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Immunomodulatory Activity of *Triphala* and its Individual Constituents w.s.r. to *Rasayana* - A Review Article

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Abstract

Ayurveda, the oldest healing science, focuses on treating different ailments through balancing the three pillars of life, *Vata*, *Pitta* and *Kapha*. The main target of *Ayurveda* is to maintain the health of healthy people. The use of herbs for improving the overall resistance of body i.e. health against common infection and pathogens has been a guiding principle of *Ayurveda*. Immunity is a biological term that describes a state of having sufficient biological defence to avoid infection, diseases or other unwanted biological invasion. The term 'immunomodulation' is used for describing the effect of various chemical mediators, hormones and drugs on the immune system. Various experimental trials were done for assessment of the immunomodulatory activity of *Ayurveda* drugs such as Carbon clearance test, Cyclophosphamide induced neutropenia, Neutrophil adhesion test, effect on Serum immunoglobulin level etc. But because of some latest rising issues like the adverse side effects, cost effectiveness, drug abuse etc, the modern medical world is seeking for alternative class of immunomodulatory drugs. For this, one of the best answer was hidden in *Ayurveda* which is *Triphala*, used as '*Rasayana Dravya'* from many centuries. In the recent researches of QOL (Quality Of Life), the immunomodulatory activity of *Triphala* & its individual content was separately proved effective by experimental studies. Therefore on the base of online published work done before, we compiled the review article on immunomodulatory activity of *Triphala* and its constituents individually w.s.r. to *Rasayana Karma*.

Keywords

Triphala, *Rasayana*, *Immunomodulatory activity*

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INTRODUCTION

Rasayana is one of the eighth branch of *Ayurveda*[1] which deals with healthy life and longevity of human being. It plays vital role in maintaining the health and equally important in treating diseases. In last few decades the medical science has developed in various directions. The plenty of techniques have been incorporated and many diseases are being invented. Along with its classical importance and utility this therapy is useful in different medical problem of present era. *Rasayana Dravya*s are supposed to have the ability of protecting the body against external factor that induced diseases. This implied resistance against disease may represent the modern concept of immunity. These active components play crucial role in enhancing body's resistance towards various diseases, boost memory and energy, which ultimately balances the health of the individual as a whole. A number of Indian medicinal plants and various '*Rasayana*' have been claimed to possess immunomodulatory activity[2,3].

Triphala

One of the important *Rasayana Dravya* in *Ayurveda* is *Triphala*, consisting of *Amalaki* (*Emblica officinalis* Gaertn.), *Bibhitaki* (*Terminalia bellerica* Roxb.) and *Haritaki* (*Terminalia chebula* Retz.). These three *Dravya*s are widely used not only in combination but also separately as they are easily available globally, known for its well established safety aspects as well. The immunomodulatory activity of *Amalaki*[4,5], *Haritaki*[6,7] and *Bibhitaki*[8,9] was proved by experimental study so that it would be used in various *Ayurveda* preparations. Now a days, *Chyavanprasha Avaleha* is the most popular *Ayurveda Kalpa* (preparation) used as immunity booster supplement. But all the contents of *Chyavanprasha Avleha* are difficult for collection, having issues of adulteration and also not as much cost effective. So considering all this, there may be one of the suitable and simple option for *Chyavanprasha Avleha* is *Triphala* which may also be used in the form of *Avleha* due to their various *Guna – Karma*[10].

