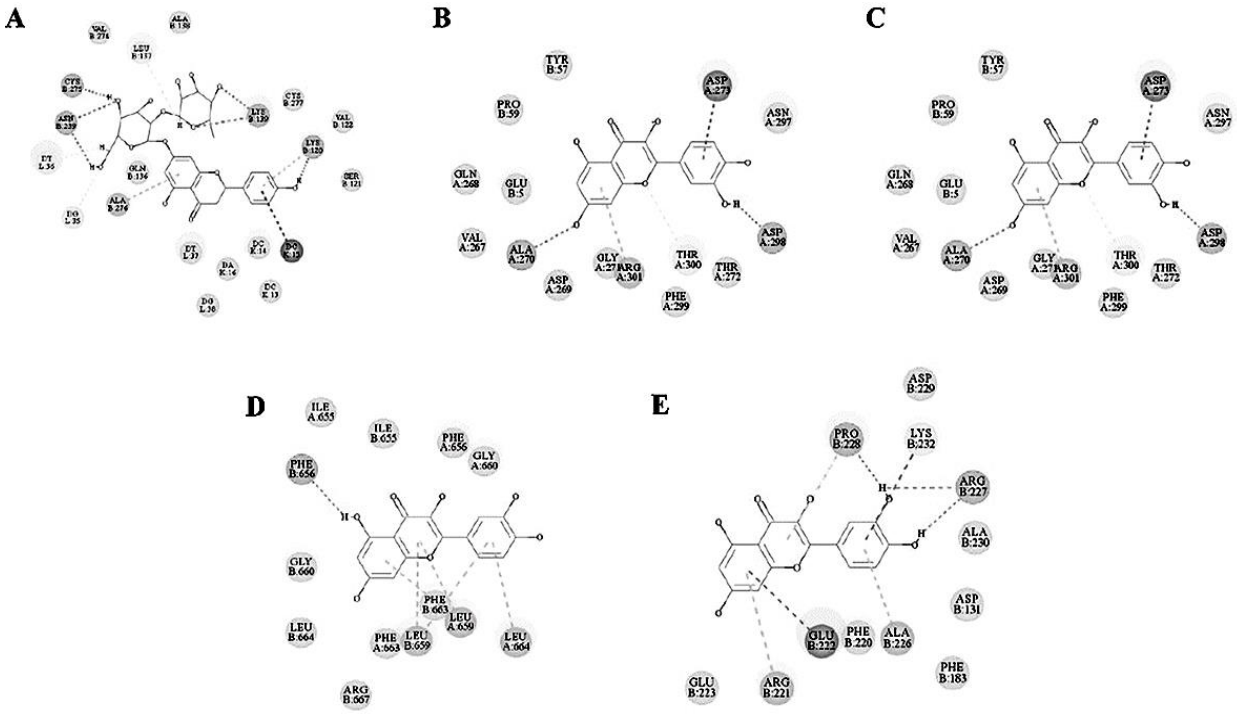

Figure 5: Docking diagram of core targets and corresponding components. A: diagram showing interaction between CDKNIA and nacingin; B: diagram depicting interaction between PLAT and quercetin; C: interaction between SPP1 and quercetin; D: interaction between ERBB3 and quercetin, and E: interaction between ICAM1 and quercetin

Table 4: Comparison of the general conditions of the two groups (n = 40)

Group	Age (years)	Weight (kg)	Fluid supplement volume (mL)	Anesthesia time (min)	Operation time (min)
Control	55.63±5.74	63.11±4.38	1384.7±133.84	166.18±7.39	134.78±8.94
Treatment	57.90±4.67	65.33±5.63	1379.23±67.90	162.65±9.34	137.30±7.83
T	-1.946	-1.962	0.231	1.837	-1.344
P-value	0.055	0.053	0.818	0.065	0.183

Table 5: Comparison of incidence of PONV between the two groups at various time points post-surgery (n (%); n = 40)

Group	T_0	T_1	T_2	T_3
Control	14(35)	11(27.5)	9(22.5)	6(15)
Treatment	10(25)	9(25)	2(5)	2(5)
χ^2	0.952	0.267	5.166	0.222
P-value	0.329	0.606	0.023*	0.136

*$P < 0.05$, compared to the control group

The KEGG enrichment pathway analysis identified the involvement of PI3K-Akt and other signaling pathways, indicating that the pathogenesis of PONV is associated with the regulation of related neuropeptides and other signal routes. Numerous studies have found that the PI3K-Akt pathway is a pro-survival pathway which regulates neuronal survival. In particular, when hypoxic/ischemic neurons are damaged, activation of the pro-survival pathway is essential for nerve cell protection [13,14]. The occurrence of PONV is strongly correlated with the nervous system. The nervous system is rich in the P13K-Akt signaling pathway [15]. Autophagic cell survival and apoptosis in the nervous system occur through regulation of the nervous system, while activation of the PI3K-Akt signaling pathway promotes endothelial cell survival and attenuates and blocks neural injury [16]. Molecular docking software was used to dock the screened core targets of *Yi Tieqing*. The resultant scores showed that the docking was stable. The high content of quercetin provided neuroprotective effect against PONV by preventing decreases in nerve and glial density [17].

CONCLUSION

The bioactive components of *Yi Tieqing* used in the prevention and treatment of PONV are luteolin, apigenin, vogonine, asaxetine, lutein, quercetin, kaempferol, and naringin. These bioactive components exert therapeutic effects by acting on PI3K-Akt and other signaling pathways through targets such as CDKNIA, SPP1, G6PC, and ERBB3.

This study has used only the bioactive components screened at the molecular level to predict the potential signaling pathways that are involved in the use of *Yi Tieqing* for prevention and treatment of PONV. The beneficial effect of *Yi Tieqing* has been verified, and good clinical results have been obtained. However, the main pathways underlying its effects have not been characterized. Thus, there is need for further *in vitro* studies on this aspect. Overall, the results indicate that *Yi Tieqing* may be a new and promising treatment for PONV.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shining Xun, Mingna Jiao and Jia Li performed the data analysis, wrote the first version of the manuscript, and processed the graphs and tables in the manuscript.

Xiaqing Zhang and Afen Zhang prepared the final version of the manuscript. Huali He and Junbo Zou collected the data. Chongzhen Duan and Xiaofei Zhang (corresponding author) conceived and coordinated the study. All authors read and approved the final manuscript.

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