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PERSPECTIVE

AUSTRALIAN HERBAL INSIGHT

Metabolic similarity of plant and human: implications for efficacy and regulatory compliance of herbal therapies

Abstract

Forecasting what biological effects an active metabolite compartmentalized in a medicinal plant may have on human health requires intensive research and clinical trials. There are inconsistencies in the application of herbal medicine in the treatment of disease mostly due to the metabolic composition. Different herbal strains, growing media and environmental conditions will alter the metabolite composition of herbal extracts. A modeling approach to identify the genes, enzymes and signaling pathways involved in the biosynthesis of the medicinal plant metabolites could harmonize the process of predicting the metabolite composition. The structural similarity of primary and secondary plant herbal metabolites does not always provide complete assurance of what pharmacological effect they may have on the respective human metabolic system. Many of the medicinal plant metabolites are either unknown or not searchable through current computational resources. In this review, we have discussed that a system based biological approach comparing human and plant metabolic signaling networks that could be additionally productive to ascertain the regulatory and biological processes conferred by a metabolite and their bioactivity pathways in living systems. A combination of a both systems and structural based approaches can generate new models that render a better metabolite composition bioactivity reduction, thereby enhancing the efficacy, safety, and toxicity of herbal medicine processing.

Key words: System Pharmacology, Herbal medicine, Bioactivity, Safety, Efficacy, Toxicology

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Introduction

Herbal medicines are a mixture of active and inactive primary and secondary metabolites, some of which are useful and safe while others are rather toxic. Components that can be readily metabolized through existing biochemical routes in humans may be regarded as pharmacologically safe. A combination of these components can provide synergistic, additive, complementary and compensated effects that should be analyzed on human metabolism to determine the patient specific pharmacology of herbal medicine. A comprehensive interaction can be obtained through the biochemical interactions to assess their biological efficiency and toxicity in human. The problems associated with this kind of interaction analysis are (Klamt et al., 2007), herbal medicine include many other effective compounds (Becker et al., 2007), there is little information on the drug effect of each component (Schuster et al., 1999), there is inconsistent quality of herbal medicines (due to variability of metabolite concentrations) (Zhao and Kurata, 2009) and environmental factors and differences in processing can effect the metabolite composition. In this theoretical investigation, we present some examples of selected exciting biochemical routes that are transforming how we can assess the patient specific bioactivity of herbal medicine and related safety, toxicity and highlight the side effects. Although the pa

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-ce of progress during recent years has initiated as structure activity relationship analysis, there are still challenges to be met in order to fully define the safety of herbal medicine.

Due to the complexity of herbal medicine, the determination of efficacy remains a significant hurdle in the field compared to modern drugs. Despite the lack of robust regulations, use of herbal medicine has been growing continuously. Many scientific approaches have been initiated to normalize herbal therapies over the years including structural similarity, QSAR and SAR. However, studying the structural similarity of primary and secondary plant herbal metabolites does not always give information on what pharmacological effects they may have on humans. The metabolic pathways discovered in plants that overlap with similar pathways in humans, with regards to similar energy consumption/metabolic pathways, could potentially be used to determine the efficacy of herbal therapies. This was assessed in this study through existing KEGG database and literature information. Determining the biological effects of a medicinal plant through utilization of similar metabolic pathways could provide the potential pharmacological efficacy or adverse effects and thus an integrated biological approach comparing human and plant metabolic signaling networks could be additionally productive to ascertain the regulatory and biological processes conferred by the metabolites and their bioactivity pathways in living systems.

If a new herbal extract has no information on the entire metabolic pathways, the essential reactions should be compared with human metabolic pathways for the demonstration of efficacy, safety and toxicity. Further, herbal extracts have another problem with inconsistencies of their ingredients which lead to quality control problems for the herbal industry in achieving compliance through the regulatory guidelines. The elucidation of metabolic pathways could determine the involvement of environmental factors and growing media for herbal medicines that act to control the production of beneficial plant metabolites independent of any seasonal variation. The understanding of the extracellular and intracellular parameters could facilitate the proper time of plant harvesting to insure a consistent level and combination of metabolites is produced for a herbal medicine. The characterization of plant secondary metabolic pathways are essential to assure the safety, quality compliance and efficacy of herbal products for contemporary medicinal applications.

