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Details of the Collaborative Activity

2019-20

Name of the Collaborating Institute: Sushrutha Ayurveda Hospital, Puttur 574201, Karnataka, India.

Name of the Collaborating Department from YDU: Yenepoya Research Center

Joint Research and Publication

Dr. T Keshav Prasad and Dr. Prashant Modi from Center for Systems Biology and Molecular Medicine, Yenepoya Research Center collaborated with the Sushrutha Ayurveda Hospital, Puttur, Karnataka for joint research in the area of neuroprotective functions of ayurvedic medicines in neurodegenerative diseases. They have done the following joint publications from this research

Karthikkeyan G, Pervaje R, Pervaje SK, **Prasad TSK, Modi PK**. Prevention of MEK-ERK-1/2 hyper-activation underlines the neuroprotective effect of Glycyrrhiza glabra L. (Yashtimadhu) against rotenone-induced cellular and molecular aberrations. *Journal of Ethnopharmacology*. 2021; 274:114025.

Karthikkeyan G, Pervaje R, Subbannayya Y, **Patil AH, Modi PK, Prasad TS.** Plant Omics: Metabolomics and Network Pharmacology of Liquorice, Indian Ayurvedic medicine Yashtimadhu. *OMICS: A Journal of Integrative Biology*. 2020; 24, 743-755

Karthikkeyan G, Najar MA, Pervaje R, Pervaje SK, **Modi PK, Prasad TSK**. Identification of Molecular Network Associated with Neuro protective Effects of Yashtimadhu (Glycyrrhiza glabra L.) by Quantitative Proteomics of Rotenone-Induced Parkinson's Disease Model. *ACS Omega*. 2020; 5(41):26611-26625.

Deolankar SC, Modi PK, Subbannayya Y, Pervaje R, **Prasad TSK**. Molecular targets from traditional medicine for neuroprotection in human neurodegenerative diseases. *OMICS: A Journal of Integrative Biology*. 2020: 7, 394-403.

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Prevention of MEK-ERK-1/2 hyper-activation underlines the neuroprotective effect of *Glycyrrhiza glabra* L. (Yashtimadhu) against rotenone-induced cellular and molecular aberrations

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ABSTRACT

Ethnopharmacological relevance: Yashtimadhu choorna (powder) is prepared from the dried root of *Glycyrrhiza glabra* L., commonly known as licorice. The Indian Ayurvedic system classifies Yashtimadhu as a *Medhya Rasayana* that can enhance brain function, improves memory, and possess neuroprotective functions, which can be used against neurodegenerative diseases like Parkinson's disease (PD).

Aim of the study: We aimed to decipher the neuroprotective effects of *G. glabra* L., i.e., Yashtimadhu, in a rotenone-induced PD model.

Materials and methods: Retinoic acid-differentiated IMR-32 cells were treated with rotenone (PD model) and Yashtimadhu, and were assessed for cellular toxicity, live-dead staining, cell cycle, oxidative stress, protein abundance, and kinase phosphorylation.

Results: Yashtimadhu conferred protection against rotenone-induced cytotoxicity, countered cell death, reduced expression of pro-apoptotic proteins (cleaved-caspases-9, and 3, cleaved-PARP, BAX, and BAK) and increased anti-apoptotic protein, BCL-2. Rotenone-induced cell cycle re-entry (G2/M transition), was negated by Yashtimadhu and was confirmed with PCNA levels. Yashtimadhu countered rotenone-mediated activation of mitochondrial proteins involved in oxidative stress, cytochrome-C, PDHA1, and HSP60. Inhibition of rotenone-induced ERK-1/2 hyperphosphorylation prevented activation of apoptosis, which was confirmed with MEK-inhibitor, highlighted the action of Yashtimadhu via ERK-1/2 modulation.

Conclusions: We provide the evidence for neuroprotection conferred by *G. glabra* L. (Yashtimadhu) and its mechanism via inhibiting MEK-ERK-1/2 hyper-phosphorylation, prevention of mitochondrial stress, and subsequent prevention of apoptosis. The study highlights Yashtimadhu as a promising candidate with neuroprotective effects, the potential of which can be harnessed for identifying novel therapeutic targets.

1. Introduction

Parkinson's disease (PD) is an age-related progressive neurodegenerative motor disorder associated with selective loss of dopaminergic neurons from Substantia Nigra pars compacta (SNpc). PD is reported to affect 2–3% of the world elderly population (>65 years) of age (Poewe et al., 2017). The onset of Familial PD is generally due to genetic mutations, while sporadic PD is attributed to environmental, biochemical, and molecular aspects that dysregulate neuron functions (Kalia and Lang, 2015; Zeng et al., 2018). The known molecular mechanisms that lead to the death of dopaminergic neurons are; protein aggregation (Lewy body formation), mitochondrial stress, oxidative stress, dopamine quinones, microglial activation, and subsequent neuroinflammation (Kalia and Lang, 2015; Poewe et al., 2017). Management of PD primarily includes levodopa, dopamine agonists, monoamine oxidase inhibitors, and deep-brain stimulation (Poewe et al., 2017; Ray Chaudhuri et al., 2016). Prolonged usage of these PD medications has been reported to

