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Original article

Efficacy of Valerian Root Extract on Anxiety via Bioinformatics

Somayeh Esmaeili[1], Mostafa Rezaei-Tavirani[2*], Mohhamadreza Razzaghi[3], Farshad Okhovatian[4]

[1]Traditional Medicine and Materia Medica Research Center, Department of Traditional Pharmacy, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[3]Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[4]Physiotherapy Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract

Background and objectives: Treatment with root extract of valerian can reduce anxiety neurosis symptoms. To investigate the related therapeutic impacts, bioinformatics analysis of proteome profile of rat's hippocampus tissue was carried out. **Methods:** Cytoscape V.3.9.1, and its plug-ins were applied for the construction of protein-protein interaction network of the treated subjects. NetworkAnalyzer and ClueGO+CuePedia were used to study centrality and gene ontology of the protein-protein interaction (PPI) network. **Results:** Results indicated that seven central proteins Actb, Alb, Akt1, Egfr, Tp53 as hub-bottlenecks and Th and H2afx as hub and bottleneck differentially expressed proteins DEPs are present in the PPI network and four corresponding biological processes. Among these seven proteins, two including tyrosine 3-monooxygenase (Th) and Histone H2A (H2afx) are differentially expressed proteins in the exposure of valerian. **Conclusion:** It was found that the histone H2A and tyrosine 3-monooxygenase as central nodes are the main targets of valerian which are associated with anti-anxiety effects of the herb.

Keywords: anxiety; biomarkers; protein-protein interaction network analysis; valerian

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Introduction

Anxiety is a prevalent psychiatric condition among the populations in the today modern life which in many cases is complex with other disorders [1,2]. Available treatments are pharmacotherapy and cognitive behavioral therapy (CBT) [3,4]. Medical treatments for this condition are serotonin and norepinephrine reuptake inhibitors (SNRIs) while they are not effective in some patients [5]. Natural sources including lavender, chamomile, saffron, Damask rose, Ashwagandha, and valerian have shown promising as alternatives with lower side effects [6-8]. In this regard, *Valerian sp.* has demonstrated health benefits for different conditions including anxiety, premenstrual syndrome (PMS), and insomnia [9-11]. *Valerina officinalis* is also used for the treatment of gastrointestinal spasms [12]. Valerian key compounds such as valerenic acid and valerenol modulate gamma-aminobutyric acid (GABA) receptors as the highlighted mechanism of sedative effects in valerian [13]. Furthermore, glutamergic receptors are also in interactions with valerian compounds in the treatment of

*Corresponding author: tavirani@sbmu.ac.ir

anxiety. What is more, other constitutions including isovaleric acid, didrovaltrate, borneol, and some lignans are noteworthy in promoting health condition [12]. Symptoms of premenstrual syndrome could subside via the impact of valerian treatment [10]. In sleep disorders, the rhythm of non-rapid eye movement (NREM) can be modulated with valerian. Valerian can develop the duration of sleep time even after caffeine intake. In NREM sleep, the delta wave rises and eventually the quality of sleep promotes [11]. However, excessive application of even herbal medicine could result in adverse effects [14], one of which is an example of overdose of valerian with GABA supplement that causes encephalopathy in the treatment of sleep disorder [15]. In this respect, some types of herbal medicine accompanied with drugs could result in unfavorable outcomes due to herb-drug interactions [14]. For instance, consumption of haloperidol and valerian are reported to result in some complications in human body via increment level of some processes that can increase liver and kidney impairment [16]. Moreover, in comparison with some applied medicine, valerian expresses some similarity to diazepam effect [9]. Patients suffering from anxiety could have other comorbidities such as depression. These cases are prone to many other complications including committing suicide [17]. Therefore, studying anxiety is eminent especially in the field of biomarker discovery. Identification of biomarkers related to anxiety, could assist the understanding of molecular basis for diagnosis and treatment approaches of this condition. By monitoring the biomarkers before and after exposure to the treatment of interest, it is feasible to detect mechanism of effectiveness [18,19]. Proteomics can be applicable in this respect by analyzing large-scale protein profile of different subjects in different conditions [19]. The dysregulated proteins in the presence of the specific treatment are considered as potential biomarkers. Furthermore, complementary bioinformatics evaluation of these biomarkers could detect more promising targets for clinical usage [20]. Set of genes or proteins can be analyzed via protein-protein interaction network analysis to find the critical individual as biomarker panel [21]. Consequently, in this study proteome profile of rats after exposure to valerian was analyzed via protein-protein interaction network analysis.