Advances in structure activity relationship

Inexpensive and time effective computational models together with compound database for bioactivity profiling, have revolutionized screening technology in medicine. The relatively structural similarity of the known and unknown components (compounds) revealed bioactivity of a single component in the current drug discovery approach. For the screening of lead components, fragments and substituents are aligned with the scaffold hopes using different computational languages encoded by various algorithms to coordinate a better drug design process. Specialized herbal medicine formulations can be screened in combination with various components to determine the patient specific bioactivity as being either safe or toxic. The breadth of the biochemical interaction of herbal formulation is not known, in part because of a lack of available metabolic studies that combine the various metabolite components. For example, *Orthsosiphone stamineus* is a kind of traditional folk herbal medicine which contains rosmarininc acid, sinensetin, euparorin and tetramethoxyflavonid as principle marker compounds. The structural similarity of these components are identical to human cancer cell chemicals such as ubiquinol, and upregulation of these metabolites might provide benefit through the apoptosis of cancer cells. A human metabolic database describes metabolites similar to plants that may be highly informative to elucidate a similarity in biochemical pathways for prospective research validation. Some proteins are structurally similar between plants and animals and may have similar activities, however this is not always reliable as an example the *Arabidopsis* (plant) deetiolated 2 (DET2/DDM1) gene and human 5α-Reductase are 40% identical (Li et al., 1996), yet their bioactivity is quite different. Therefore, the current drug discovery approaches cannot be exploited to identify agonistic and antagonistic effect of the metabolites. Even the structure based information is not able to determine the competitive biological effect of a metabolite component. A combined integrated of computational methods, structure based information and proven clinical data can help to link the pharmacological activity to a known metabolic pathway, but it is almost impossible to reveal the metabolic effect for combined components such as in *Orthsosiphone stamineus*. Although the substructure component is recognized to show similar properties, their polypharmacological effects in a combined formulation (herbal medicine) cannot be revealed by current drug discovery approaches.

Accelerating determination of combined components bioactivity

Development of biochemical interaction database of human and plant metabolites (components) and comparative analysis of their interaction will enable functional profiling of a combined metabolite component bioactivity. The required approach aims to validate and exploit the similarity of the interaction routes of complex human and plant metabolic pathways. Similarity analysis of the biochemical routes as well as structural identity determinations of the efficacy, safety, and toxicity of herbal medicine can advance herbal medicinal applications (Agren et al., 2014, Hebar et al., 2013). Recent examples of similarity in the interaction routes of human and plant proteins show some identity for tyrosine, tryptophan and phenylalanine biosynthesis (Fig. 1). This similarity could be used to elucidate the safety or toxicity of a herbal medicine. The proteins are useful for both plant and human metabolic functions by providing secondary metabolites for stress protection in plants and

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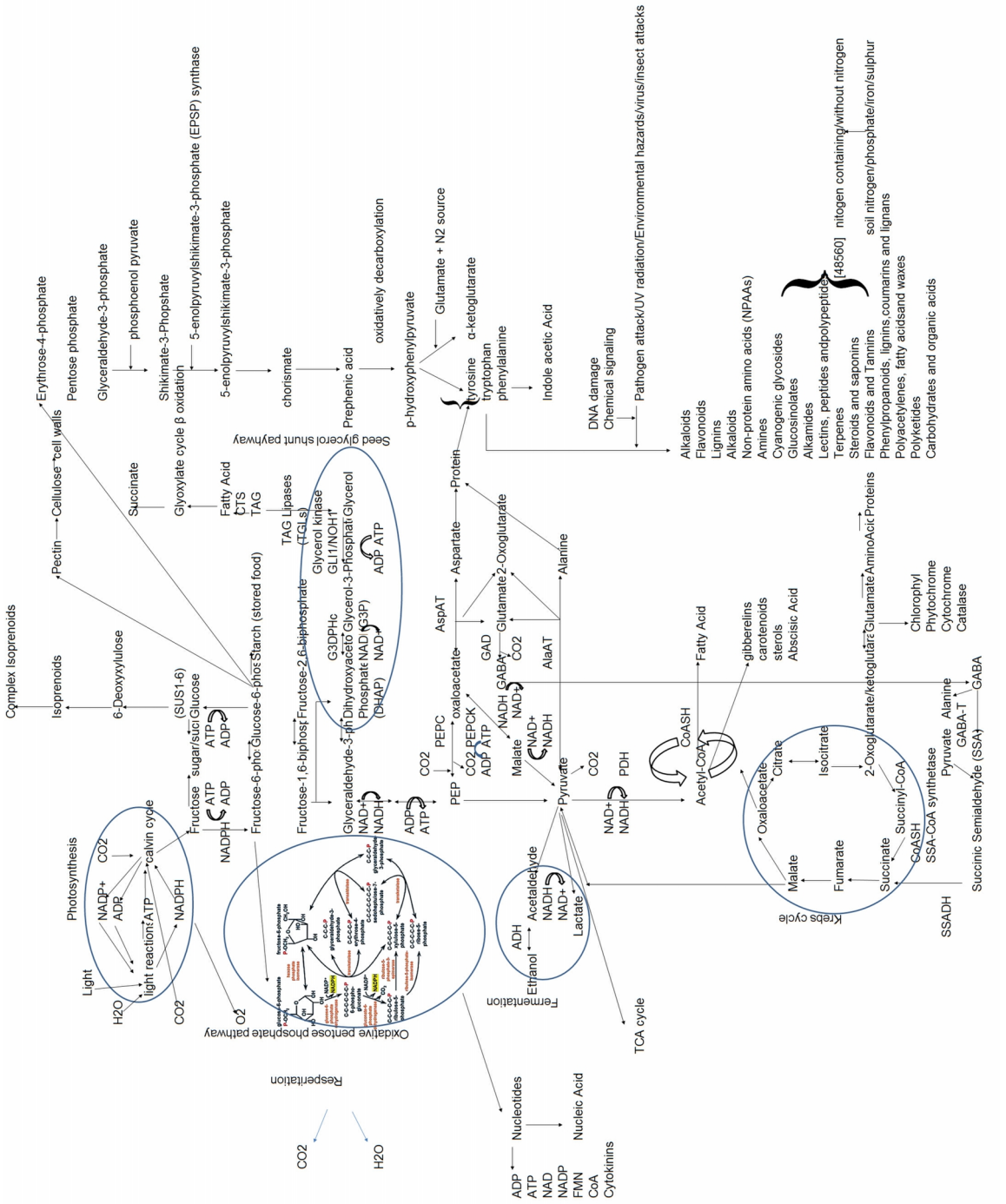