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Plant Omics: Metabolomics and Network Pharmacology of Liquorice, Indian Ayurvedic Medicine Yashtimadhu

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Abstract

Plant omics is an emerging field of systems science and offers the prospects of evidence-based evaluation of traditional herbal medicines in human diseases. To this end, the powdered root of Yashtimadhu (*Glycyrrhiza glabra* L.), commonly known as liquorice, is frequently used in Indian Ayurvedic medicine with an eye to neuroprotection but its target proteins, mechanisms of action, and metabolites remain to be determined. Using a metabolomics and network pharmacology approach, we identified 98,097 spectra from positive and negative polarities that matched to ~1600 known metabolites. These metabolites belong to terpenoids, alkaloids, and flavonoids, including both novel and previously reported active metabolites such as glycyrrhizin, glabridin, liquiritin, and other terpenoid saponins. Novel metabolites were also identified such as quercetin glucosides, coumarin derivatives, beta-carotene, and asiatic acid, which were previously not reported in relation to liquorice. Metabolite–protein interaction-based network pharmacology analyses enriched 107 human proteins, which included dopamine, serotonin, and acetylcholine neurotransmitter receptors among other regulatory proteins. Pathway analysis highlighted the regulation of signaling kinases, growth factor receptors, cell cycle, and inflammatory pathways. *In vitro* validation confirmed the regulation of cell cycle, MAPK1/3, PI3K/AKT pathways by liquorice. The present data-driven, metabolomics and network pharmacology study paves the way for further translational clinical research on neuropharmacology of liquorice and other traditional medicines.

Keywords: liquorice, metabolomics, Ayurvedic medicine, Yashtimadhu, neuropharmacology, bioinformatics

Introduction

Traditional herbal medicines have been used in human diseases since time immemorial. Yet, their molecular mechanisms of action are not adequately characterized or remain unknown. There is a need for data-driven and evidence-based evaluation of traditional medicines and their systems scale effects.

Plant omics is an emerging field of systems science and offers veritable prospects for discovery and translational clinical research. In addition, the World Health Organization (WHO) has launched the WHO Traditional Medicine strategy 2014–2023 to strengthen and develop the role of traditional medicine practices in health. In the United States, National Center for Complementary and Alternative Medicine (NCCAM) and Food and Drug Administration (FDA) are involved in the regulation of complementary and alternative medicinal (CAM) practices (Ventola, 2010), and in the European Union, CAMBrella European research network is involved in the regulation of CAM (Wiesener et al., 2012). In India, the Ministry of AYUSH acts as the regulatory body that regulates and promotes the use of Ayurvedic medicines.

The powdered root of Yashtimadhu (*Glycyrrhiza glabra* L.), commonly known as liquorice, is frequently used in Indian Ayurvedic medicine for human diseases, and in neurology and psychiatry-related clinical contexts in particular (Hosseinzadeh and Nassiri-Asl, 2015; Hwang et al., 2006; Shen et al., 2013; Yu et al., 2008). On the contrary, liquorice target proteins, mechanisms of action, and metabolites remain to be determined. Recently, we have shown that

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Article

Identification of Molecular Network Associated with Neuroprotective Effects of Yashtimadhu (*Glycyrrhiza glabra* L.) by Quantitative Proteomics of Rotenone-Induced Parkinson's Disease Model

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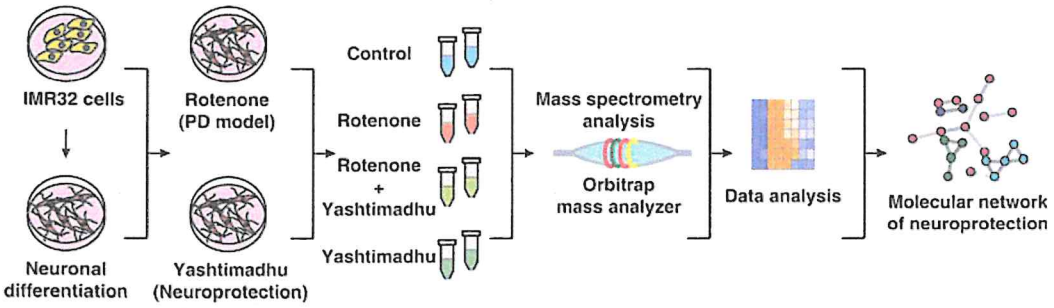

