Original Article

In Silico Identification of Drug Targets for Antifertility from Natural Products by Differential Reaction Content Analysis of Metabolic Pathways

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Abstract

***Background*: One of the major concerns of governments of developing and developed countries is to have a check on their population increase. Realising the importance of avoiding the harmful effects of synthetic compounds, scientists and researchers throughout the world are cooperating in efforts to design new and effective contraceptives from compounds of plant origin.**

***Methods*: In this paper, we compared 11 plant species by analysing compounds showing antifertility properties with respect to the metabolic pathways involved. The Kyoto Encyclopedia of Genes and Genome pathway database was the source of metabolic pathway information. Protein sequences and classification numbers of unique enzymes exclusively present in certain species were identified using the Expert Protein Analysis System.**

***Results*: Two enzymes, namely, L-aspartate dehydrogenase (EC no. 1.4.1.21) and trans-hexaprenyltranstransferase (EC no. 2.5.1.30), were identified as novel drug targets from the metabolic pathway analysis. Validation of the essential proteins identified through metabolic pathway comparison was done based on the literature information.**

***Conclusion*: The *in silico* analysis resulted in identification of 2 enzymes that are predicted to be the targets for putative antifertility drug. These enzymes can further be modelled to obtain their 3-dimensional structures with the help of various protein structure modelling softwares.**

Keywords: *contraceptives, databases as topic, drug discovery, enzymes, metabolic pathways, natural products*

Introduction

In the developing world, the population is projected to increase rapidly. By 2025, about three-quarter of the 3.2 billion increase in the world's population is expected to take place in developing countries; the global increase is expected to double by 2050 to approximately 7.8–12 billion. China and India are the two most populous countries, with around half of the world's population living there (1,2). The rise in population can be a serious problem, as it has damaging economic and environmental consequences. The world's population has increased exponentially since the industrial revolution because of the modern technologies and medicines available. Thus, the population carrying capacity has increased. The planet is experiencing depletion of the world's net resources due to the requirement of food and other materials for sustenance. The increased use of fossil fuels presumably contribute to global warming resulting in the melting of glaciers, the rising ocean levels, and the more frequent hurricanes. Due to the ever-increasing demands for food and palatable water from the population increase, deaths due to hunger and thirst will potentially be at higher numbers if the demands are not met. However, the lands are becoming infertile because of overcropping and urbanisation, considering that the constructions as well as the inflow of household wastes and industrial effluents increase the acidity of the soil (2). Population explosion is alarming; it results in the exploitation of natural resources and affects the economic growth of a country.

Population control is the practice of curtailing the population increase, usually by reducing birth rate. India was the first country with an official family planning programme, which commenced in the late 1950s and early 1960s, because the government could foresee the adverse effects of an increased population on economic growth

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and living standards. A careful examination of history reveals that family planning has been practised since the dawn of literature. Initially, the family planning programme started with many contraceptive methods, but due to the side effects of most of the contraceptives such as oral steroidal contraceptive and abortifacient drugs, the use of these drugs has been prohibited due to their side effects such as myocardial infarction and breast cancer in females (3–5). Several very efficient synthetic drugs have been introduced in recent years, but despite their benefits, there are certain side effects that may result in severe disorders. Therefore, it has become necessary to synthesise contraceptive drugs of natural origin that are more effective, with negligible side effects and minimum cost.

The World Health Organization estimated that approximately 75% to 80% of the world's population uses natural products as a source of drugs, derived from either full plants or certain parts of plants, such as roots, stems, leaves, and flowers. Nowadays, due to growing health concerns, most people prefer natural alternatives to synthetic drugs, as they are cheaper and have fewer side effects (6–8). Since the ancient times, medicines obtained from various parts of plants, such as barks, leaves, and roots, provide efficient and long-lasting cure against several diseases, with negligible side effects. Some common examples are quinine, which is extracted from *Chinchona oficinalis*, ephedrine from *Ephedra giardiana*, and reserpine from *Rauwolfia serpentine*. Ancient Ayurvedic and Unani literature reveals the use of plant species for fertility regulation. Several plant species with antifertility activity have been reported in the literature, such as *Musa paradisiaca*, *Hibiscus rosa-sinensis, Beta vulgaris*, *Allium cepa*, *Carica papaya*, *Catharanthus roseus*, and *Populus trichocarpa* (9–13). The knowledge of the mechanisms of action of most medicinal plants is scant and exploration of their use as therapeutic agents is limited; therefore, there is a need to implement newer techniques to determine their potential uses. With the advent of proteomics and genomics, this problem can be partially alleviated with these efficient methods for rapid identification of protein targets of herbal ingredients (14).

Several bioinformatics tools and software have been used to develop efficient methods for facilitating target identification, as the first step in drug discovery. Metabolic pathway analysis is one approach to identify the potential drug targets, as it involves the study of organism metabolism. The targets are evaluated using 2 criteria: essentiality and selectivity, that is, essential proteins that may effect the specific metabolic activity and are not synthesised inside human body, and thus have to be taken from outside source. These essential proteins selectively bind at their binding sites. Therefore, the essential proteins are identified by pathway analysis and can then be taken as novel drug targets (15). Due to a lack of information in databases, only a restricted number of drug targets have been identified.

