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Original Research Article

Mechanism of pharmacological effect of *Angelica essential* oil on anxiety based on network pharmacology and molecular docking

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Abstract

Purpose: *To investigate the pharmacological effect of Angelica essential oil (AEO) on anxiety disorder and the mechanism of action involved, using network pharmacology and molecular docking.*
Method: *Chemical compositions of AEO were determined using gas chromatography-mass spectrometry (GC-MS). The potential targets involved in the effect of AEO on anxiety were predicted by searching Bioinformatics databases. Cytoscape software was applied for networks construction. Analysis of Gene Ontology (GO) and pathway was done via R Programming Language, while molecular docking analysis was conducted by Autodock vina software.*
Results: *Network analysis showed that there were 10 bioactive components and 34 potential targets. The potential targets were distributed in cerebellar cortex, basic ganglia, hippocampal formation, amygdala, pons and medulla and liver. AEO was involved in multiple biological processes (BP), cell components (CC) and molecular functions (MF) associated with anxiety disorder. Molecular docking revealed good binding affinity between the major bioactive components and targets.*
Conclusion: *This study has revealed the pharmacological mechanism involved in the effect of AEO on anxiety, through network pharmacology and molecular docking. The pharmacological mechanism provides a firm theoretical basis for further studies in this area of research.*

Keywords: *Angelica Essential oil, Anxiety, Network pharmacology, Molecular Docking, Pharmacological*

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INTRODUCTION

Anxiety disorder as the most prevalent mental symptom [1], it is caused by genetic, mental and social factors, and manifested in various fancies and conjectures, restlessness and abnormal suffering, which can cause fear and even panic to a certain extent. Anxiety disorder is due to disproportionate activation of the sympathetic and parasympathetic systems, with obvious influence on the cardiovascular system. The main cardiovascular manifestations are

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palpitation, increased heartbeat, chest pain, dizziness, and numbness. In the acute coronary syndrome population, anxiety disorder leads to a high risk of subsequent myocardial infarction [2].

Since the emergence of coronavirus disease in 2019 (COVID-19), more than 100 million people have been infected worldwide (as at February 2021). The COVID-19 pandemic has led to increased economic pressure and stress due to death, leading to increases in anxiety disorders. Therefore, it is necessary to find more effective ways of treating anxiety disorders.

At present, there are two ways of treating anxiety disorder: psychological treatment, based on cognitive behavioral therapy, computer-assisted and internet-based treatments; and pharmacological treatments using certain inhibitors which would involve in the anxiety pathway, such as serotonin-reuptake inhibitors, serotonin–noradrenaline-reuptake inhibitors, tricyclic antidepressants and monoamine oxidase inhibitors. These treatments are 60 - 70% effective [1]. However, the associated side effects such as withdrawal symptoms, safety problems, complexity of medication, and high costs have limited the clinical application of psychological and pharmacological treatments. Therefore, it is of great significance to seek treatment methods or drugs with little or no side effects.

Essential oils regulate various mental discomforts through their fragrance. Different essential oils have different therapeutic effects [3]. AEO is a volatile oil extracted from *Angelica sinensis*. It is composed of small-molecule compounds with the unique aroma of *Angelica sinensis*. Studies have shown that certain concentrations of AEO exhibited obvious anti-anxiety effects in male Swiss mice [4]. Some research revealed that AEO has potential usefulness on disorders caused by anxiety and social inadequacy [5]. Therefore, AEO is a novel therapeutic approach to anxiety disorders, However, the pharmacological mechanism underlying its effect has not been elucidated.

In this study, the pharmacological mechanism of which AEO on anxiety disorders was uncovered using network pharmacology, and verified via molecular docking. The compositions of AEO were determined using GC-MS. The bioinformatics databases were explored to get the targets of AEO on anxiety disorders. Cytoscape software was applied to discover the relationship of component with target, protein with protein and target with tissue distribution, while R was applied to perform the GO and conduct KEGG pathway of the targets. Autodock vina software was applied to figure out the interaction between the main active chemical components and protein targets.