ABSTRACT: Parkinson's disease (PD) is a progressive neurodegenerative disorder, whose treatment with modern therapeutics leads to a plethora of side effects with prolonged usage. Therefore, the management of PD with complementary and alternative medicine is often pursued. In the Ayurveda system of alternative medicine, Yashtimadhu choorna, a *Medhya Rasayana* (nootropic), prepared from the dried roots of *Glycyrrhiza glabra* L. (licorice), is prescribed for the management of PD with a favorable outcome. We pursued to understand the neuroprotective effects of Yashtimadhu choorna against a rotenone-induced cellular model of PD using differentiated IMR-32 cells. Cotreatment with Yashtimadhu choorna extract rescued rotenone-induced apoptosis and hyperphosphorylation of ERK-1/2. Quantitative proteomic analysis of six peptide fractions from independent biological replicates acquired 1,561,169 mass spectra, which when searched resulted in 565,008 peptide-spectrum matches mapping to 30,554 unique peptides that belonged to 4864 human proteins. Proteins commonly identified in biological replicates and >4 PSMs were considered for further analysis, leading to a refined set of 3720 proteins. Rotenone treatment differentially altered 144 proteins (fold $\geq$1.25 or $\leq$0.8), involved in mitochondrial, endoplasmic reticulum, and autophagy functions. Cotreatment with Yashtimadhu choorna extract rescued 84 proteins from the effect of rotenone and an additional regulation of 4 proteins. Network analysis highlighted the interaction of proteins and pathways regulated by them, which can be targeted for neuroprotection. Validation of proteomics data highlighted that Yashtimadhu confers neuroprotection by preventing mitochondrial oxidative stress and apoptosis. This discovery will pave the way for understanding the molecular action of Ayurveda drugs and developing novel therapeutics for PD.

INTRODUCTION

Neurodegeneration is a progressive phenomenon at old ages, which occurs as a result of neuronal loss or the inability of neurons to transmit the signals. Parkinson's disease (PD) is one such age-related progressive neurodegenerative disorder whose prevalence accounts for 3% of the population, above 65 years.[1] PD is caused by loss of dopaminergic neurons from the substantia nigra pars compacta (SNpc), resulting in loss of the neurotransmitter dopamine, and leads to the development of motor symptoms. The death of dopaminergic neurons is caused as a result of mitochondrial dysfunction, endoplasmic reticulum (ER) stress, neuroinflammation, and accumulation of protein aggregates.[2,3] The management of PD relies on the alleviation of the symptoms, and levodopa is one of the most commonly prescribed medicines, which is useful in the early stages of the disease. However, with prolonged usage, the efficacy of PD medications decline and is reported to cause many side effects.[4] Thus, there is a need for the use of a sustainable alternative and complementary management

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Review Article

Molecular Targets from Traditional Medicines for Neuroprotection in Human Neurodegenerative Diseases

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Abstract

Neurodegeneration is one of the greatest threats to global public health. Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease are among the major causes of chronic neurological conditions in the elderly populations. Hence, neuroprotection is at the epicenter of the current 21st-century research agenda in biomedicine. Yet, novel molecular targets are limited and solely needed for neuroprotection. Marked person-to-person variations in outcomes require a deeper understanding of drug targets in neurology and clinical neurosciences. In this context, traditional medicines offer untapped potentials for discovery and translation of novel molecular targets to human neurodegenerative disease research and clinical neurology. This expert review offers a synthesis of the prospects and challenges of harnessing new molecular targets from traditional medicines, with a view to applications for neuroprotection in human neurodegenerative diseases.

Keywords: traditional medicine, neuroprotection, neurodegeneration, multi-OMICS, experimental evidence

Introduction

NEURODEGENERATION IS IRREVERSIBLE DAMAGE of neurons leading to dementia and dementia-related disorders (Sancesario and Bernardini, 2018). Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) are among the major causes of chronic neurological conditions in elderly populations. These conditions have a subtle onset with a gradual decline in health at mid to late age groups, making the early diagnosis a real challenge (Blennow et al., 2015; McKhann et al., 1984).

The clinical management of these conditions within the context of the present therapeutic strategies is limited to symptomatic remedies, as the molecular mechanisms of the disease progression and cross-talks in these conditions remain unclear. In addition, the available treatment options display limiting side effects, especially upon long-term use (Bidzan and Bidzan, 2012; Jankovic and Clarence-Smith, 2011; Kaur et al., 2016; Thanvi et al., 2007). Person-to-person variations in treatment outcomes require a deeper understanding of the molecular basis and the targets for human neurodegenerative diseases as well.

Practices with traditional medicine systems such as Ayurveda, Yoga, Unani, and homeopathy have been documented as early as the 2nd century BC (Jaiswal and Williams, 2017). Ayurveda is reported to be the most ancient system, which is thought to serve clinical end points dealing with real-world patient outcomes in particular. Ayurveda relies on the concept that every individual possesses a unique biological and ecological constitution (Prakriti and Tridosha), which can be harnessed for personally tailored treatments. Thus, the concept is somewhat similar to "personalized medicine" in its ethos.

For the past two decades, traditional medicines have gained in popularity as complementary and alternative medicine. This was enabled in part by advances in molecular methods that helped unravel the mechanism of action of traditional and herbal medicines (Thomford et al., 2018).

In all, traditional medicines offer untapped potentials for discovery and translation of novel molecular targets to

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