Fig. 1. Pathway map that shows the metabolic similarity of plant and human. The significantly identical similarities is observed in the primary metabolites of plant and human and the percentage of similarity is varied in the metabolic routes of secondary metabolites of plant.

antioxidants that benefit human health (Hermann, Janice). For instance, rosmarinic acid (RA) is widely used medicinal components found in many plant species (Shashank and Abhay, 2013). The biosynthetic pathways of RA in human and plants are nearly identical and as such can be regarded as safe component for herbal medicine (Fig. 2). Many chemical interactions and alternative biosynthetic pathways are active during the metabolism of RA, which are found as sulfoglucuronide conjugates of RA, methyl RA, 1-O-(2, 4, 5-trimethoxycinnamoyl, caffeic acid (CAA), ferulic acid (FA) and m-coumaric acid (COA) (Radutoiu et al., 2003, Indrasumunar et al., 2009).

A schematic metabolism of RA (Fig. 4), showed that RA could be converted into glucuronide conjugate of 1-O-(2, 4, 5-trimethoxycinnamoyl, flavone, flavonol and isoflavone into their glucuronide and/or sulfate conjugates by hydrolytic cleavage in the digestive tract and these metabolites are found in the plasma and urine. The metabolism of RA is initiated

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by the esterase presence in the digestive tract which hydrolyzes the ester linkage of the RA and make *p*-dehydroxylation of CAA. The produced CAA and COA from RA are then absorbed, conjugated and methylated in tissues. Such herbal polyphenols are necessary for human health, being metabolized in the intestine, liver and kidneys. The metabolic route of caffeic acid in the plant is similar to RA which reveals the requirement for similar enzymatic activities and metabolite pathways in plants and animals (Fig. 2A, 2C). As such the chemicals involved in RA metabolism in both humans and plants are unlikely to be toxic of have detrimental side effects on human health. Some chemicals produced by plants are not in the human body and therefore considered to be a toxic metabolite. Plants store these chemicals in specific organelles, tissues or organs and in some cases release them when attacked to detour insect or animal feeding such as jasmonic acid, salicylic acid, ethylene, and reactive oxygene species etc (War et al., 2012). Some of these toxic chemicals can be beneficial in the correct dose of application towards human health problems, but generally they are difficult to administer and considered unsafe for human consumption (Wikipedia, Chemcompound, Vandenberg et al., 2012). The most toxic compound known to man is the naturally-occurring botulinum, 10 gm equivalent of which would kill one fourth of the world's population. The normal level of monosodium glutamate can cause attention deficit hyperactivity disorder (ADHD) risk and autism symptoms. Adversely, toxic metabolites can be produced by humans and excreted through urine and stool, sometimes being safe for plants while in other instances quite toxic for plant health (Mara and Gee, 2010).