Material and Methods

Ethical considerations

This project was approved by ethical committee of Shahid Behheshti university of Medical Sciences (IR.SBMU.RETECH.REC.1401.126).

Data collection

In this study, proteome profile of rats' hippocampus samples was evaluated in a protein-protein interaction network scale. In the first research, in order to examine the sedative effects of *Valeriana jatamansi* Jones, male rats were exposed to the treatment of root extract with different combinations. In a way that, the stress-induced rats were tested with ten different combinations of the ingredients (iso-chlorogenic acid A, isochlorogenic acid B and isochlorogenic acid C and chlorogenic acid) to assess the best outcome. At the 21st day of the treatment, the rats were examined with behavioral tests and consequently prepared for the final investigation that was the iTRAQ-based proteomics evaluation [9]. Moreover, bioinformatics study of the differentially expressed proteins (DEPs) was handled and the gene ontology (GO) and pathways of the constructed PPI was analyzed in the main study.

Protein-protein interaction network analysis

In our analysis, further bioinformatics examination of the DEPs was pursued in terms of centrality prioritization and biological process detection of hub-bottleneck proteins. In the protein-protein interaction network proteins expressing highest values of degree (K) and betweenness centrality (BC) are the hub-bottlenecks [22]. In order to perform PPI network analysis, String database was explored in Cytoscape V.3.9.1. (https://cytoscape.org/). String database (http://string-db.org/) provides different query sources including Disease query, PubMed query, STITCH query, and protein query [23]. To gain interaction network analysis of the differentially expressed proteins from the main study, protein query (Rattus norvegicus) was used with the confidence score cut off of 0.4 as the default option. Furthermore, centrality analysis of PPI network was handled by NetworkAnalyzer [22] plug-in that is embedded in Cytoscape software.

Gene anthology analysis

Finally, enrichment analysis of hub-bottlenecks

and their predicted action type in an interaction network was assessed with ClueGO 2.5.8+ CluePedia 1.5.8 [24,25].

Statistical analysis

For the biological process determination, certain criteria were designated as follows: kappa score cutoff for term grouping was set to 0.4. Protein number and percentage per term were 2 and 3, respectively. P-value correction method was the default option of Bonferroni step-down test. The statistical significance was P ≤0.05. The statistical criteria for this analysis was kappa score statistics. The kappa score cut off for activation, expression, and inhibition determination were set to 0.5.

Results and Discussion

In the first study, 20 dysregulated proteins were determined in proteome group of rats treated with optimum proportion of valerian. These identifiers were searched against Cytoscape with two criteria, first network without additional neighbors, and second network with the addition of 50 nodes. In the first network, with the confidence score cut off=0.4, proteins were not in a notable interactions and only two links were present among four nodes separately. By adding 50 nodes to these DEPs, a network of 70 nodes with 650 links was obtained with the same confidence score; however, four DEPs still remained not connected to the network. A network of protein connections is shown in Figure 1 with respect to the expression changes of query proteins. Since expression value of the first neighbors were not accessed, these nodes are not connected with expression change links. The bigger the node, the higher the expression of that protein in the treated sample is.

As mentioned in the Figure 1, the highlighted nodes are query proteins that are expressed differentially. The linkages of these nodes with their neighbors are also highlighted in red. Other nodes are the neighbors that don't reflect any expression changes in this pattern.

The hubs, bottlenecks, and hub-bottlenecks of the PPI network were calculated based on 20% of the highest values of degree and betweenness centrality after analysis with "NetworkAnalyzer" (Table1).

Five nodes were assigned as hub-bottlenecks in table 1 that none were among the query proteins. Actb is the leading valued node (degree: 41 and BC: 0.1)

H2afx is a bottleneck with a betweenness centrality of 0.2 and degree of 16. This node is not a hub since it has a low value of degree. However, it has the highest values of betweenness centrality in the network that makes it as a central node that is in the control of information flow in the system of interaction.