The plants taken for the analysis are used in traditional medicines; however, there are many properties of these plants yet to be investigated. The present study involves 13 plant species: *Allium cepa* (onion), *Beta vulgaris* (sugar beet), *Brassica napus* (rape), *Nicotiana tabbacum* (tobacco), *Populus trichocarpa* (black cottonwood), *Prunus persica* (peach), *Helianthus annuus* (sunflower), *Vitis vinifera* (grapes), *Glycine max* (soybean), *Hordeum vulgare* (barley), *Oryza sativa* (rice), *Triticum aestivum* (wheat), and *Zea mays* (maize). Some of these plants have been experimentally tested for antifertility (9–13). The differential reaction content of the above plant species was analysed by comparing the metabolic pathways taken for in silico analysis.

Materials and Methods

Plant species taken for pathway comparison

Metabolic pathway analysis is a novel approach for identification of drug targets. In this study, metabolic pathway analysis is performed by identifying the unique metabolic reaction content in 13 plant species, out of which 8 species are assumed to have antifertility properties, based on the literature. Although these plant species are used as traditional medicines, some of them lack research evidence to support their antifertility properties.

These 13 plant species were divided into 2 groups. The first group include 8 plant species with antifertility properties (9–13): *Allium cepa* (onion), *Beta vulgaris* (sugar beet), *Brassica napus* (rape), *Nicotiana tabbacum* (tobacco), *Populus trichocarpa* (black cottonwood), *Prunus persica* (peach), *Helianthus annuus* (sunflower), and *Vitis vinifera* (grapes). The other group consist of 5 species assumed to be without antifertility properties: *Glycine max* (soybean), *Hordeum vulgare* (barley), *Oryza sativa* (rice), *Triticum aestivum* (wheat), and *Zea mays* (maize).

Metabolic pathways analysis

Twelve different metabolic pathways, namely, alkaloid biosynthesis, alkaloid biosynthesis II, androgen–oestrogen metabolism, steroids biosynthesis, caffeine metabolism, C-21 steroid hormone biosynthesis, fatty acid biosynthesis, flavonoid biosynthesis, nicotinate and nicotinamide biosynthesis, linoleic acid metabolism, sulphur metabolism, and tetracycline biosynthesis were analysed comparatively. These pathways were chosen from the Kyoto Encyclopedia of Genes and Genome (KEGG). Metabolic pathway comparison of these species provides facts regarding enzymes exclusively present in species that showed antifertility properties. Further mapping with human proteome was conducted using the Basic Local Alignment Search Tool (BLAST); the enzymes that are non-homologous to the human proteome were taken as novel drug targets. The information regarding the classification of enzymes was taken from various databases and web servers, namely, Braunschweig Enzyme Database (BRENDA), KEGG, and Expert Protein Analysis System (ExPASy).

Identification of essential proteins by metabolic pathway comparison

Each class and subclass of enzyme is described by its unique enzyme classification number (EC no.), which is designated by the International Union of Biochemistry and Molecular Biology according to the reaction catalysed. The KEGG pathway database was used as a source of metabolic pathway information. The Comparative Pathway Analyzer, an online web server tool (16), is referred for the comparative study. The differential pathway analysis of the selected species is performed, and their reaction content was visualised with the respective pathway in order to obtain enzymes showing antifertility properties. The enzymes uniquely present in plant species showing the antifertility property were identified.

Validation of essential enzymes by searching similarity against Database of Essential Genes

Protein sequences of unique enzymes (essential proteins) exclusively present in plants species were identified using ExPASy, a proteomics server of the Swiss Institute of Bioinformatics. The essential protein sequences were compared using BLAST against Database of Essential Genes for validating the essentiality of the proteins identified through metabolic pathway comparison.

Drug target identification: Mapping essential proteins on human proteome

The essential proteins were searched for similarity against human proteins. Mapping of the essential proteins identified was done against human proteome by a sequence similarity search using BLAST-Protein (BLAST-P). The e-value, $e = Kmn(e\text{-}\lambda s)$, which gives the number of entries required in the database for a match to happen by random chance, was set to default; all other parameters were also set to default values in order to predict probable drug targets.

Results

Comparisons of metabolic pathways of the 13 plant species led to the identification of 2 unique enzymes, which may be responsible for the antifertility property: trans-hexaprenyltranstransferase or heptaprenyl diphosphate synthase (EC no. 2.5.1.30), which is involved in the biosynthesis of steroids, and L-aspartate dehydrogenase (EC no. 1.4.1.21), which is involved in the biosynthesis of nicotinate and nicotinamide. The intermediate reactions in which these enzymes are involved in their corresponding pathways are as follows:

Trans-hexaprenyltranstransferase (EC no. 2.5.1.30)

Trans-hexaprenyltranstransferase is involved in the biosynthesis of steroids in onion. This enzyme catalyses the condensation reactions, resulting in the formation of all-trans-heptaprenyl diphosphate, isoprenoid side-chain of ubiquinone-7, and menaquinone-7. The enzyme adds 4 isopentenyl diphosphate molecules sequentially to farnesyl diphosphate with trans-stereochemistry.