Immune activation is an effective as well as protective approach against emerging infectious diseases. The immunomodulatory activities of *Triphala* were assessed by testing the various neutrophil functions like adherence, phagocytosis, phagocytic index (P.I), avidity index (A.I)) and nitro blue tetrazolium (NBT) reduction in albino rats. In recent years much attention is being

focused on the immunological changes occur during stress. In one of the study, noise (100dB) stress for 4h/d for 15d, was employed to alter the neutrophil functions. The neutrophil function tests and corticosterone levels were carried out in different groups of animals. It was found that, food and water intake were significantly increased in the *Triphala* administrated groups. Chawla (1982) reported that *Triphala* improves digestion, this may be one of the reason for an increased food and water intake. The animal body weight was reduced in the initial stage of the drug administration, may be due to hypocholesterolaemic action of *Triphala*, however animal body weight was gradually increased from 4th week of drug administration. The outcome of this result confirms that, treatment with *Triphala* for 48 days enhanced the A.I in *Triphala* group and appears to stimulate the neutrophil functions with decreased corticosterone level. When rats were exposed to noise-stress for 15 days, neutrophil functions were significantly suppressed and the corticosterone levels were increased. This noise-stress induced suppression in the neutrophil functions and increased corticosterone levels were significantly prevented by *Triphala*[11].

In order to simplify the formulation, one was taken all 3 herbs in equal quantity (w/w) and mixed to from MegaHerb. This research plan envisages the very narrow and specific therapeutic benefits which could be meant as *Rasayana* benefits. *Triphala* MegaHerb was subjected to successive extraction in soxhlet apparatus, using non polar to polar solvent (Pet.ether, Benzene, Choloroform, Ethyl acetate, 70% ethanol and water). Each of all six extracts was concentrated by distilling the solvent and air dried. All six extracts were mixed together to prepare *Triphala* megaExtract (megaExt). Now Immunomodulatory activity of *Triphala* megaExt was determined by using different experimental models as carbon clearance test and Delayed Type Hypersensitivity (DTH) response [Foot Pad Swelling]. *Triphala* megaExt was administered orally at low dose and high dose of 500 mg/kg and 1000 mg/kg. This result was shown significantly ($p<0.01$) increased in phagocytic index when compared to control group. This indicates stimulation of the reticuloendothelial system and increase in DTH response or cell mediated immunity indicated of increase mean paw oedema value. According to this study, it is clearly

indicating that *Triphala* megaExt show potent Immunomodulatory activity[12].

Nowadays, alternative medicine for the treatment of various diseases, including immunological disorders, is becoming more popular. Research interest has been focused on various herbs that possess immunomodulatory properties that may be useful in reducing the risk of various diseases and cancers. Immune activation is an effective as well as a protective approach against emerging infectious diseases. In one of the study, the extracts with different proportions of *Bibhitaki*: *Haritaki*: *Amalaki* (w/w/w), i.e.12:8:4 (F1), 4:12:8 (F2), 8:4:12 (F3) and 8:8:8 (F4), were prepared by decoction in water and dried under vacuum. Gallic acid, a major compound in *Triphala*, was detected by high performance liquid chromatography (HPLC). The effect of the extracts on IFN-γ and IL-10 cytokine production produced by MOLT-4 cells was determined by ELISA. The result was found that the different proportions of *Triphala* extracts and induction conditions affect cytokine production, with a predominant Th1 response. F4, the equal proportion *Triphala* extract, could be applied as a healthy herbal drink. F1, containing a high proportion of *Bibhitaki*, was a promising extract as an effective therapeutic intervention against Th2 imbalance diseases such as allergy and autoimmune disease or for use with cancer vaccines. A previous study reported that a *Triphala* aqueous extract had a cytotoxic effect on the human breast cancer cell line MCF-7 and barcl-95 transplantable mouse thymic lymphocytes, but it was not toxic to normal cells such as breast epithelial cells, MCF-10F cells, human peripheral blood mononuclear cells, and mouse liver and spleen cells[13].

In another research study, the effect of *Triphala* was investigated on complement activity, humoral immune response, and cell mediated immune response in mice, and in mitogen (phytoheamagglutinin)-induced T-lymphocyte proliferation in vitro. In this study *Triphala* powder (1:1:1) was taken and its aqueous suspension in 2% gum acacia was used. The arthritis was induced by 0.1 ml of Complete Freund's Adjuvant (CFA) in the right hind paw. *Triphala* was orally administered (500/1000mg/kg/b.wt) by gavage needle, 1 h before induction of adjuvant, then daily for 5 days. *Triphala* administration was significantly inhibited the complement activity, humoral and cell mediated immune response, delayed type hypersensitivity reaction (DTH)), and

mitogen (phytohaemagglutinin) induced T-lymphocyte proliferation in a dose dependent manner[14].