EXPERIMENTAL

Identification of chemical components and screening of targets

The components of AEO were recognized using GC-MS. The gas chromatographic requirements were as follows: capillary column was Agilent HP-5 (0.25mm×30m, 0.25μm); the carrier gas was Helium (99.999%), the sample volume used was 1μL, the shunt ratio was 40:1, while the flow rate was 1mL/min. The temperature was programmed viz: initial temperature of 80 ℃ with a heating rate of 3 ℃/min up to 167℃ in 2.5min; and thereafter at 2 ℃/min up to 202 ℃, followed by 4 ℃/min up to 260 ℃in 2min. The mass spectrometry requirements were: EI ion source, electron energy of 70 eV, temperature of ion source and MS quadrupole were 230 ℃ and 150 ℃, respectively; 3.0 min solvent delay, and full scan pattern. Chemical components with quality greater than 80 were screened with Database of SwissTargetPrediction [6] (http://swisstargetprediction.ch) and TCMSP [7] (http://lsp.nwu.edu.cn/tcmsp.php) to gain the targets.

Screening for anxiety targets

The TTD [8] (https://db.idrblab.org/ttd/), DrugBank [9] (https://www.drugbank.ca/) and DisGeNET [10] (http://www.disgenet.org/) databases were searched for anxiety targets.

Networking the interaction of bioactive component and target

The intersection of the chemical component targets and anxiety targets were taken as the potential targets, and the chemical components corresponding to the potential targets were considered as the potential bioactive components [11]. UniProt database [12] (http://www.uniprot.org/) was utilized to obtain the UniProt ID of targets. The bioactive components and potential targets were imported into Cytoscape 3.7.1 software so as to network the bioactive component and potential target.

Networking and analysis of the interaction of potential targets

The potential target was imported into the STRING database [13] (https://string-db.org/); the species was defined as human, and the result was saved in the TSV format. Then, the

information on the interaction of protein and protein was analyzed by Cytoscape 3.7.1 software.

Networking and analysis of the tissue distribution and targets network

Human Protein Atlas [14] (https://www.proteinatlas.org/) was searched for tissue data on potential targets. Tissues corresponding to expression values (NX) greater than 5 in the data were analyzed using Cytoscape 3.7.1 software in order to get the tissue distributions of potential targets.

GO and KEGG pathway analysis

Analyses of GO (including BP, MF and CC) and KEGG pathway were done using clusterProfiler(R). This served to reveal the pharmacological mechanism with the effect of AEO on anxiety.

Molecular docking of the main bioactive components on targets

Autodock vina1.1.2 software was used to determine the interaction/binding between the main bioactive chemical components of AEO and protein targets. The structure of protein targets and main bioactive chemical components were uploaded through PDB database [15] (www.rcsb.org/) and PubChem Database [16] (https://pubchem.ncbi.nlm.nih.gov/) respectively, and the molecular docking analysis was conducted.

RESULTS

Chemical components in AEO

In the results from GC-MS (Figure 1), a total of 31 chemical components of AEO were identified via searching the database NIST14.L in Agilent 7890A/5975C. Chemical components with quality value greater than 80 are shown in Table 1. Cis-ligustilide was the major component of the AEO, accounting for an area percentage of 78.96%, relative to the other 10 components.

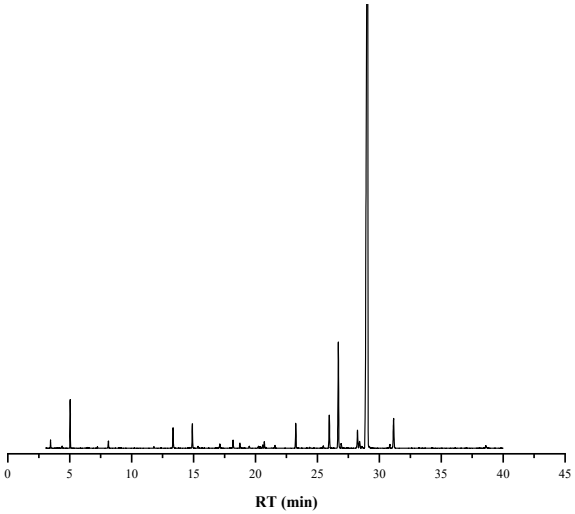

Figure 1: GC-MS chromatogram of AEO

Acquisition of potential targets

The Targets of 11 chemical components were searched via the TCMSP and SwissTargetPrediction. Anxiety-related targets were obtained using the TTD, DrugBank and DisGeNET. 34 potential targets (Table 2) were acquired, from the intersection targets of chemical components targets and anxiety targets.

