The mechanism of guarana regulating central nervous system based on network pharmacology and molecular docking

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Abstract. This study aims to explore the mechanism of guarana on central nervous system based on network pharmacology and to verify the results by molecular docking technique. The chemical constituents of guarana were collected through the chemical specialty database and TCMSP database, and the targets were predicted, the component-target network map was constructed, the protein interaction network map (PPI) and target-pathway map were constructed, the targets contained in guarana and common targets related to the regulation of central nervous system were screened, GO and KEGG analysis of common target were performed. In the result, the component-target network contains 13 components, 79 targets, 8 of these targets may be the core targets of guarana in regulating the central nervous system. The main biological processes include cAMP signalling pathway, P13K-Akt signalling pathway, calcium signalling pathway, Rap1 signalling pathway and so on. In conclusion, guarana plays a role in regulating the central nervous system through multi-target and multi-pathway. Through the analysis of its core network, it is found that the core efficacy of guarana may focus on anti-fatigue.

1. Introduction

Guarana (Paullinia cupana) is an aconite-like shrub, fruit and seed used in medicine, which is widely distributed in in the Amazon basin in northern Brazil, known as Brazil's "national drink" [1]. Guarana as a painkiller, aphrodisiac, astringent, stimulant diuretics and supplements [2,3], used to prevent arteriosclerosis treatment of rheumatism, hypertension, heart disease, fever, migraine neuralgia, regulation of the central nervous system and weight loss [4-9].

Guarana, like many traditional Chinese medicine, is complex in composition and involves many diseases, involving many genes and signalling pathways in the body's regulatory network [10]. In order to study the core efficacy of guarana, the drug research method of single target is obviously not suitable for systematic interpretation [11].

Therefore, from the point of network pharmacology, we will study the complex network relationship between guarana component-target-gene-disease [12, 13]. The core function of guarana is described from the point of view of multi-component and multi-pathway action and verified by molecular docking technique [14].
2. Materials and methods

2.1. Establishment of a pool of key chemical constituents of guarana
The guarana components were collected based on Shanghai Chemical Professional Database (http://www.organchem.csdb.cn/) and TCMSP Database (https://tcmspw.com/tcmsp.php), and the molecular structure was recognized by Pubmed (https://pubchem.ncbi.nlm.nih.gov/) database.

2.2. Screening of major chemical constituents and target prediction
Chemical composition through absorption, distribution, metabolism, excretion (ADME) process to reach the target organs, tissues to play a role. The absorption of drugs is the basis of drug preparation. Pharmacokinetic analysis is the basic analysis method to evaluate absorption characteristics. In the resulting guarana composition database, OB>30%, DL> 0.18, was chosen to get the main components of guarana. Using PubChem databases (https://www.ncbi.nlm.nih.gov/pubmed), Querying the corresponding SMILES number according to the CAS number of the molecule, predicting possible targets for each component in the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) and collating relevant data.

2.3. Prediction of regulatory targets in the central nervous system
The keywords "central nervous system" were retrieved in OMIM database (https://omim.org/) and Gene Cards database (https://www.gene_cards.org/) to obtain the relevant targets of regulating central nervous system.

2.4. Construction of a network of guarana active ingredients and potential targets
The target of guarana active component and the related target that can regulate the central nervous system are taken as the potential target of guarana regulating the central nervous system. The corresponding relationship between component and target is constructed based on Excel, and cytoscape 3.7.2 software is used to visualize component-target network. The Cytoscape (NCA) plug-in is used to analyse the correlation, and the eight components with high degree are docked to further verify the relationship between the components and the targets.

2.5. Construction of protein-protein interaction network (PPI)
The effect of drugs may be the result of multiple protein interactions. Therefore, in order to better study the core efficacy of guarana, it is necessary to analyze the protein interaction of guarana components and construct the interaction network. We used online interaction tools String platforms (https://string-db.org/) to introduce common targets into String, species selection as human (Multiple proteins), and use highest confidence=0.900 as screening condition to obtain protein phase interaction relationship.

2.6. Topological analysis of core targets of guarana
For studying the core efficacy of guarana, it is particularly necessary to screen out the core targets by topological analysis of its PPI network. Therefore, based on the NCA plug-in in the cytoscape3.7.2, the core network analysis is carried out, and the top 8 targets of the degree value (degree) are screened out.

2.7. GO and KEGG analysis
This study applied Metascape database (https://metascape.org/) for enrichment analysis. The Common targets were introduced into the Metascape website, GO BP, GO CC, GO MF enrichment analysis and KEGG pathway analysis were carried out, and the pathway analysis Gene in GO and was visualized by cytoscape 3.7.2 software of top 5.