Structural similarity of human and plant chemical metabolites cannot provide the same bioactivity to identify the mechanism for human safety (Hyun et al., 2015). For example, plant synthesized genistein was elegantly demonstrated as an agonist and antagonist for estrogen receptor activity (NTP-CERHR, 2006). Similar structures of phytoestrogen lignans, isoflavones, and coumestans were found to be identical to human estrogen. Chemical information and structural analysis of this plant chemicals may not help to elucidate the proper biological effect with safety or toxicity. The biological activity of genistein is found in puberty, proliferation, pro- and anti-estrogenic, which is dependent on the applied dose of these chemicals. In breast cancer patients, genistein was found to proliferate the cells. Some chemicals such as daidzein and glycitein are similar to genistein in structure and the bioactivity might be similar according to current drug design theory. The biochemical routes for genistein in plants showed similar metabolic functions to that of humans (Fig. 3A) (Hughes 1998, Sarkar et al., 2006). Genistein was found in plant to produce more rhizobial nodule cells in plant roots, protecting the plants from stress and interfering with animal reproductive function (Hughes 1998). In humans, the biological form of genistein is the biologically active glucoside genistin (bound to sugar molecule) which at first, needs to be released from the genistein through the acid hydrolysis in stomach before it can act as an antibacterial enzyme in the intestine (Fig. 3A). The absorption and metabolic rate of gensitein infer higher bioavailability than genistin which is similar to the unconjugated form of aglycone and conjugated glucoside. After activation of genistein as hydroxylated and methylated form, it is located on the cell surface to modulate the intercellular activity to affect the several enzymes of cell growth (Figure 3A) (Sarkar, 2006). Plant (in particular, legume family) uses genistein to fix the atmospheric nitrogen to ammonia and form the nodules (root organ) with the help of bacteria. In such way, ammonia converted into glutamine and asparagine to produce carbohydrate, i.e. malate for plant energy. This nodulation process is initiated by the signaling molecule genistein to activate the bacterial nod gene which produces Nod factors (NF) by binding with two receptor-like kinases, Receptor kinases 1 and 5 (NFR1 and NFR5) at the root epidermis (Fig. 3B, Fig. 4A, 4B) (Redmond et al., 1986, Spanik 2000, Stacey et al., 2006, Dénarié et al., 1996, Limpens et al., 2003, Madsen et al., 2003, Indrasumunar et al., 2011). These factors are required to activate the potassium ion-channels, nucleoporins, Nodulation Signalling Pathways 1 (MtNSP1 and MtNSP2), ERF (Ets2 repressor factor) for nodulation (MtERN) and Nodule Inception (LjNIN) and thereby expression of *ENOD* genes in the epidermis. The cortical and pericycle cell divisions and continued mutation occur as a result of cytokinin signaling. During the plant mutation, cell proliferation and amino acid production was severely affected by the oxygen molecules specially the nitrogenase enzyme complex. To avoid this oxidation, oxygen is carried out by the monomeric leghemoglobin protein in the plant root, which is similar to human hemoglobin. In human, genistein participates in the antiproliferation activity by downregulating the protein kinase VEGF, and other signaling factors NF-kβ, COX-2, AP-1, HIF-1α, matrix metalloproteinase, FAK, Bcl-2, and Bcl-XL (Fig. 3B). In line with this, genistein metabolic function is related to cell growth, regulation and nutrient supply which is found both as pro- and anti-proliferation in human and normal cell proliferation in plants. In plants, any pathogenic invasion causes the plant to produce phytolexins, which are similar to genistein and help to kill pathogens. The signaling mechanism triggered by genistein in plants is not characterized, yet it appears to differentiate a balance between phytohormone action and cellular homeostasis. Genistein works with bacteria (rhizobia) to mutate the plant cell whereas lexins works to protect them. Similarly, human physiological and pharmacolgoical activities are modulated by the different physical and genetic signals to produce metabolic enzymes which activate the genes against particular metabolic process to provide nutritional benefits.

Aristolochia fangchi, is a Chinese herb that contains aristolochic acid and shows severe side effects in the patients who use it for the weight loss (Nortier et al., 2000). In patients suffering from renal failure, nephropathy, urothelial carcinoma by