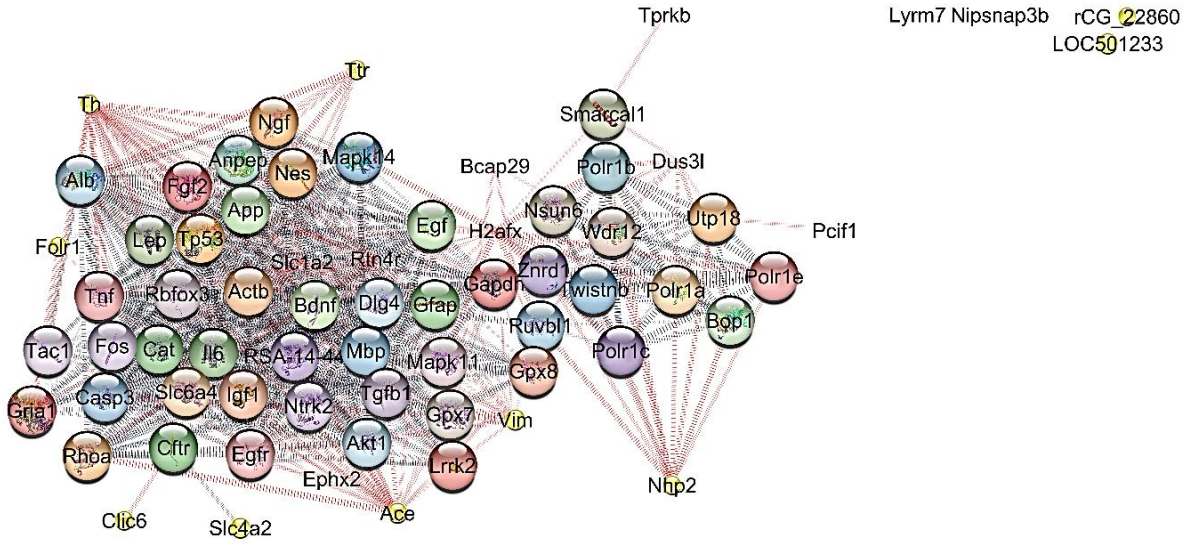

Figure 1. Expression profile of protein-protein interaction network in the presence of valerian; highlighted nodes are the query proteins. The red links are interactions of DEPs with their surroundings. The isolated nodes are shown in the left side of schema.

Table 1. The list of hub-bottleecks ranked based on degree values (K). Betweenness centrality (BC) values are normalized.

Row	Display Name	K	BC
1	Actb	41	1.00
2	Alb	41	0.67
3	Akt1	37	0.00
4	Egfr	33	0.17
5	Tp53	32	0.00

The query proteins were examined for their centrality perspective in the PPI network via "NetworkAnalyzer" as listed in Table 2.

Tyrosine 3-monooxygenase (Th) and Histone H2A (H2afx) are characterized with the highest values of degree and betweenness centrality, respectively. Th could be accounted as marginally valued hub of the PPI considering 20% highest rank determination. In the next step, enrichment study of central nodes (hub-bottlenecks and hub and bottleneck DEPs) was conducted via ClueGO+CluePedia. The biological process identification is depicted in Figure2.

In Figure 2, biological process analysis for central nodes is addressed. These highlighted groups are the main related terms. Four groups were identified and all of them are statistically significant. Regulation of cyclin-dependent protein serine/threonine kinase activity, response to osmotic stress, and circadian behavior are the most leading groups in terms of relation to the most linked proteins.

Action type analysis in view of activation, expression, and inhibition were also designated for the central nodes evaluations as presented in Figure 3.

Studies show that valerian can influence the connection in brain in the presence of stress and anxiety [26]. One of the reported mechanisms by which valerian shows anti-stress effects is by incrementation of GaBRB3 expression [25]. It has also beennoted that valerian and diazepam use the same mechanisms to promote anti-anxiety effects [25].

The samples were treated with the designated optimal concentrations of compounds of valerian for the proteomics study in the main research. Among the 6818 detected proteins, 80 were expressed differentially. In the original study, bioinformatics evaluation was conducted and a number of biological processes were recognized [9].

Table 2. The list of query terms with the corresponding degree value (K), normalized betweenness centrality (BC), and fold change (FC) are presented.