2.8. Molecular docking validation
The 2D structure information of four core genes (CYP19A1, PTGS2, AR, NR3C1, DRD2, ESR1, CNR1, AKT1) were downloaded from the Pubchem database and unified into 3D structures by using
Chem3D software. Search the Uniprot database (https://www.uniprot.org/) and download the 3D structures regulating the candidate target proteins of the central nervous system in PDB database (http://www.rcsb.org/). Ligands and water in the target protein were removed by Pymol software. AutoDock Vina software, 4 core components were docked to protein receptors. The lowest binding energy of docking is the best binding position. Pymol software is used to visualize the conformation with the best binding position.

3. Results

3.1. Analysis of guarana ghemical composition and Adventist

By confirming the structure of the downloaded components, 43 guarana components were obtained. The 13 components meet the screening conditions of OB > 30 and DL > 0.18, and the screening results are shown in Table 1.

Table 1. Chemical constituents of guarana

| CAS       | Molecule Name       | Structure | MW     | OB (%) | DL  |
|-----------|---------------------|-----------|--------|--------|-----|
| 80356-14-5| CLR                 |           | 386.73 | 37.87  | 0.68|
| 1406-18-4 | vitamin-e           |           | 490.69 | 32.29  | 0.7 |
| 5779-62-4 | beta-sitosterol     |           | 414.79 | 36.91  | 0.75|
| 83-46-5   | ZINC03982454        |           | 414.79 | 36.91  | 0.76|
| 19044-06-5| poriferast-5-en-3beta-ol |       | 414.79 | 36.91  | 0.75|
| 64997-52-0| sitosterol          |           | 414.79 | 36.91  | 0.75|
| 474-62-4  | campest-5-en-3beta-ol |        | 400.76 | 37.58  | 0.71|
| 474-62-4  | campesterol         |           | 400.76 | 37.58  | 0.71|
| 6869-99-4 | delta 7-stigmastenol|           | 414.79 | 37.42  | 0.75|
| 481-19-6  | stigmast-7-enol     |           | 414.79 | 37.42  | 0.75|
| 83-48-7   | Stigmasterol        |           | 412.77 | 43.83  | 0.76|
| 50770-19-9| anethole            |           | 148.22 | 32.49  | 0.03|
| 83-67-0   | theobromine         |           | 180.19 | 69.29  | 0.06|
3.2. Establishment of a library of component targets and disease targets
The SMILES characters of guarana active component structure information were input into the Swiss Target Prediction website for target prediction, and 79 potential component targets were obtained under the condition of probability greater than 0.1. The Gene Cards database and the OMIM database were input into the “Central nervous system” search to obtain the relevant targets that can regulate the central nervous system, and the disease targets obtained from the two database were combined and reprocessed. A total of 16911 targets related to the regulation of the central nervous system were obtained. The potential component targets and the targets related to the regulation of the central nervous system were intersected to obtain 79 common targets of the two, and 79 potential targets of regulating the central nervous system. As shown in Fig. 1, these 79 common targets are used as the core targets for subsequent enrichment analysis and molecular docking.

3.3. Construction of guarana component-target action network
According to the corresponding relationship, the "component-target" network diagram (Fig.2) was constructed by using Cytoscape 3.7.2 software. It can be seen from the diagram that one component can act on multiple targets, and one target is also associated with multiple components, which also reflects the characteristics of multi-target and multi-pathway action of guarana.

3.4. Construction of protein interaction network (PPI)
Biological function may not be the result of the interaction of a single protein. Based on the target of Guarana component, the protein interaction network of Guarana component target was further studied. The 79 component targets of Guarana component targets of Guarana were introduced into the online protein interaction analysis platform STRING, the protein interaction network (PPI) was obtained under the screening condition of highest confidence-0.900. It was found that these proteins may be related to the core efficacy of Guarana.
Figure 2. Composition - Target Network

Figure 3. Schematic diagram of protein interaction network of potential target
3.5. Core network analysis

The protein interaction network constructed by the PPI network is downloaded from its "string_interactions.tsv" format file. The relationship between Cytoscape 3.7.2 (NCA) plug-in and R language is topologically analyzed, the value of core protein (Degree) is further calculated including genes have CYP19A1, PTGS2, AR, NR3C1, DRD2, ESR1, CNR1, AKT1, its screening process and network relationship of genes are shown in figure 4.