Networking of bioactive component and action target

The component - target network (Figure 2) was constructed. There were 44 nodes in the network, including 10 compound nodes (trans-beta-ocimene was not associated and removed) and 34 target nodes.

Table 1: Information of AEO components

No.	Retention time (min)	Area（%）	Components	CAS	Quality
1	3.4518	0.3941	Alpha-pinene	000080-56-8	95
2	5.0372	2.0762	Trans-beta-Ocimene	003779-61-1	97
3	13.3462	0.7718	2-Methoxy-4-vinylphenol	007786-61-0	90
4	17.1224	0.3317	Germacrene D	023986-74-5	87
5	23.2552	1.3232	Espatulenol	006750-60-3	96
6	26.6983	6.4862	3-butylidenephthalide	000551-08-6	96
7	29.0728	78.9609	Cis-ligustilide	1000365-98-5	91
8	31.1631	1.9236	Trans-ligustilide	1000365-98-8	94
9	38.6207	0.4373	N-Hexadecanoic acid	000057-10-3	95
10	44.3068	0.1579	Methyl linolelaidate	002462-85-3	87
11	45.9618	0.2560	Linoleic	000060-33-3	94

Table 2: Profile of potential targets

S/no.	Gene	Protein	UniProt ID	Degree
1	CYP2C19	Cytochrome P450 2C19	P33261	1
2	BCHE	Butyrylcholine esterase	P06276	1
3	CYP1A2	Cytochrome P450 1A2	P05177	1
4	CYP1A1	Cytochrome P450 1A1	P04798	1
5	ESR1	Estrogen receptor	P03372	2
6	DAO	D-amino-acid oxidase	P14920	1
7	ADRA1B	Alpha-1B adrenergic receptor	P35368	2
8	GABRA1	Gamma-aminobutyric acid receptor subunit alpha-1	P14867	4
9	CYP17A1	Cytochrome P450-C17	P05093	1
10	GABRB2	Gamma-aminobutyric acid receptor subunit beta-2	P47870	3
11	PDE10A	cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A	Q9Y233	3
12	ADRA1A	Alpha-1A adrenergic receptor	P35348	1
13	GABRB3	Gamma-aminobutyric acid receptor subunit beta-3	P28472	3
14	GABRG2	Gamma-aminobutyric acid receptor subunit gamma-2	P18507	3
15	MAOA	Monoamine oxidase type A	P21397	4
16	KCNH2	Potassium voltage-gated channel subfamily H member 2	Q12809	1
17	CYP2A6	Cytochrome P450 2A6	P11509	3
18	IL10	Interleukin-10	P22301	1
19	SLC6A2	Sodium-dependent noradrenaline transporter	P23975	2
20	SLC6A4	Sodium-dependent serotonin transporter	P31645	5
21	PTGS1	Prostaglandin G/H synthase 1	P23219	5
22	CNR1	Cannabinoid receptor 1	P21554	2
23	PTGS2	Prostaglandin G/H synthase 2	P35354	6
24	SLC6A3	Sodium-dependent dopamine transporter	Q01959	4
25	FAAH	Fatty-acid amide hydrolase 1	O00519	4
26	ADRA2A	Alpha-2A adrenergic receptor	P08913	5
27	ADRA2B	Alpha-2B adrenergic receptor	P18089	5
28	ADRA2C	Alpha-2C adrenergic receptor	P18825	3
29	MAPT	Microtubule-associated protein tau	P10636	2
30	CHRM2	Muscarinic acetylcholine receptor M2	P08172	8
31	CHRM4	Muscarinic acetylcholine receptor M4	P08173	4
32	CHRM5	Muscarinic acetylcholine receptor M5	P08912	4
33	CHRM1	Muscarinic acetylcholine receptor M1	P11229	7
34	CHRM3	Muscarinic acetylcholine receptor M3	P20309	5

The size of the nodes represented the degree value, and the "degree" represented the action intensity. It had 107 edges, and each edge represented the relationship between a compound and target. The dark blue V nodes represented the bioactive component, while the green ellipse nodes represented the potential target. In the entire network, the chemical components 3-butylidenephthalide, trans-ligustilide and cis-ligustilide were highly correlated with potential targets, with degree values were 20, 19, 19 respectively. The targets CHRM2, CHRM1 and PTGS2 were highly correlated with the chemical components, and the degree values were 8, 7, 6 respectively.