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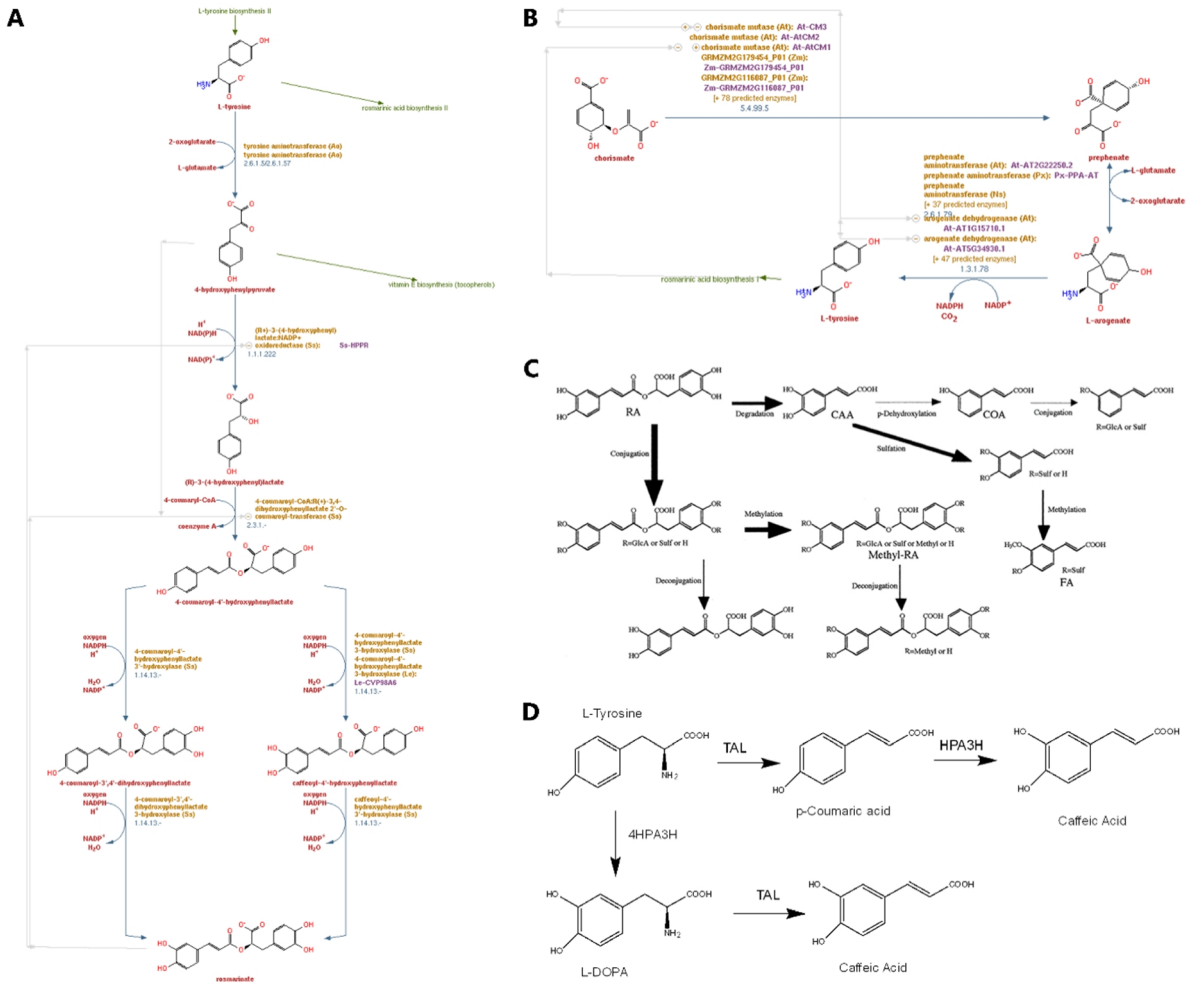

Fig. 2. Metabolic route of rosmarinic acid (RA) in plant (A); metabolic route of tyrosine in plant (B); metabolic route of rosmarinic acid in human (C); metabolic route of caffeic acid (CA) in plant (D). The similarity of metabolic pathways of RA, CA and tyrosine in living systems could be utilized to determine the reaction mechanism that reaction similarity is useful to determine the eventual metabolites in plant and human. The end point of the reaction and similarity of the involved catalysts could confirm the biological efficacy, and interaction of the metabolites.

the formation of DNA adducts, herbal medicines such as those containing pyrrolizidine alkaloids, cause hepatotoxicity and bleeding of the gingko biloba (Stephen, 2008). These side effects and reactions are the result of the interactions with the intercellular enzymes, proteins or genes in the human cell. Herbal components must have same kind of metabolic interaction in the plant involving intercellular or extracellular factors to avoid any side effects. An advance level of comparison of these biochemical interactions may help to identify a similar kind of mechanism in humans. Plant metabolic network maps can focus on the synthesis and degradation of plant and human components to elucidate the biochemical routes and interaction for bioactivity. The interactions can be further elucidated by examining the site of synthesis in the organelle, tissues or organs in both humans and plants in order to identify the biological efficacy of herbal medicine. In systems biology, SBML coding can be sued to identify the biochemical routes and interactions (Klamt et al., 2007, Becker et al., 2007) thereby interrogating the similarities in metabolic activity. Flux balance analysis can be used to estimate the safety or toxicity of the desired metabolism and consistency of the metabolic interactions in the herbal extract. In addition, a stoichiometric matrix and elementary modes (EMs) (Schuster et al., 1999) can also, be used to identify the combined metabolite effect. The EM analysis can be used to explore all possible pathways from primary metabolites to a herbal secondary metabolite as well as its degradation products. Since components of the herbal extract are degraded in human and act to inhibit or control enzymes as well as effect gene expression. A pathway-based mathematical method