Trans,trans-farnesyl diphosphate
+ 4 isopentenyl diphosphate
↔ All-trans-heptaprenyl diphosphate
+ 4 diphosphate

$$C_{15}H_{28}O_7P_2 + 4C_5H_{12}O_7P_2 \leftrightarrow C_{35}H_{60}O_7P_2 + 4P_2H_4O_7$$

Aspartate dehydrogenase (EC no. 1.4.1.21)

This enzyme is involved in the biosynthesis of nicotinate and nicotinamide in black cottonwood. The enzyme is strictly specific for L-aspartate as the substrate, and catalyses the first step in biosynthesis of NAD from aspartate. The enzyme has a higher affinity for NAD^+ than $NADP^+$.

L-aspartate + $NADP^+$
↔ Iminoaspartate + NADPH + H^+

$C_4H_7NO_4 + C_{21}H_{29}N_7O_{17}P_3$
↔ $C_4H_5NO_4 + C_{21}H_{30}N_7O_{17}P_3 + H^+$

Both plants have some antifertility properties, as observed in the ethanolic extract of onion (9) and the flavonoids of black cottonwood (13). Consequently, it can be predicted from the intermediate complexes formed by these enzymes in the biosynthesis of steroids, nicotinate, and nicotinamide that these enzymes have antifertility properties. The above 2 enzymes had no homologues in the human proteome, and therefore are putative drug targets.

Discussion

The inherent hazards of drugs of chemical origin has drawn attention to the search for better treatment options, such as drugs of natural origin with negligible side effects. Most contraceptive pills used today are potent steroidal ovulation inhibitors, but the constant use of steroidal pills can disturb the hormonal balance in the body. Synthetic drugs, being foreign to the human system, may induce foreign-body responses and cause damages, thus repressing and paralysing the functions of that particular organ or tissue. On the other hand, drugs of natural origin are more suitable and effective because they contain compounds, such as phytoproteins, flavonoids, and saponoids, that help to protect the body from harm. Furthermore, all synthetic drugs available up to now are modelled after the structures of natural compounds isolated from plants, and thus are related to plant drugs in several ways. Some drugs of natural origin have been proven effective against several fatal diseases, for example, the anticancer drugs Vincristine and Vinblastine obtained from the rosy periwinkle, *Catharanthus roseus* (17,18). Therefore, researchers are showing more interest on drugs derived from plants to obtain better results with negligible side effects compared with synthetic drugs. There are various types of pharmacological and physiological activities and substances of known structure in plants with demonstrated antifertility activity (19–21). In this paper, the differential reaction content of the 13 plant species was visualised using the Comparative Pathway Analyzer. Of these selected plants, only 2 (onion and black cottonwood) showed antifertility properties in identified enzymes. However, there may be many other essential proteins that can be taken as putative drug targets. Due to restricted information in databases, we analysed only 2 essential enzymes: L-aspartate dehydrogenase (EC no. 1.4.1.21) and trans-hexaprenyltranstransferase (EC no. 2.5.1.30). These identified enzymes were not found in various plant databases, likely because the information available is limited. Based on the literature, onion and black cottonwood shows the antifertility properties (9,13), and thus these enzymes can be validated to be putative drug targets for antifertility regulation.

Conclusion

With the increased pace in technology, newer *in silico* methods of drug target identification are being evaluated frequently. Target identification is the first step of the drug discovery process, and the use of plants to derive drug candidates is desirable due to negligible side effects of these herbal drugs over synthetic drugs. Moreover, the approach used is also novel, as we used differential pathway analysis of metabolic pathways to visualise the differential reaction content of the various plant species. We identified 2 unique enzymes L-aspartate dehydrogenase (EC no. 1.4.1.21) and trans-hexaprenyltranstransferase (EC no. 2.5.1.30) showing antifertility properties from 2 different metabolic pathways, namely, nicotinate and nicotinamide metabolism and steroids biosynthesis. Due to restricted information in databases for different plant species, only a few drug candidates were traced from the plant species in the present study. These were validated based on information present in various databases and literature. The literature contains information on these plant species that indicates their terpenoids and flavonoids have antifertility properties. These enzymes were involved in the biosynthesis of steroids and the biosynthesis of nicotinate and nicotinamide; therefore, we can assume that these enzymes are potentially novel antifertility drug targets. In future, in vivo experiments can be performed using extracts of these plants to validate the results. The target obtained can be exploited for rational drug design, which includes ligand-based and structure-based drug designing approaches. Furthermore, we can design analogues by using or modelling the inhibitors for these enzymes.

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Authors' Contributions

Conception and design, collection, assembly, analysis, and interpretation of the data, drafting of the article: SS
Critical revision and final approval of the article, administrative, technical, or logistic support: SD

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