Networking and analysis of protein-protein interaction networks

Figure 3 shows the constructed network of protein-protein interactions (PPI) of the 34 potential targets. The PPI network contained 33 node proteins (one protein target was not associated, and so was removed) and 104 interaction lines.

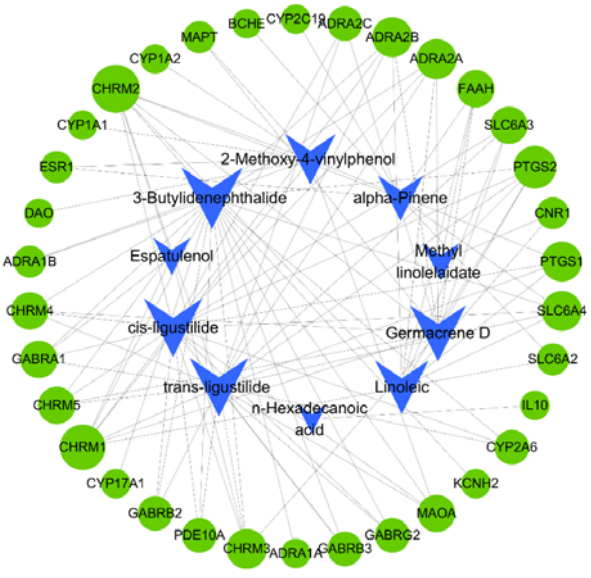

Figure 2: Component-target network of AEO (components of dark blue V nodes were connected to targets in green ellipse nodes)

The node size represented the degree value. In the protein-protein interaction network, the targets with degree exceeding 10 were SLC6A4 (degree 14), MAOA (degree 13), CNR1 (degree 10), ADRA2A (degree 10) and ADRA2C (degree 10).

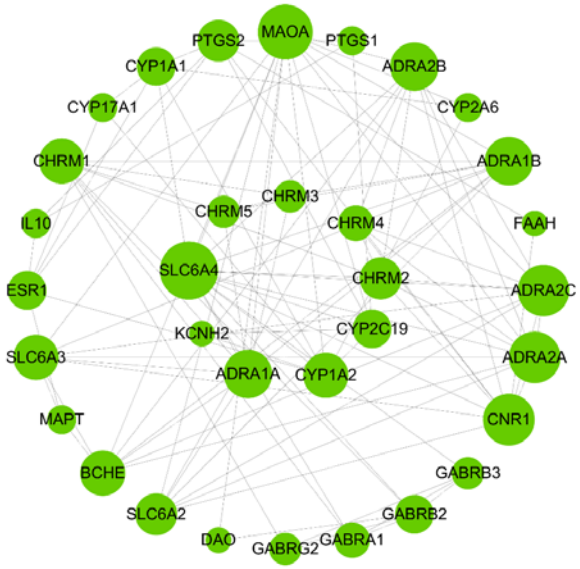

Figure 3: Protein-protein interaction network of potential targets

Networking and analysis of tissue distribution and potential targets

The network of tissue distribution - potential targets was constructed through The Human Protein Atlas database and Cytoscape 3.2.1. The network is shown in Figure 4. After removing the unrelated nodes, there were 90 nodes and 380 edges (the green ellipse nodes represented potential targets, the blue round rectangle nodes represented organ or tissue, while the node size represented the degree value). Each edge represented the relationship between the potential target and tissue or organ. The network analysis results showed that 34 targets were distributed in the cerebellar cortex (degree 16), basic ganglia (degree 15), hippocampal formation (degree 12), amygdala (degree 12), pons and medulla (degree 12), and liver (degree 12). In particular, MAOA (degree 45) and FAAH (degree 43) were the targets most closely related to organ or tissue.

GO and KEGG pathway analysis

GO and KEGG pathway analysis ($p \leq 0.05$) of potential targets were carried out via ClusterProfiler (R). In the results of GO analysis, there were 294 items (BP) that were related to response to ammonium ion, adenylate cyclase-modulating G-protein-coupled receptor signaling pathway, and G-protein-coupled receptor signaling pathway coupled to cyclic nucleotide second messenger. There were 47 CC items that involve synaptic membrane and postsynaptic membrane. There were 85 items in MF that involved G-protein-coupled amine receptor activity and neurotransmitter receptor activity. As indicated in Figure 5, the first 10 items in analyses of BP, CC and MF are shown.

