emergent
Life Sciences Research

Review Article

Immunomodulatory potential of synbiotics and phytochemicals – A Review

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Abstract

In the present time, the cost and side effects of commonly used synthetic medicines raise a need to develop alternative therapeutic agents. Natural/ medicinal plants and their products/ secondary metabolites are most acceptable, cost effective and safer alternatives to synthetic drugs. Various plant extracts and purified compounds have been evaluated for their biological activities like Piperine, Quercetin, Genistein, Ellagic acid and terpenoid for immunomodulatory activity. Experimental and clinical work revealed that probiotics and many medicinal plants are safe immunomodulators. Moreover, immunomodulatory efficacy of probiotics can be enhanced by prebiotics and that of medicinal plants by using their effective bioactive components rather than the whole extract. Phytosynbiotics (Prebiotic+ probiotics+ phytochemical) are a new class of food grade supplements which promote metabolic health and improve living. This review article highlights immunomodulatory activity of synbiotics and phytochemicals and their combinatorial use in the form of Phytosynbiotics, proving it a novel therapeutic agent in treatment of various diseases like diabetes, cholesterol and liver diseases. However, the undesirable side effects of medicines have been observed that have caused concern about their long term therapeutic use. This paper may throw some light to prove the ability of the Phytosynbiotics as a novel alternative or adjuvant to chemical drugs to help fight the diseases.

Keywords immunotherapy, phytochemical, phytosynbiotics, synbiotics

Received: 29 July 2018
Accepted: 12 November 2018
Online: 19 November 2018

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Emer Life Sci Res (2018) 4(2): 22-30

E-ISSN: 2395-6658
P-ISSN: 2395-664X

DOI: https://doi.org/10.31783/elsr.2018.422230

Introduction

Since ages, plants or their products have been employed in medicine and health benefits. The increasing awareness about the side effects and cost of synthetic medicines makes the interest of consumer shifts towards alternative therapeutic agents. The advancement in knowledge about immunotherapy makes it as one of the alternative way of modification of not only infectious diseases, but also non-infectious diseases related to immune response like diabetes, cholesterolemia, neurological, cardiovascular disorders etc. Immunotherapy includes modulation of disease by immune response alterations through potentiation or suppression. Over a period of time the experimental and clinical work revealed that probiotics (the microbes with GRAS) and many medicinal plants [1] are safe immunomodulators. Moreover, immunomodulatory efficacy of probiotics and medicinal plants can be enhanced by prebiotics and by using their effective bioactive components, respectively. Plants like *Zizipus maruritiana* , *Withania somnifera*, *Prunus cerasus* have been proved to be effective immunomodulators.[2] Various plants and their purified compounds including Quercetin, Rutin, Daidzein, Genistein, Ellagic acid and Betulinic acid have been studied for their

immunomodulatory efficacy *in vitro* and *in vivo*. This brought the thought that like synbiotics there may be synergistic effect of plant extracts and synbiotics which may improve their bioactivities including immune activity. Less literature is available in this regard except Tat (2013) who first time reported the use of phytosynbiotics for diseases like diabetes, cholesterol, hepatotoxicity, but the mechanism of working of PSB has not been elucidated.

Synbiotic

Synbiotics are defined as the mixtures of probiotics and prebiotics that beneficially effect the host by improving the survival and implantation of alive microbial dietary supplements in the gastrointestinal tract of the host [3]. Probiotics which are alive bacteria, intend to colonize the large intestine and confer physiological health benefits to the host by promoting a beneficial balance of microbes to live in the gastrointestinal tract [4]. Example of probiotics are *Bifidobacterium bifidum, Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium breve*, *Lactobacillus rhamnosus* etc. Probiotics areinterpreted as viable microorganisms, whose adequate amounts reach the intestine in an active state and thus, employ positive health effects and possess immunomodulatory functions. Studies reveal that treatment of infectious diseases including viral, bacterial or antibiotic associated diarrhea and altered intestinal microbiota is possible with probiotics [5]. Numerous studies have revealed that probiotics can encourage the immune system, produce vitamins, decline serum cholesterol [6], lessen lactose intolerance, act as antibiotics, destroy tumors and defend against colon cancer mend intestinal microflora, reduce the oxidative stress, immune modulatory and antidiabetic effects [7].
On the other hand, Prebiotics are non-digestible food ingredients that excite the growth and activity of bacteria in the digestive system [8]. The growth and properties of probiotics are enhanced by prebiotics such as fructo-oligosaccharides (FOS), inulin, lactulose and galacto-oligosaccharides (GOS) and enhance the gastrointestinal functions and immunity [9]. Health effects of prebiotics include lowering the incidences of clinical allergy, infections, preventing cancers, infections, allergies, inflammatory bowel diseases [10]. Relating probiotics with prebiotics could mend the endurance of the bacteria crossing the upper part of the GI tract, thus increasing their effects in the large bowel. Various types of substances that can be used as Prebiotics are Fructo-oligosaccharides (FOS), Galacto-oligosaccharides (GOS), Inulin, Polydextrose, Lactulose, Acacia Gum etc [11].
Synbiotics are not only a mixture of probiotics and prebiotics but a synergy between the two components [12]. Few examples of Synbiotics showing synergistic effect are- *Bifidobacteria* and fructo oligosaccharides (FOS), *Lactobacillus rhamnosus* GG and Inulin, *Bifidobacteria* or *Lactobacilli* with FOS or Inulin or galactooligosaccharides (GOS), *Lactobacilli* and lactitol.