One study was conducted to evaluate the immunomodulatory effect of *Triphala* based on the analysis of the antibody titre, Pan-T, CD4+/CD8+ lymphocyte phenotype in spleen and different cytokines like IL-2, IL-4 and IFN-γ. Four groups of rats were employed namely, control, *Triphala* (1g/kg), noise stress (100dB for 4 h/day for 15 days), *Triphala*+noise stress immunized by sheep RBC (5×109 cells/ml). The results showed elevation in serum antibody titre and IL-4 levels accompanied by decreased IL-2, IFN-γ levels and reduction in Pan-T, CD4+/CD8+lymphocyte phenotype in spleen induced by noise stress. However, these effects were significantly prevented in the rats those were exposed to noise stress after being treated by *Triphala*, thus suggesting its therapeutic effectiveness[15].

Amalaki (*Emblica Officinalis*)

Aqueous extract of dried *Amalaki* (*E. officinalis* Gaertn.) pulp powder was evaluated for immunomodulatory effect on male Swiss Albino mice. The mice were divided into three groups. The first group received vehicle alone to serve as control. The second and third groups received the extract orally at 100 and 200 mg/kg body weight dose levels respectively per day for a period of 19 days. There was significant dose dependent increase in haemagglutination antibody titre, sheep red blood cells induced delayed type of hypersensitivity reaction, macrophage migration index, respiratory burst activity of the peritoneal macrophages, total leukocyte count, percentage lymphocyte distribution, serum globulin and relative lymphoid organ weight in group II and III compared to group I on 12th and 19th day of experiment on in *Amalaki* treated mice. In this study, the aqueous extract of *Amalaki* has shown promising immunomodulatory activity. The concept of immunomodulation relates to nonspecific activation of the function and efficacy of macrophages, be due to it stimulant effect on cell mediated and humoral immune responses respectively[16].

One of the study reveals that the alkaloid fraction from *E. officinalis* was evaluated for its potential ability as an adjuvant effect on the immune responses to hepatitis and diphtheria-pertussis-tetanus (DPT) antigen on human whole blood using flow cytometry. Cells were treated with variable doses of alkaloid fraction (0.625 – 2.5 mg)

in presence or absence of hepatitis and DPT vaccine antigen. Hepatitis and DTP vaccine containing alum used as standard for the studies. The results showed that the DPT mediated blood counts were significantly enhanced by alkaloid fraction at lower doses (0.625 mg) compared with DPT control group. Moreover, alkaloid containing hepatitis antigen showed no adjuvant effect on human whole blood. Alkaloid fraction is a potent enhancer of antigen-specific humoral immune responses, thus showing promise as immune adjuvant for vaccines against extracellular infectious agents such as bacteria, protozoa etc[17].

Immune activation is an effective as well as protective approach against emerging infectious diseases. *Amalaki* has been reported to inhibit chromium induced free radicle production, and it restored the antioxidant status back to control level. I also inhibited the apoptosis and DNA fragmentation induced by chromium. It relived the immunosuppressive effect of chromium on lymphocyte proliferation, and even restored the IL2 and Gamma IFN production. *Amalaki* was assessed for immunomodulatory activity in adjuvant induced arthritic rat model. Complete Freund's adjuvant was injected in right hind paw of the animals induced inflammation. Lymphocyte proliferation activity and histopathological severity of synovial hyperplasia were used to study the anti-inflammatory response of the extract, which show a marked reduction in inflammation and oedema, and caused immunosuppression in AIA rats, indicating that this drug may provide an alternative approach for the treatment of arthritis[18]. One of the study was also proved for *Amalaki* as a potent immunosuppressant as that of Dexamethasone and can be used in arthritis (Ganju et al., 2003)[19].