Row	Query Term	K	BC	FC
1	Th	31	0	0.58
2	Ace	26	0	0.66
3	Slc1a2	22	0	1.24
4	Rtn4r	17	0	1.24
5	H2afx	16	0.2	1.38
6	Vim	15	0.05	0.68
7	Nhp2	11	0	0.58
8	Ttr	9	0	0.61
9	Dus3l	8	0	1.35
10	Folr1	4	0	0.6
11	Bcap29	3	0.01	1.26
12	Ephx2	2	0	1.6
13	ae2	1	0	0.66
14	Clic6	1	0	0.59
15	Pcif1	1	0	1.23
16	Tprkb	1	0	1.23
17	LOC100911130	0	0	0.55
18	LOC501233Â	0	0	0.68
19	Lyrm7	0	0	1.29
20	Nipsnap3b	0	0	1.4

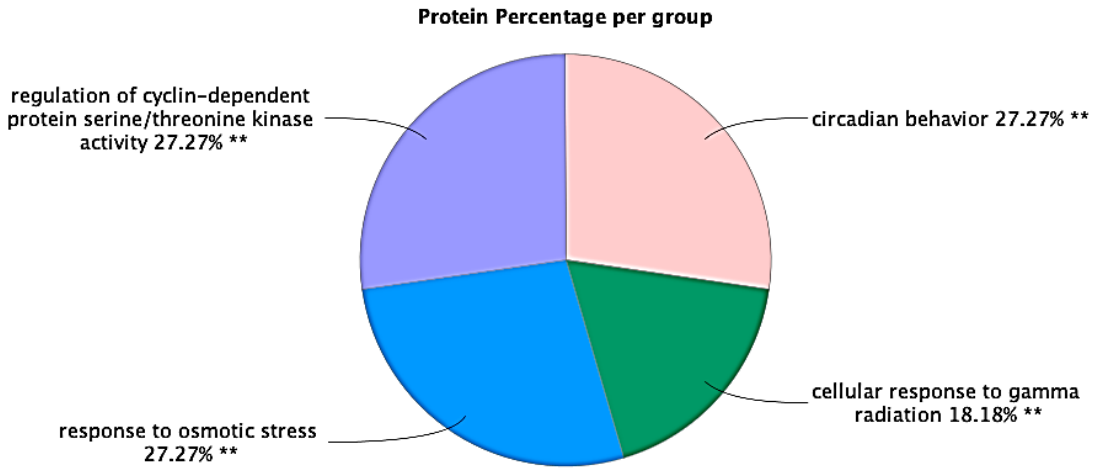

Figure 2. Pie chart view of biological processes linked to central proteins p≤0.05

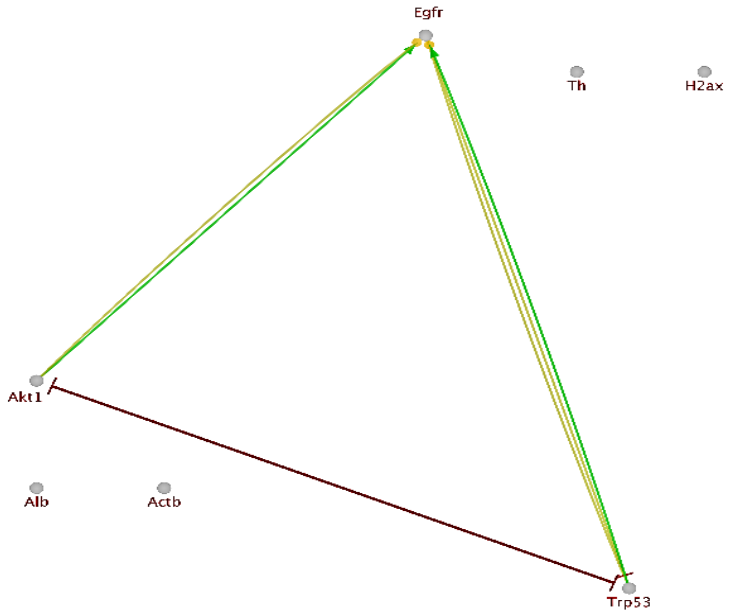

Figure 3. Action type analysis via CluePedia of the central nodes with kappa score cut off=0.5; green: activation yellow: expression red: inhibition; among the central first neighbor nodes, only three of them are in interactions in different types. These nodes are Akt1, Trp53, and Egfr.