Mechanism of action of Synbiotics

There can be different mechanisms involved in synbiotics action Gibson [13] determined that Synbiotics has direct antagonistic effects on pathogens. It has been reported by Gourbeyre [14] that synbiotics improved the immune function and stimulation of appropriate immunomodulatory cells. Prebiotics are fermented by the probiotic bacteria and other bacteria that reside in the colon, and butyrate and other SCFAs are formed resulting in butyrate, influence histone deacetylation which is responsible for decreasing the proinflammatory cytokine secretion[15]. Katharina et al [16] reported that synbiotics, because of their prebiotic content, may raise the levels of calcium and magnesium in the colon and may enhance the growth of *Bifidobacteria* and *Lactobacilli* in the large intestine. Watson and Preedy [17] confirmedthat synbiotics stimulate the host immune response by increasing the phagocytic activity, by the synthesis of IgA and activation of T and B lymphocytes and alteration of physicochemical conditions of colon with decreasing pH.

In vitro analysis signifies that the cell wall element of lactic acid bacteria stimulate immune cells such as lymphocytes and macrophages. The cells of both the innate and acquired immune system are activated by bacterial DNA. Study done by Liong et al [18] showed that the synbiotic diet that enclosed *L. casei* ASCC 292, fructooligosaccharide, and maltodextrin constructively changed cholesterol levels and

formed a healthier bowel microbial population without movement of *Lactobacilli* to other organs. These synbiotics act as original substitute or adjuvant to chemical drugs to support fighting the hypercholesterolemic problem [19]. Experimentally, synbiotics have been shown to have proven effects on treatment of allergy, modulation of microbial ecology of gut [20], anti-inflammatory effect [21], effectsagainst carcinoma [22] and modulating immune responses. Synbiotic treatment had immuno modulatory effect [23] and cholesterol lowering effects, reducing necrotizing enterocolitis [24]. Synbiotics have shown numerous benefits to health including resistance to infection, antibacterial activity, antioxidant activity and enhancing the immunity as showed in Figure1.

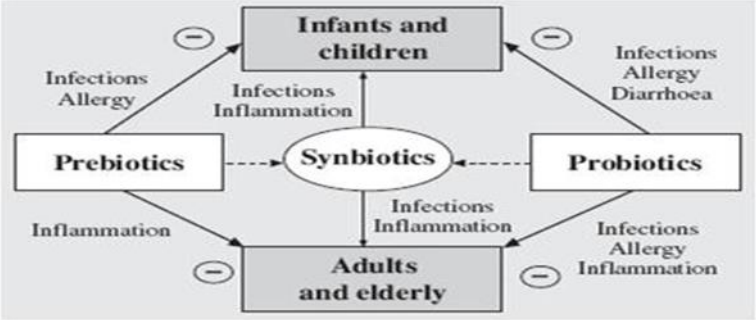

Figure 1. Role of Synbiotics (25)

Several researchers have focused on balanced colonic microflora, restoration of immune response, glycemia and cholesteremea. There is additive and synergistic effect observed while combining the study of probiotic and prebiotic. Synbiotic is beneficial for both micro-organisms and substrates. On the upper part of intestine the probiotic bacteria may colonise to prevent the adhering of pathogens to the intestinal tract and help in digestion. Prebiotic like FOS, GOS consumption stimulates the microflora in large intestine. This combination work of probiotic and prebiotic improve the gut health [26].

Probiotics are very effective in normalizing pH balance in the colon, reducing inflammation. They provide enzyme activity and improve digestion. Hoffman et al [27] explained that aproperly acidic colon is required for keeping the beneficial lactic acid bacteria healthy and suppressing the pathogenic bacteria. As compared to the probiotic, the combination of probiotic and prebiotic are more effective that helps to stimulate the growth and activity of specific friendly bacterial strain. Hanaway et al [28] explained that synbiotics are more effective at lower dosage. Japanese researcher and Lichiroh Ohhira [29] developed the new fermentation process that helps to create short chain naturally occurring oligosaccharides, so that synbiotic can be created at natural temperature. However, the long chain oligosaccharides of probiotics are synthetically created by adding fungal enzymes to white cane sugar.