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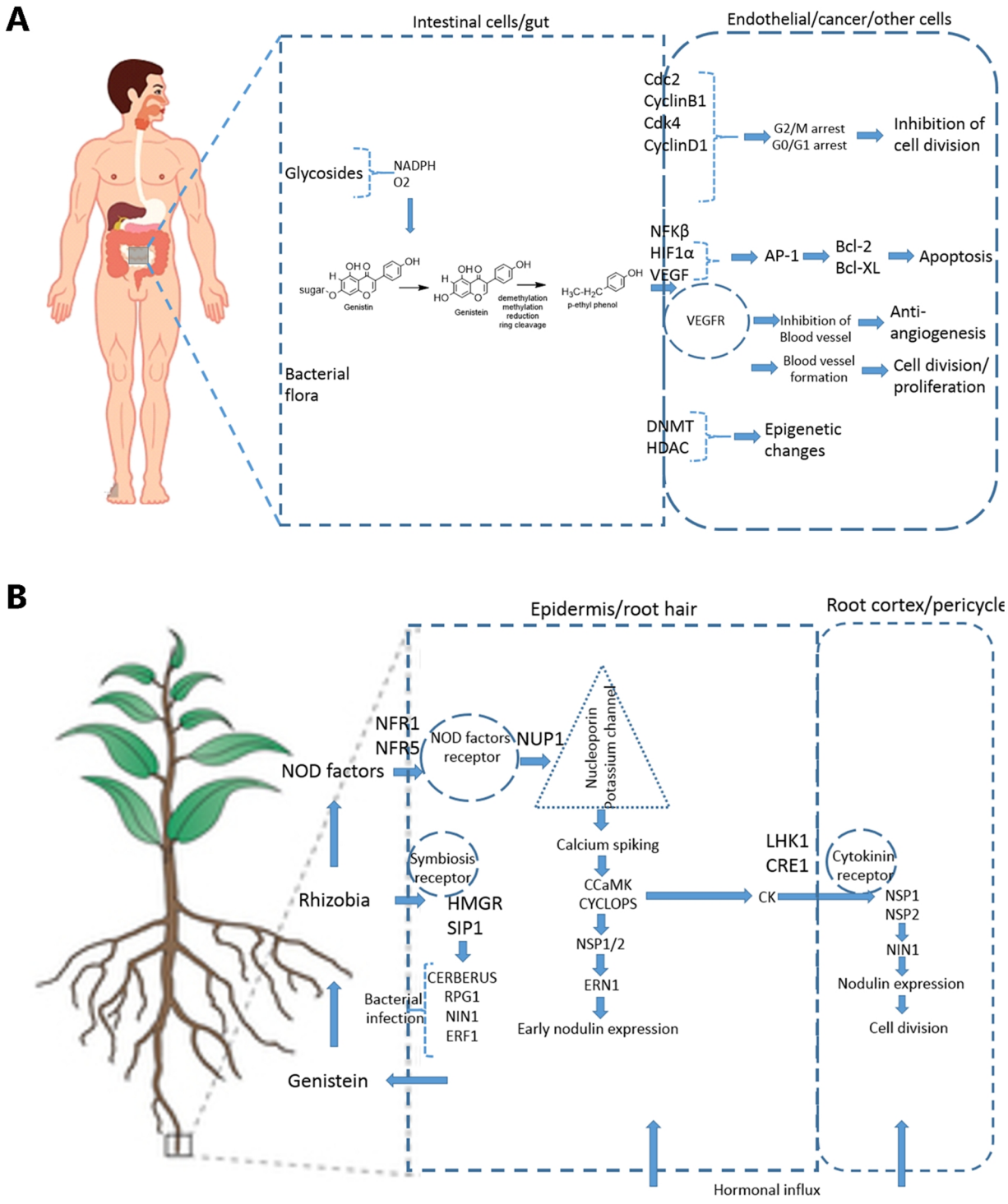

Fig. 3. Metabolic route of gensitein in human (A), and metabolic route of genistein in plant (B). The similarity of genistein metabolic function confirms the pharmacological efficacy in plant and human and thereby we could endorse that genistein is useful for human use.