Bibhitaki (*Terminalia bellerica*)

One study reveals the Immunomodulatory activity of ethanolic extract of *T. bellerica* in mice. Immunomodulatory activity of ethanolic extract of T. bellerica (150 and 350 mg/kg) was carried out by testing delayed type hypersensitivity (DTH) reaction, phagocytic index, cyclophosphamide induced neutropenia and relative organ weight. Pre-treatment with both the doses of ethanolic extract of *T. bellerica* showed significantly ($p<0.01$) potentiated the DTH reaction by facilitating the footpad thickness response to SRBC's in sensitized mice. Moreover, pre-treatment with ethanolic extract of *T. bellerica* (350

mg/kg) showed significant ($p<0.01$) increase in phagocytic index and significant ($p<0.05$) protection against cyclophosphamide induced neutropenia. Furthermore, significant ($p<0.01$) increase in relative weight of spleen at 350mg/kg was observed but there was no remarkable change in thymus index was observed in tested doses of plant extract. This study have demonstrated non-specific and specific immunostimulatory properties of the ethanolic extract of *T. bellerica* fruits. This suggests its therapeutic usefulness in immunocompromised conditions[20].

The immunomodulatory activity of an acetone extract of *T. bellerica* fruit on the mouse immune response in vitro was done. For this, Mitogen induced-lymphocyte proliferation using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] technique, Th1- and Th2-related cytokine production by lymphocytes using ELISA and peritoneal macrophage function in ICR mice were assayed. At the end of the study, production by lymphocytes using ELISA and peritoneal macrophage function in ICR mice were assayed. At the end of the study, the results show that the extract had a mild inhibitory effect on the generation of oxidase enzyme (Phagocytic Index 0.8, 100 µg/ml) but did not influence acid phosphatase enzyme function during phagocytosis. The extract stimulated the proliferation of both T and B lymphocytes. The maximal activation (Stimulation Index 3.2, 100 µg/ml) was presented with concanavalin A induction, indicating a major effect on T lymphocyte proliferation. The extract reduced the production of IFN-γ (89%, 100 µg/ml) and IL-2 (98%, 100 µg/ml) but increased IL-10 secretion (231%, 100 µg/ml) compared to concanavalin A. Gallic acid, a pharmacological component contained in this plant, presented a similar effect as that of *T. bellerica* extract and may contribute to the immunomodulatory activity of *T. bellerica* fruits in cooperation with other phytocompounds. The decrease in the IFN-γ/IL-10 ratio indicated a shift in the Th1/Th2 balance towards a Th2-type response, which might lead to a treatment for Th1mediated inflammatory immune diseases. It shows that the acetone extract of *T. bellerica* fruit possesses immunomodulatory activity, both in cell mediated immunity (CMI) and humoral immunity (HI), but the response inclined to CMI dominance since the major effect of the extract was on T cell proliferation[21].

In one of the study, the oral administration of the ethanolic extract of bark of *T. bellerica* was given at the doses of 100 mg/kg in mice. The dose-dependent potentiated the delayed-type hypersensitivity reaction induced by sheep red blood cells (SRBC). Cyclophosphamide induced suppression of humoral as well as cell mediated response were significantly attenuated by daily oral treatment with alcoholic extract of *T. bellerica*. This extract at the dose of 100 mg/kg was found to suppress delayed type hypersensitivity reaction induced by SRBCs in mice. The standard control, Vitamin E treated group exhibited similar attenuation of the suspension in immune responses. Cyclophosphamide at a dose of 50 mg/kg, showed significant inhibition in antibody titre response, while ethanolic extract of *T. bellerica* was found to significantly enhance the production of circulating antibody titre. This indicates the enhanced responsiveness of macrophages and T and B lymphocyte subsets involved in antibody synthesis[22].