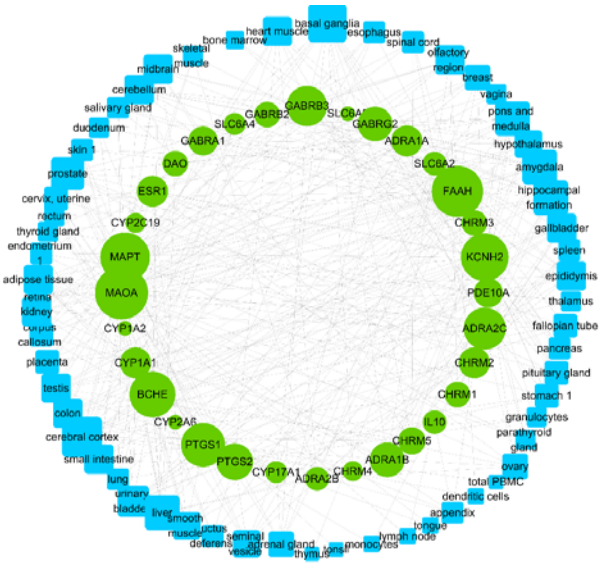

Figure 4: Tissue distribution - potential targets network (targets in green ellipse nodes were connected to tissues or organs in blue round rectangle nodes)

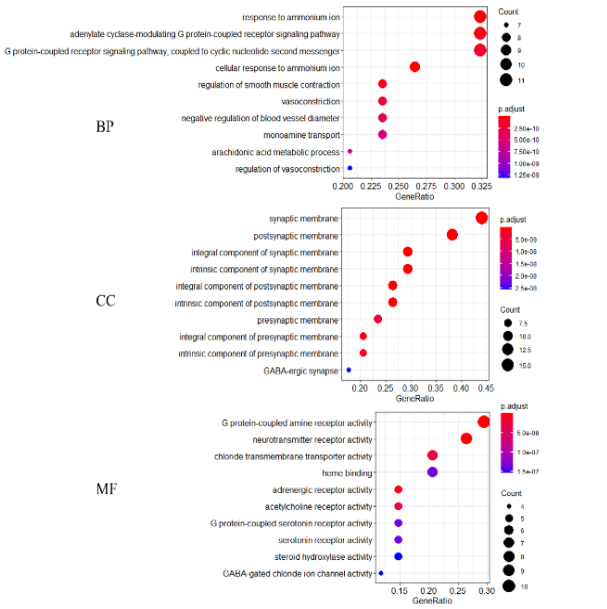

Figure 5: Bubble diagram of Gene Ontology enrichment analysis

KEGG enrichment analysis mapped 22 signal pathways. The first 10item pathways are shown in Figure 6. The main pathway was neuroactive ligand-receptor interaction.

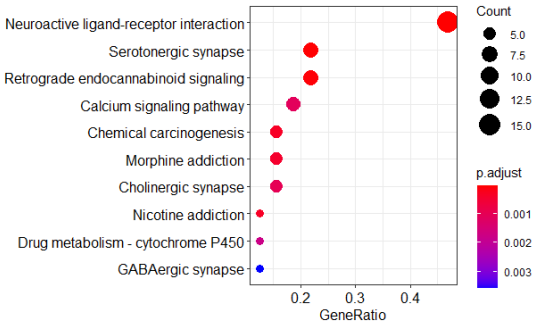

Figure 6: Bubble diagram of KEGG enrichment analysis

Molecular docking of main active ingredients to targets

The unique ligands of the main active targets of CHRM2, CHRM1 and PTGS2 in PDB database were glycerol, methscopolamine and 1,2-ethanediol, respectively. The binding abilities of the main active targets to glycerol, methscopolamine, and 1,2-ethanediol (as control) were compared with the binding abilities of the main active targets to the main bioactive components (3-butylidenephthalide, trans-ligustilide and cis-ligustilide. The binding energies of ligands (control and three active ingredients) to the receptor (the active targets) were analyzed using Autodock vina1.1.2 software. The binding energy (affinity value) results are listed in Table 3. The smaller the binding energy, the better the binding ability. Compared with the control, the main active components showed good binding ability. Trans-ligustilide and cis-ligustilide are isomers of one another and 3-butylidenephthalide has a similar chemical structure to the two isomers (trans-ligustilide and cis-ligustilide). Thus, they had the same binding energy.

