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PUNARNAVADI GHRITA - A POTENTIAL IMMUNOMODULATORY DRUG

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ABSTRACT

In the present era, Infectious diseases are leading causes of illness and death. The world is currently witnessing a dramatic disruption of everyday life owing to rapid progression of COVID-19 disease. Hence, huge emphasis is being given on immunomodulatory effect of drugs which could enhance the immune system and eventually combat the disease or infection by modulating the immune response. A significant part of Ayurvedic therapeutics aims at prevention of diseases. This is the concept of *vyadhikshamatva* or immunity. Immunity can be defined as the body's ability to identify and resist large numbers of infectious and potentially harmful microorganisms. An immunomodulator is a substance, biological or synthetic, which can stimulate (immunostimulants), suppress (immunosuppressors) or modulate any of the components of immune system. Many synthetic immunomodulators are in use but they have potential side effects like nausea, mood alterations, arthralgia, etc. Hence there is a need for safe and potent herbal formulation. In ancient ayurvedic texts concept '*Rasayana*' (drugs reputed to enhance body resistance) and various indigenous plants and formulations with immunomodulatory effect has been mentioned. One such *Rasayana* formulation is '*Punarnavadi Ghrita*'. It is a polyherbal formulation consisting of *Punarnava*, *Yashtimadhu*, *Godughda* and *Goghrita*. This formulation is said to increase '*ojas*' in the body. According to Ayurveda, *ojas* is an essence present in every *dhatu* (tissue)and is considered as *sara* of all the seven *dhatus* starting from *Rasa* to *Shukra* and is responsible for the strength, vitality and immunity of the body. Various researches have been done in recent times which has further validated the immunostimulatory activity of the four constituents of the *ghrita.*

KEYWORDS: COVID-19, Immunity, Immunomodulatory substances, Infectious diseases, *Ojas*, Pandemic, *Rasayana*.

INTRODUCTION

With the rise in global population and Substantial change in the living environment of humans (climate extremes, rising pollution, issues on food security, increasing diseases etc.), maintainence of healthy status of individuals has become very important. The number of individuals surrendering to any diseased condition is increasing alarmingly day by day. There is therefore a greater need to support the health and well being, primarily the immune system of individuals at different stages throughout their life. These concerns regarding immunity have become more important in recent times because of periodic outbreaks of various infectious diseases such as SARS (Severe acute respiratory syndrome), MERS (Middle east respiratory syndrome), and now the Covid-19 pandemic, that within a few months, has led to more than three million cases across the world.

Adequate nutrition is an important way to help build a resilient immune system over the long term. But today's population is undergoing a major nutritional shift where their diet mainly consists of high-fat, high-salt, high-sugar and low-fiber content. Since the diet is not balanced hence their levels of vitamins and minerals are lower than what is required for optimal immune function. Moreover, Sedentary lifestyle, chronic mental stress due to hectic schedules and lack of proper sleep, malnutrition, environmental toxins, excess weight, old age, all these factors add to the problem leaving the population vulnerable to various bacterial and viral infections. Hence there is a need for safe, potent,

affordable herbal nutritional supplement to help support the immune system.

MATERIAL AND METHOD

Source of data

Literature related to immunity, immunomodulation, *Punarnavadi ghrita* and its components have been referred from various ayurvedic texts, modern medical books, research papers and journals.

Methods

- To define the concept of immunity and immunomodulation.
- To emphasize the need of herbal immunomodulatory supplement.
- To correlate immunomodulators with *Rasayana*.
- To compile the available literature on *Punarnavadi ghrita* and its ingredients.

CONCEPT OF IMMUNITY AND IMMUNOMODULATORS

- **Immunity**: The ability of an organism to resist a particular infection or toxin by the action of specific antibodies or sensitized white blood cells.
- **Immunomodulators:** These are the medications used to help regulate or normalize the immune system.

Classified into 3 Types

a. Immunostimulants: These agents are inherently non-specific in nature and are envisaged to increase body's resistance against infections. Thus, used in treatment of cancers, chronic infections.

b. Immunosuppressants: These are structurally and functionally heterogenous group of drugs which are used to suppress the immune system. Anti-rejection medicine in organ transplant, treatment of auto-immune disorders.

c. Immunoadjuvants: These agents are used for enhancing vaccines efficacy and therefore, could be considered specific immune stimulants.[1]

NEED FOR HERBAL IMMUNOMODULATORY SUPPLEMENT

Herbal immunomodulatory supplements will not have the side effects of synthetic immunomodulators i.e Isoprinosine, levamisole, thalidomide, inosiplex etc. Various side effects of these synthetic immunomodulators include nausea, arthralgia, drowsiness, weakness, mood alteration etc. The herbal immunostimulant can help to prevent various infections. These can also be used as Adjunctive therapy in Malignancy, AIDS, Chronic infectious diseases.

CORRELATION OF IMMUNOMODULATORS WITH "RASAYANA" IN AYURVEDA

The word *Rasayana* is a combination of two words (rasa & ayana) which refers to nutrition and it's transportation throughout the body. It's a Stream of medication for immune promotion, anti-degenerative and rejuvenating health care. *Rasayana* can be a drug, diet or even a lifestyle conduct which may be helpful in increasing immunity and youthfulness.[2]

Acharya Charaka in Charak Samhita Chikitsa sthana Chapter-1 "*Rasayanadhyaya*" first pada "*Abhayamalakiya Rasayana pada*" has described two types of medicine, "*Ojovardhaka*" i.e. health promoting and "*Roganut*" i.e disease curing. *Rasayana* comes under *Ojovardhaka* medicine.[3]

The *Rasayanas* are supposed to strengthen *oja* and *bala* i.e vitality and bio strength with natural resistance against aging and diseases. It contributes to the integrity of body tissues and thus increases longevity. It also promotes memory, intelligence, youth, lusture, complexion and voice.[2]

Ayurveda describes a number of drugs as *Rasayana* which are claimed to possess immunomodulatory effect.