Haritaki (*Terminalia chebula*)

One of the study described that *T. chebula* alcoholic extract shows immunomodulatory activity. The various parameters determined were differential leukocyte count (DLC), phagocytic activity and zinc sulphate turbidity (ZST) test. Oral administration of T. chebula alcoholic extract (100 mg/kg) was found to increase the neutrophils and lymphocytes as compared to vehicle and cyclophosphamide treated groups. *T. chebula* alcoholic extract showed linear time dependent significant phagocytic activity as compared to SRBC sensitized and cyclophosphamide treated group. In zinc sulphate turbidity test *T. chebula* treated rats serum showed more turbidity (cloudy) which indicate the increase in the immunoglobulin level as compared to vehicle, SRBC sensitized and cyclophosphamide treated group. So it is revealed that the alcoholic extracts of *T. chebula* obtained from the dried ripe fruits possess good immunomodulatory activity[23]. Further continuing to above study, the immunomodulatory activity of the alcohol extract of *T. chebula* dried ripe fruits at the cellular level was also studied after sacrificing animals. For antioxidant study, the liver mitochondria were separated and used for the estimation of enzymes catalase (CAT) and superoxide dismutase (SOD) - as well as lipid peroxidation (LPO) and reduced glutathione (GSH); Melatonin secretion was characterized using sodium

dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) while spleen lymphocyte proliferation assay was performed by measuring optical density at 570 nm using ELISA reader. The cytokines, namely, IL-2, IL10 and TNF-α expression in spleen cells, were determined by real time polymerase chain reaction (RTPCR). It was found that *T. chebula* extract (100 mg/kg/p.o.) increased the level of liver mitochondrial enzymes CAT and SO) as well as GSH but decreased the level of LPO in the liver when compared to the vehicle, sheep red blood cells (SRBC) and cyclophosphamide-treated groups. Secretion of melatonin by pineal gland was enhanced by T. chebula treatment. The extract also increased spleen lymphocyte proliferation. Based on RT-PCR analysis, the expression of cytokines, viz, IL-2, IL-10 and TNF-α, was more in *T. chebula* treated than in vehicle and cyclophosphamide treated groups[24].

Typhoid is a worldwide problem today due to the emergence of multidrug resistance to *Salmonella typhi* and limited scope of vaccine against this disease. So one of the researcher investigated the immunomodulatory activity of *T. chebula* against *salmonella typhi* in mice. The author has already reported the protective effect of aqueous extract of the fruits of this plant against *S. typhimurium* in vitro as well as in vivo and also the antioxidant activity against these bacteria. In this study the same extract was evaluated for its immunomodulatory activity against *S. typhimurium* in vivo. Animals pre-treated with the same extract at a dose 500 mg/kg body wt orally showed an increase in WBC count by 3x103/cu mm and lymphocyte count by 4 % as compared to saline treated control challenged with 50000 colony forming unit of *S. typhimurium*. The drug showed the proliferation of lymphocyte by 102% and increase in food pad thickness by 28.87% as compared to infected control in delayed type of hypersensitivity test. The fruits of *T. chebula* are known for their pharmacological activity and in this study it was shown that the extract can be used as an effective immunomodulator against *S. typhimurium*. Aqueous extract of *T. chebula* showed an increase in WBC and lymphocyte count against *S. typhimurium*. The extract was also proliferate lymphocyte and exhibited delayed type of hypersensitivity[25].

The aqueous fruit extract of *T. chebula* was investigated for its effect on cell mediated and humoral components of the immune

system in mice. Administration of *T. chebula* extract produced an increase in humoral antibody (HA) titre and delayed type hypersensitivity (DTH) in mice. So it was concluded that the *T. chebula* extract is a promising drug with immunostimulant properties[26].

DISCUSSION

Above various studies indicate that, *Triphala* has a good Immunomodulatory property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin, glycosides and phenolic compounds[12]. *Triphala* extract has proved immunomodulatory activity, supporting the Th1 response more than the Th2 response. *Triphala* is safe for normal cells and might have an anti-cancer effect. Gallic acid and other phenolic compounds might be responsible for this activity[13]. Also *Triphala* caused immunosuppression in experimental induced inflammation, indicating that they may provide an alternative approach to the treatment of inflammatory and autoimmune diseases[14].