Roller et al [30] did a clinical study on 34 colon cancer patients who had sustained ‘healing surgery’ and 40 polypectonized patients to study the effect of synbiotics in the immune system. The authors stated that synbiotic could be beneficial in the study of colon cancer. Gibrilovich and Pisarev [31] stated that in the rats treated with Azoxymethane (a tumor promoter), synbiotics reduced the cancer precursors. In the immune system, the effective anti-tumor immune response depends upon the interaction of several elements that include T cells, B cells and NK cells. Furrie et al [32] showed that synbiotics reduced intestinal inflammation in humans. Synbiotics can be used to stimulate the immune system. The presence of externally gram positive bacteria can encourage the secretion of anti-inflammatory cytokines depending upon the species [33-34]. In case of cancer, this type of reactions can be useful where the immune response helps to eliminate cancerous cells. For example, anti-inflammatory cytokines secrete IL-10, which prevent the production of other cytokines promoting inflammation [35] and it would be helpful to reduce hypersensitivity, allergy and other inflammatory disease, where the inflammatory reaction is overactive [36]. Mice inadequate in IL-10 develop colitis as a result of their failure to down-regulate the inflammatory response. It has been found that the nourishment of Lactobacilli can restore IL-10 levels and prohibit the onset of colitis [37]. In a study done by Rosenfeldt et al [38], the interleukins were unaffected, but the

serum eosinophil cationic protein (released by granules in eosinophils during inflammation) level decreased.

With the help of immune stimulation the pathogens in the gut can be cleared. When treated with lactic acid bacteria, *E.coli* and *Salmonella typhimurium* clearance happened more rapidly in murine models with rise in intestinal IgA specific to the pathogen. It was also noted that the effects of probiotic authority on the immune system can expand beyond in the gastrointestinal tract and into other systems of the body, [34].

Lactobacilli are expressed to be easy to activate murine macrophage [39]. In mice, the NK activity of spleen cells increased by the lactic acid bacteria [40]. Lactic acid bacteria stimulate the generation of cytotoxic macrophage ancestors in bone marrow and generate their differentiation in spleen. Numerous studies in animals and some in humans have examined the immunomodulatory effects of probiotics [41] as shown in Table 1. In humans, the oral consumption of probiotics increased *ex vivo* cytokine interferon secretion of peripheral blood mononuclear cells [42]. Intake of probiotics changed the respective capacity of leucocyte subsets and increased phagocytic activity as well as NK cell cytotoxicity.

Table 1. Summary of studies reporting effects on immunity with prebiotics, probiotics and synbiotics

Prebiotic/Probiotic/synbiotics	Effect on immunity	Reference
L. casei ssp. casei ,dextran	Increase of NK cell activity	Ogawa et al [43]
Lactobacillus rhamnosus GG, *Bifidobacterium breve Bb99*, GOS	Probiotic treatment tended to reduce IgE-associated atopic diseases , eczema, respiratory infection incidence after synbiotic treatment	Kukkonen et al.[44]
Lactobacillus casei and *Bifidobacterium lactis* Bb-12	Higher percentages of CD3+ and CD3+CD4+ lymphocytes only in the placebo group	Hol et al. [45]
Lactobacillus sporogenes, inulin	significant effects on serum insulin, decrease hs-CRP, increase plasma total GSH levels.	Asemi et al.[46]
Lactobacillus casei and lactulose used as synbiotics .	Immunomodulatory ,blood glucose and cholesterol lowering effects.	Bhatia et al.[19], Anandhraj et al.[23]

Immunomodulatory plants and their phytochemicals

A huge number of Indian medicinal plants have been described to hold immune-stimulatory action and thus, can work as potential source of drug in various immunocompromised states including AIDS, cancer and for treatment of various chronic infections, cancer, diabetes etc. [47]. Naturally produced plant products serve as alternate immunomodulatory and therapeutic agents to overcome non-availability of drugs in developing countries [48]. The bioactivity of any medicinal plant depends upon its components known as phytochemicals. Phytochemicals are classified as primary metabolites i.e. sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc or secondary metabolites which include alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides etc. [49]. Many purified compounds of plants as shown in Table 2 have been proved to be immunomodulators e.g Quercetin (flavonoid), Rutin (glycoside), Daidzein (isoflavonoid), Genistein (phytoestrogen), Ellagic acid (phenolic) and Betulinic acid i.e. triterpenoid [2]. Piperine, an alkaloid from *Piper nigrum* and *Piper longum* was shown to be immunomodulatory.