DISCUSSION

Studies have shown that AEO exerts an anti-anxiety effect. This work applied network pharmacology to systematically determine the pharmacological mechanism involved in the effect of AEO on anxiety, at the molecular level. In GC-MS test, eleven chemical components were selected for analysis, with cis-ligustilide being the most abundant component. In component-target network, the main bioactive components were 3-butylidenephthalide, trans-ligustilide and cis-ligustilide, while the main active targets were CHRM2, CHRM1, PTGS2. The three main bioactive components have sedative, anti-spasmodic, anti-asthmatic and strong pharmacological effects on cardio-cerebrovascular and immune systems [17,18]. It has been revealed that CHRM2 is muscarinic acetylcholine receptor M2 involved in neuronal excitability, advanced cognitive processing, and memory and cognitive impairment in many neuropsychiatric disorders [19,20].

Muscarinic acetylcholine receptor M1 (CHRM1) improves cognitive deficits in schizophrenia [21]. Prostaglandin G/H synthase 2 (PTGS2) inhibits apoptosis and immune surveillance, and enhances angiogenesis [22]. Anxiety is caused by excessive activation of nervous system, and often occurs in cognitive impairment [2]. Long term anxiety affects the cardiovascular and immune systems. Therefore, AEO may exert sedative, neuroprotective and immune-regulating effects in anxiety disorder through interaction with its active targets.

From the PPI network of screening 34 potential targets, the core targets were SLC6A4, MAOA, CNR1, ADRA2A and ADRA2C.Sodium-dependent serotonin transporter (SLC6A4) is a serotonin transporter gene. Study has shown that SLC6A4 is associated with the etiology of anxiety disorder [23]. Monoamine oxidase type A (MAOA) gene is associated with emotional stability and neuroticism [24].

Through analysis of tissue distribution/potential targets network, it was revealed that the potential targets were mainly distributed in the cerebellar cortex, basic ganglia, hippocampal formation, amygdala, pons and medulla, and liver. This result is consistent with that the fact that the major areas involved in anxiety reactions are the cerebral cortex, hippocampus and amygdala.

Table 3: Analysis results of the optimal binding in Autodock vina

Active target	Binding energy (kcal/mol)			
	Control	***Active componet***		
CHRM1	Glycerol -3.5	Trans-ligustilide -6.0	Cis-ligustilide -6.0	3-butylidenephthalide -6.0
CHRM2	Methscopolamine -6.0	Trans-ligustilide -6.0	Cis-ligustilide -6.0	3-butylidenephthalide -6.0
PTGS2	1,2-Ethanediol	Trans-ligustilide	Cis-ligustilide	3-butylidenephthalide

-3.4	-6.7	-6.7	-6.7

From KEGG pathway results, neuroactive ligand-receptor interaction was the main pathway through which AEO affected anxiety.

Autodock Vina software has the advantages of speed and accuracy in analysis of molecular docking. This study compared the binding capacities of the controls to the main active targets, with those of the main bioactive components in Autodock Vina. The results showed that the bioactive components had the same or lower binding energies with the controls, which indicated that the bioactive components had good binding ability to the active targets.

CONCLUSION

The pharmacological mechanism involved in the effect of AEO on anxiety has systematically been explored using network pharmacology. The analysis has identified 10 bioactive components and 34 potential targets. The results obtained suggest that AEO acts on anxiety through multi-component, multi-target, multi-channel and multi-organization pathways. Molecular docking reveals low binding energies of the bioactive components to their protein targets. Thus, this study provides a theoretical basis for further studies on the effect of AEO on anxiety.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims related to the content of this article are borne by the authors. Xiaoli Liu designed the study and analyzed the data. Xiaofei Zhang interpreted the data; Fang Wang prepared the manuscript for publication. Jie Xu supervised the data collection, while Ming Yang reviewed the draft of the manuscript. All authors read and approved the manuscript.

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