Triphala has been reported to be a rich source of Vitamin C, Ellagic acid, Gallic acid, Chebulic acid, Bellericanin, β-sitosterol, Ascorbic acid and Flavonoids. Spectroscopic techniques including mass spectroscopy, nuclear magnetic resonance and infrared spectroscopy showed Gallic acid as the major component. *Triphala* also contains about 20% tannins of both condensed and hydrolysable type. Other constituents identified in the fruits include lipids, sitosterol, saponins, cardiac glycoside and various carbohydrates (Bali chouhan et al. 2013).

According to *Ayurveda* point of view i.e. *Guna* (property) and *Karma (action)* of *Triphala*[27], it is basically a *Kashaya Rasa* (astringent) *Pradhan Dravya* i.e. *Prithvi* and *Vayu Mahabhuta Pradhana*. It is *Laghu* (Light) and *Dipana* (Digestive) in *Guna* which acts on *Jatharagni* as well as *Dhatwagni* for *Sukshma Pachana Karma* (Metabolism) to produce *Prakrut Dhatu*[28] (seven primitive matter). It may ultimately results in *Ojas Nirmiti* (governing the immune system) which will break the pathology and may help to maintain the strength of the body. Also in classical *Ayurveda* literature, *Triphala*[29] along with *Amalaki*[30], *Bibhitaki* and *Haritaki*[31] are well described for its *Rasayana Karma*.

Amalaki is traditionally used for range of severe diseases. The antioxidant, immunomodulatory, anticancer, cytoprotective, analgesic, antimicrobial,

antipyretic, antitussive and gastro protective are the important properties of *Amalaki*. Vitamin C, Tannins and flavonoids present in *Amalaki* have very powerful antioxidant activities. The fruit of *Bibhitaki* had been found to contain gallic acid as an active component, along with other phytochemical compounds such as ellagic acid, ethyl gallate, chebulagic acid, β-sitosterol, lignans and flavan. Gallic acid has a wide range of biological activities, including anti-oxidant, anti-inflammatory, anti-microbial and anti-cancer activities. Gallic acid may contribute to the immunomodulatory activity of T. bellerica fruits, or may cooperate with other phytocompounds.

The immunomodulatory activity of *Haritaki* was may be by inhibition of lipid peroxidation and increased levels of antioxidant enzymes catalase and superoxide dismutase; increased melatonin secretion by pineal gland which play a role in immunomodulatory action by exerting direct and/or indirect stimulatory effect on both cellular and humoral immunity; and proliferation of lymphocytes as indicated by the increase in the number of β and T cells which release cytokines and growth factors that regulate other immune cells and secretion of antibodies in the blood. Other mechanisms such as increased levels of cytokines IL-2, IL-10 and TNF-α which play important role in immunomodulatory actions such as T and B lymphocyte proliferation, natural killer cell activation, elevation of Th2 cells and modulation of cytokine gene expression also seem to be in operation. Also *Triphala* appears to be a very safe compound as none of its ingredients has reported any toxicity ever[32-37].

CONCLUSION

The results of this review article are very encouraging & indicate that *Triphala* should be studied more extensively to confirm these results & reveal other potential therapeutic effects also. According to modern pathophysiology, multiple immunomodulatory actions including modulation of cytokine secretion, histamine release, immunoglobulin production, immunoglobulin class switching, cellular co-receptor expression, lymphocyte proliferation and phagocytosis promotion (Spelman et al.,2006). The studies with new immunomodulatory *Dravya*s like *Triphala* are important for the discovery of drug with less side effects, less costly, more potent and effective treatment developed for immune and their related diseases. This type of study will contribute to the benefit of the

populations who needs pure herbal treatment to treat immune diseases without being used of synthetic drugs and prevent or reduce its side effects.

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