![Core Gene Screening Process](image)

**Figure 4.** Core Gene Screening Process

3.6. Biological process enrichment analysis

The use of R language for biological processes (biological process) of guarana and common genes regulating the central nervous system BP, cellular components (cellular component); and CC, molecular function (molecular function), and MF Enrichment Analysis, BP analysis of 10 biological processes obtained. BP enrichment analysis showed that guarana biological processes were mainly in steroid metabolism process, regulation of small molecule metabolism process, regulation of inflammatory response, regulation of lipid metabolism process, regulation of steroid biosynthesis process, regulation of lipid biosynthesis process, intracellular receptor signaling pathway, negative regulation of external stimulus response, etc. BP enrichment analysis showed that guarana was involved in the whole component of the cell component presynaptic membrane, the component of the synaptic membrane, the intrinsic component of the synaptic membrane, etc. CC enrichment analysis showed that the molecular
function of guarana mainly regulated nuclear receptor actively, ligand-activated transcription factor actively, steroid binding, transcriptional coactivator binding, transcription cofactor binding, etc. The enrichment results are shown in Fig. 5 (a, b).

**Figure 5.** GO BP, GO CC, GO MF enrichment results (a, b).
3.7. **KEGG pathway enrichment analysis**

For each given gene list, enrichment analysis was performed using KEGG pathways. All genes in the genome are used as enrichment backgrounds. The \( p \) value <0.01, the minimum count is 3, and the enrichment factor >1.5 (the enrichment factor is the ratio of observation count to accidental count) is collected and grouped according its membership similarity. For visualization of the first 8 pathways in the degree value (Fig. 6). The correlation is shown in Fig. 7. These pathways include neuroactive ligand receptor interactions, cancer pathways, cMAP signaling pathways, human cytomegalovirus infection, PI3K-Akt signaling pathways, calcium signaling pathways, human papillomavirus infection, Rap1 signaling pathways, etc.

![Figure 6. KEGG pathway enrichment](image)

**Figure 6.** KEGG pathway enrichment

![Figure 7. Relationship between Pathway and Gene.](image)

**Figure 7.** Relationship between Pathway and Gene.
3.8. Molecular docking
To test our results, we use molecular docking technology to further explore the mechanism of Guarana's target on central nervous regulation. Choose the 3D structure of the core gene as ligand. These include β sterols, sterols, β7-bean sterols, vitamins E, stigma steroids. The protein molecules obtained from the PDB database are composed of 3EQM, 5F19, 1XOW, 5EMP, 6CM4, 2JFA, 1LVR, 2UVM. Auto Dock_Vina software was used to simulate the docking of core components with protein ligands. A unified size_X, size_Y, size_Z of 40 per cent in software, energy range 5 per cent; num_modes20. The specific coordinate parameters are shown in Table 2. The docking analysis shows, there is good binding between small molecule ligand and target protein receptor. And predicted the binding sites, See (Fig. 8)

Table 2. Coordinate parameters of ligand binding to receptor

| Receptor | ligand | Center_x | Center_y | Center_z |
|----------|--------|----------|----------|----------|
| CYP19A1  | 3EQM   | 55.602   | 29.44    | 30.42    |
| PTGS2    | 5F19   | 22.594   | 40.999   | 39.56    |
| AR       | 1XOW   | 21.587   | 3.815    | 10.839   |
| NR3C1    | 5EMP   | 14.116   | 22.282   | 16.048   |
| DRD2     | 6CM4   | 44.095   | 20.73    | 25.862   |
| ESR1     | 2JFA   | 17.58    | 23.676   | 19.21    |
| CNR1     | 1LVR   | 21.08    | 3.729    | 10.575   |
| AKT1     | 2UVM   | 20.793   | -0.007   | 10.614   |

![AKT1 and 2UVM](image1)

![AR and 1XOW](image2)

![CNR1 and 1LVR](image3)

![CYP19A1 and 3EQM](image4)
4. Discussion

Network Pharmacology is the understanding of the interaction between drugs and the body from a holistic perspective, a philosophy and research model of modern biomedical research in line with the overall concept of traditional Chinese medicine [15], for the modernization of traditional Chinese medicine to provide a new research ideas. Although Guarana does not belong to traditional Chinese medicine, it is still within the scope of this herb in a broad sense [16,17]. Therefore, we can still use network pharmacology to study the core efficacy of Guarana in traditional Chinese medicine research [18]. This study uses the network pharmacology method to study the core efficacy of Guarana [19]. The experiment first studied Guarana's material base, constructed its component target protein interaction network, and screened out is core network [20].

5. Conclusion

In this study, the enrichment of biological processes, metabolic pathways and molecular functions was carried out, and the effects of the core network on the central nervous system were finally verified. Enrichment results showed that its core targets were associated with steroid metabolic processes, regulation of small molecular metabolic processes, regulation of inflammatory responses, regulation of lipid metabolic processes, steroid biosynthesis processes, regulation of lipid biosynthesis processes, intracellular receptor signaling pathways, and negative regulation of external stimuli responses, all of which were associated with regulation of the central nervous system, which also confirmed some of the roles played by guarana in antifatigue, uplifting, and increasing attention. This also points out the direction for our further research on Guarana in the later period, and provides theoretical basis and reference for the later experimental verification.

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