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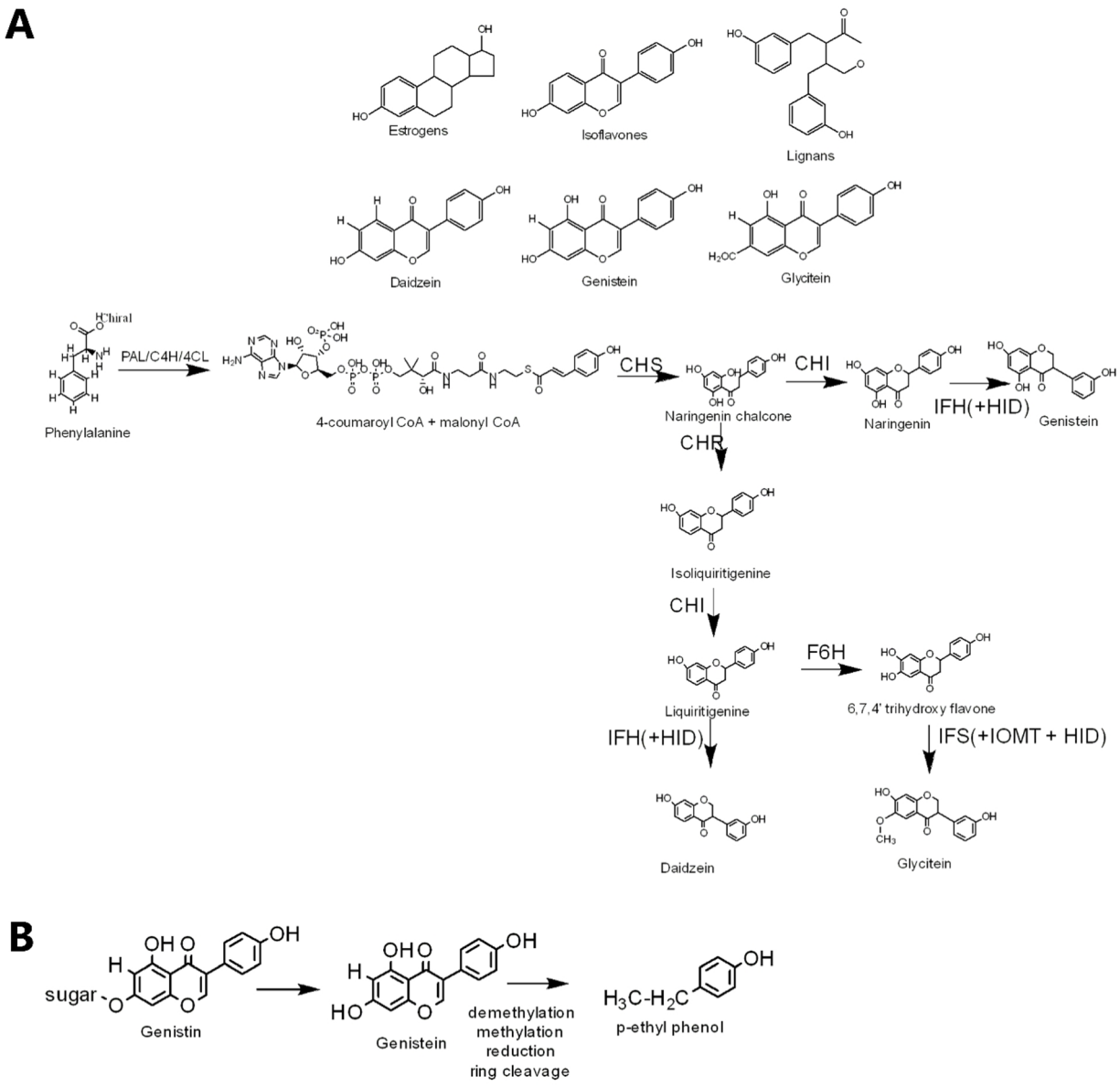

Fig. 4. Metabolic reactions of gensitein in plant (A) and human (B). The metabolic reaction profiling might determine the drug-drug interaction which mechanism could make a rational prediction formula.

which integrates the altered enzyme activity distribution into the metabolic flux distributions (Zhao and Kurata, 2009, Li et al., 1996) would be useful to predict how the administration of multiple herbal components changes the physical and pharmacological efficacy of the combined metabolites at the molecular level.

Tracing similarities between plants and human

All living substances on earth widely share the same instruction molecule living unit deoxyribonucleic acid (DNA) which forms the chromosome with similar housekeeping genes (Arabidopsis-Genome-Initiative) but significant differences in metabolic function have been identified between different species. For example, humans have roughly 18% identical metabolic function with plants (koshland-science). Further, it has been observed that heme molecules of the blood and the chlorophyll of plants are similar in their function in energy metabolism (supplementary Fig. S3). It is assumed from the structural similarity between heme and chlorophyll, that light can penetrate the plant cell with low energy whereas much energy light source is required to pass the blood cell (Gurwitsch et al.,1923). This biological difference might be due to the composition of the organism and their survival activity. It has been reported in the literature that about 30% genes are identical in plants and human. Likewise, 48 to 60% of gene-encoded protein

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synthesis pathways have counterpart eukaryotic genes (Arabidopsis-Genome-Initiative). In most cases, plant metabolism and energy pathways are relevant to yeast and bacterial metabolic functions and their ancestors.

The noteworthy difference in metabolic processes occures at the seperation between plants and animals 1.6 billions of years ago with 30% genetic overlaps (Arabidopsis-Genome-Initiative). Thus, plant and human developed a similar "photometabolic ability. In addition, Chory and Meng described the proteins involved in the process of electron transfer chain as well as the subunits of ATPase important for photosynthesis in plants. Importantly, these proteins are encoded by similar genes in humans. They also noted the critical similarities between the genes important for mitochondrial and chloroplast function (Meng Chen, 2000). In humans, pre-photosynthetic transformation for energy production is observed, a process termed radiogenic metabolism (Luckey,1980). In this process, free radicals are precursors of photosynthesis and respiration using the active oxygen. Radiogenic metabolism is also, similar to all microbes, plants and human for the cellular growth and energy. To complete a sufficient energy cycle, cells need alternative fuel such as light, glucose and heat (Vander,1970).