In the present research, differentially expressed proteins were chosen for PPI network analysis in respect to centrality and action relationship between central proteins. Dysregulation of central nodes could impose high amount of disruption in the network system. According to the previous studies [27], exploring and understanding their roles could be essential in the mechanism of disease and the applied treatments. Vertices that show high degree have so many connections in a PPI map. Similarly, nodes with high values of betweenness centrality carry out heavy flow of information passing through. Any dysfunction towards these central nodes develops irregulations in the whole interaction network and consequently massive phenotype alterations. These changes could be in correspondence to disease progress or any external influence such as treatment applications. In addition to DEPs, other central nodes could be important to be examined. Based on Table 1, there are five nodes in the PPI network that none are among the DEPs.

Actin, cytoplasmic 1 (Actb) possess the highest value of the centrality in this PPI network analysis. The relation between this gene with bipolar disorder has been mentioned in one study [28]. What is more, based on our previous study this gene is also a hub-bottleneck in Autism disorder as well [29]. The possibility of Actb correlation with anxiety behavior is important to be studied. The next high ranked hub-bottleneck is albumin, which showed elevation in serum in patients with anxiety [30]. Akt1, the third ranked hub-bottleneck, appeared to have some linkage with anxiety and other related conditions such as depression [31]. The fourth hub-bottleneck is epidermal growth factor receptor (EGFR) that in patients with cancer, has shown correlation with depression [32]. The last hub-bottleneck is TP53 that in respect to psychiatric disorders, its polymorphism indicated associations with bipolar disorder [33]. H2afx as the central DEP bottleneck is reported to be associated with cognitive dysfunction and anxiety trigger in mice with the history of stress-induced by maternal separation [34]. Based on another study, it is noted that lack of expression of histone H2A could develop impairment in neurobehavioral functions [35]. Other studies also referred to the substantial linkage between neurological activities and the role of H2A. Absence of H2A could consequently promote neurobehavioral disorders [36]. This protein in the main study is up-regulated in the presence of valerian which indicates its potential regulatory effect. By affecting this protein, valerian could conduct its

sedative effects. Tyrosine 3-monooxygenase (Th) is the marginally hub DEP that could play a role in psychiatric disorders by modulating dopamine and norepinephrine levels [37]. Effective properties of this protein against Parkinson disease has been reported [38]. Next analysis is to investigate the centrality properties of DEPs. These proteins express no significant central role in the PPI network except for two proteins including tyrosine 3-monooxygenase (Th) and Histone H2A (H2afx). The first protein is a marginally significant hub in the PPI network and the second protein is an important bottleneck. Enrichment analysis of central nodes led to introducing four groups of biological processes. H2ax is linked to cellular response to gamma radiation and Th corresponds to the response to osmotic stress, and circadian behavior. These terms could be a part of valerian sedative mechanism in anxiety. Finally, the action type evaluation of the central nodes identified three nodes in different relations including activation, expression, and inhibition. AKt1 and Trp53 have inhibition effects on each other. These two nodes are also show activation and expression impact on Egfr. Based on the findings, valerian effects on anxiety were confirmed but further investigations are recommended to explore the related biomarkers

Conclusion

This study suggests that five first neighbor central proteins plus histone H2A and tyrosine 3-monooxygenase among the query DEPs are the main targets of *Valeriana jatamansi*. The finding describes molecular mechanism of anti-anxiety of *V. jatamansi* root extract as a nutrient. It seems that regulation of the related biological processes may be involved in the sedative mechanisms; however, more analysis is required to verify this claim.

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Author contributions

Somayeh Esmaeili, Mostafa Rezaei-Tavirani, Mohhamadreza Razzaghi and Farshad Okhovatian were involved in data collection and analysis and approved the final draft of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

PPI: protein-protein interaction; DEPs: differentially expressed proteins; CBT: cognitive behavioral therapy; SNRIs: serotonin and norepinephrine reuptake inhibitors; NREM: non-rapid eye movement; GO: gene ontology; K: degree; BC: betweenness centrality; Actb1: cytoplasmic 1; FC: fold change; EGFR: epidermal growth factor receptor