Table 2. List of plants with immunomodulatory properties

Component name	Source	Chemical class	Activity	Reference
Betulinic acid	*Betula pubescens*	Pentacyclic triterpenoid	Anti-inflammatory, anti-malarial, anti-retroviral and anticancerous properties	Chowdhury et al. [50]
Daidzein	*Prunus cerasus*	Isoflavone	Anti-estrogenic effect, antioxidant activity	Foti et al. [51]
Ginsan	*Panax ginseng*	Polysaccharide	Anticancer	Kim [52]
Tylophorine	Tylophora indica	Alkaloid	Antidiabetic effect	Swathi et al. [53]
Matrine	*Sinomenium acutum*	Alkaloid	Antiviral	Wang [54]
Quercetin	*Moringa oleifera*	Flavonol	Anticholesteremic and Antilipidemic activity	Senecha et al. [55]
Resveratrol	*Polygonum cuspidatum*	Phenol	Prevent heart diseases and cancer	TomeCarneiro et al. [56]
Piperine	*Piper longum*	Alkaloid	Antitumor, immunomodulator and bioavailabilty enhancer	Kumar et al. [57]
Artemisinin	*Artemisia annua*	Sesquiterpene lactone	Psoriasis and autoimmune disorders	Ho et al. [58]
Shatavarin	*Asparagus racemosus*	Triterpenoid saponin	Immunostimulant and vaccine adjuvant	Thakur et al. [59]

Since natural sources do not contain compounds in their purified forms, various immunomodulatory compounds are found together in plants in their crude form. Hence, the effect of one compound is always affected by the other. Therefore, it is not possible to check the effect of any one compound in the crude plant extract and it is difficult to determine which compound affects the activity of the other compound. Hence, the purified plant food derived compounds areselected for the studies.

Phytosynbiotic: Phytosynbiotics are the combination of plants with healing properties and synbiotics.

PHYTOSYNBIOTICS = PLANT NUTRIENTS + PROBIOTICS + PREBIOTICS
[PHYTO] [SYNBIOTIC]

By adding beneficial bacteria and other active compounds into the gut via dietary supplements, the phytosynbiotics help to optimize gut health and maintain an ideal balance of beneficial to pathogenic bacteria in the gut [60]. Studies showed that the combination of probiotics and essential oils both have a great potential in terms of their beneficial effect against microbial gut infection and also show a synergistic effect that is normally higher than any known drug due to their complementary actions [61]. The outcomes of Hooshmand [62] suggest that although a genistin-rich isoflavones diet can increase the Bone Mineral Density in rats with Ovx-induced bone loss, blend of genistin-rich isoflavones and FOS had larger effect in averting bone loss in this rat model. A study done by Daniells [63] reported that calcium and prebiotic combination boost the bone health than prebiotic alone. The immunomodulatory activity of prebiotics, probiotics and phytochemicals have been very well understood and widely studied *in vitro* and *in vivo*. Though a number of studies have been carried out to isolate immunomodulatory bioactive compounds, the literature lacks the reports on the synergism of the phytochemical and synbiotics especially in *in vivo*. Synergistic effect of the essential phytochemical and synbiotics will be necessarily higher than using them alone as the health products.

Modern day diets and living conditions place tremendous stress on our bodies. Metabolic syndrome ailments such as diabetes mellitus (high glucose levels), cholesterolemia (high cholesterol levels), hypertension, obesity and gout are growing at an alarming rate. People are both physically and mentally overworked, and pollutants in our environment are subjecting more stress to our bodies. Much research has been dedicated to find the natural solutions of these problems. A new type of formulations called

phytosynbiotics is showing tremendous promise to meet these challenges head-on. Phytosynbiotics are orally-administered natural formulations of synergistic prebiotics and probiotics that are co-fermented with plants to amplify their special properties. Both prebiotics and *Lactobacilli* work synergistically to strengthen the epithelial lining of the gut and to maintain its integrity. A strong and intact gut lining ensures better absorption of nutrients and bioactive compounds while preventing the harmful by-products from invading the body. A strong and intact gut lining also reduces digestive problems like diarrhea, constipation and flatulence, common in people who have metabolic imbalance. Phytosynbiotics exhibit characteristics which offer great potential to become a breakthrough solution in the fight against different diseases and its features can be summarised as follows:

- Phytosynbiotics are safe. They are made from food ingredients and their GRAS (Generally Recognized As Safe) status make them perfectly safe for consumption.
- Phytosynbiotics are effective. They can survive the stomach environment which would destroy the most commercial probiotics.
- Phytosynbiotics are condition specific. This is not a single formulation to cure all ailments. Due to the possibility of combining different plants with their specific healing properties, phytosynbiotics can be formulated to address specific metabolic conditions. Different conditions can be addressed using different combinations of plants and probiotics to achieve maximal optimisation of the microbiome. Phytosynbiotics increase the healing value of the plants by enhancing the absorption and bio-availability of their phytonutrients [60].

Acknowledgements

This work was supported by the Department of Biotechnology Punjabi University Patiala, Punjab, India 147002.

Conflicts of Interest

The author declared no competing interests.

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