Similarities between metabolites and their integrated pathways could reveal the mechanism of disease and treatment for both of plant and human. For example, the human protein HSP is not structurally similar to the RHD3 plant protein but their function is similar in neuronal development (Lee et al., 2008, Hu et al., 2009). Moreover, HSP protein are mutated through the atlastin signaling pathways and which can cause neurodegenerative disorders such as leg muscle impairment. From this point of view, malfunction of RHD3 can cause problems in membrane tubulation and vesiculation in plants. Through the impairment of RHD3, endocytic internalization is less active in plants (Zheng et al., 2004). RHD3 is also required for proper axonal maintenance and synapse development (Evans et al., 2006, Zhu et al., 2006, Lee et al., 2009). In addition, both human and plants produce the glutamate receptor which has role in cell development, root growth, morphogenesis and behavior (Tapken and Hollmann, 2008, Walch-Liu et al., 2006, Li et al., 2006). These functions infers similar mechanism despite their structure is not similar. The function of the RHD3 and Atlastin were predicted using the DALI server tools (supplementary Fig. S4). Therefore, the structure similarity is not always useful to rationalize the efficacy of herbal therapies.

Novel direction to prepare competitive standardized herbal therapies

If a new herbal extract has no information on its effects on any metabolic pathways, the essential reactions should be compared with human metabolic pathways for the demonstration of efficacy, safety and toxicity (Table S1, S2). In this analysis, any human metabolites that would be found to be structurally similar to any of the plant component compounds (from KEGG) could then be linked to the metabolic pathways to infer the efficacy potential of the medicinal plant (explanation in Supporting Information, Fig . S5). As expected due to the similarity in structure between the plant and human metabolites, there is significant overlap between these lists. The jaccard similarity scores above 0.50 were considered as significant and only those human metabolites with a structural similarity score above a given threshold. In each of the lists of structurally similar plant metabolites, very few pathways are clearly over-represented. For Genistein, "map00254", "map00980" and "map05204" were found as similar to human metabolism pathways and that can be considered as 30% safe for human. Further, herbal extracts have another problem due to the inconsistencies in their ingredients which leads to regulatory compliance problems for the herbal industry. The elucidation of important metabolic pathways could determine the involvement of environmental factors and growing media that could be used to better control the production of beneficial plant metabolites independently of seasonal variations. The understanding of extracellular and intracellular parameters could give a better indication when plants should be harvested to ensure a consistent level and combination of metabolites are produced for an herbal medicine. When omics data such as transcriptome and metabolome (i.e. KEGG etc.) are available, statistical analysis is effective (Table S2). The similarities between metabolic reactions can be explored by the principal component analysis (PCA) and clustering analyses over a variety of scales between the plant and human cells. Most-meaningful or significant changes of metabolites and critical metabolites (factors) that affect physiology can be identified via volcano plots and the least absolute shrinkage and selection operator (LASSO) or elastic net regression analysis (Badsha et al., 2016).

Conclusion

The parameters that influence the pharmacological efficacy of medicinal plants are the focus of active investigations and are important for understanding the similarities between metabolic pathways in order to determine the efficacy, safety, and toxicity of herbal medicine. The characterization of plant secondary metabolic pathways are essential to ensure the safety, quality compliance and efficacy of herbal products for contemporary medicinal applications. The study of both the system and clinical pharmacology aspects of the mechanisms underlying determination of the similarities between metabolic pathways in plants and humans could be an exciting field of research. Due to their high level of complexity, the physiological and pharmacological significance of these interactions are not yet fully clarified; however, future work will undoubtedly shed light on these important molecular interactions and their roles in metabolic pathways.

Additional Information

Author Contribution

M. S. S. Khan made substantial contributions to the conception and design of the manuscript, review of the

literature, and drafting of the manuscript and figures. C. I. Cazzonelli made equally substantial contributions to the conception and design of the manuscript, review of the literature, and drafting of the manuscript and figures. H. Kurata made significant contributions to the collection of citations related to plant and human metabolism and participated in drafting relevant sections of the manuscript. M. B. Badsha made substantial contributions to the collection of citations related to metabolism and computational information and in drafting the relevant sections of the manuscript. C. G. Li provided in depth input on the acquisition and interpretation of the literature on safety of herbal medicine and participated in the preparation of the manuscript. G. Munch provided interpretation of the literature on herbal medicine value and participated in the preparation of the manuscript. A. M. S. Abdul Majid supervised every step in the design, structure and preparation of the manuscript and gave the final approval of the version to be published. All authors read and approved the final manuscript.

Competing financial interests

The author(s) declare no competing financial interests.

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