PUNARNAVADI GHRITA AS AN IMMUNOMODULATOR

In Ayurveda objective of immune enhancement is achieved through the use of *Rasayana*. In ancient ayurvedic texts various indigenous plants and formulations with immunomodulatory effect has been mentioned. One such *Rasayana* formulation is "*Punarnavadi ghrita*". It is mentioned in *Chakradutta*, Chapter-18, *Madatya chikitsa* (Treatment of chronic alcoholism). It is a poly-herbal formulation made from *Godughda*(cow's milk), *Yashtimadhu*(Glycyrrhiza glabra), *Punarnava* (Boerhaavia diffusa) and *Goghrita*(cow's ghee). This formulation is said to increase "*ojadhatu*" in human body which is destroyed by over consumption of *Madya*. (alcohol).[4]

According to Ayurveda, *Ojas* is an essence present in every *dhatu* (tissue) and is considered *sara* of all the seven dhatus starting from *Rasa* to *Shukra* and is responsible for the strength,vitality and immunity of the body.[5]

The formulation is also mentioned in *Bheshajya ratnavali* and *Gada nigraha*. *Acharya Chakrapani* has termed the decrease in '*ojas'* due to over consumption of *madya* as '*hatojous'*.[4] *Acharya Sushruta* while explaining the pathology of *Abhinyaasjwara* (a type of fever) named it "*Hatojous*" which means in this disease "*oja*" decreases. *Acharya Dalhan* in his commentary *Nibandh sangrah* compared "*Hatojous*" with "*Oja visransa*" lakshana.

Acharya Sushruta has mentioned the symptoms of *Ojas Visransa* (first stage of abnormality of *ojas*) in chapter-15 of *sutrasthan* as- *Sandhi Vishlesh* (looseness of joints), *Gatra Sada* (weakness of the body), *Dosha Chyavanam* (provoked tri doshas move away from their normal seats), *Kriya Sannirodha* (inability to perform

normal functions), *Shrama* (lethargy in organs), *Aprachuryam Kriyanam* (It also results in the impairment of *Kayik* (physical), *Vachik* (vocal) and *Mansik* (mental) functions of the body).[6]

The dose of *Punarnavadi ghrita* is 5-10gm/dose as mentioned in *Bheshajya ratnavali* commentary by *Siddhinandan Misra*.[7]

DRUG REVIEW

Table no. 1: Constituents of *Punarnavadi Ghrit.*

S.No.	Ingredients	Botanical Name	Rasa	Guna	Virya	Vipaka
1.	Punarnava[8]	*Boerhaavia diffusa* Linn.	Madhur,Tikta, Kashaya	Laghu, Ruksha	Ushna	Madhur
2.	Yashtimadhu[9]	*Glycyrrhiza Glabra* Linn.	Madhur	Guru, Snighda	Sheeta	Madhur
3.	Godughda[10]	-	Madhur	Guru, Snighda	Sheeta	Madhur
4.	Goghrita[11]	-	Madhur	Guru, Snighda	Sheeta	Madhur

PHYTOCONSTITUENTS OF THE HERBS USED TO MAKE *PUNARNAVADI GHRITA*

PUNARNAVA (BOERHAAVIA DIFFUSA)[12]

Various chemical constituents are isolated from roots

Phenolic glycoside- Punarnavoside
C-Methyl flavone- Borhaavone
Isoflavone- 2'-O-Methyl abronisoflavone
Phenolic acid-*trans*-caftaric acid
Rotenoids-Boeravinones A, B, C, D, E, F; Boeravinones G, H; Boeravinones I, J; 9-O-Methyl-10-hydroxy coccineone E; Diffusarotenoid;6-O-Demthyl-boeravinoneH; 10-Demethyl boeravinone C; Coccineones E, B; Boeravinones M, P, Q, R, S
Triterpenoids- Ursolic acid
Xanthone- Boerhavine
Lignan-Liriodendrin, syringaresinol mono-β-D-glucoside
Purine nucleoside-Hypoxanthine-9-L-arabinofuranoside
Amino acid-Aspartic acid, Glutamic acid
Sterol – Boerhavisterol
Phytosterol- β-sitosterol
Sterol ester- Boeravilanostenyl benzoate
Ecdysteroid- β Ecdysone
Fatty acid- triacont-24-en-1-oic acid, tetracosanoic acid, icosanoic acid, stearic acid, pamitic acid
Flavonoid- 5,7-dihydroxy-3 4 -dimethoxy-6,8- dimethyl flavone
Hydrocarbon- Boeradiffusene, hentriacontane

Chemical constituents isolated from leaves

Flavonol-Quercetin, Kaempferol
Flavonoid glycoside- 3,4-Dihydroxy-5-methoxycinnamoyl Rhamnoside,Quercetin 3-O-rhamnosyl (1→6)galactoside (quercetin 3-O-robinobioside), Eupalitin 3-O-galactosyl (1→2) glucoside,Kaempferol 3-O-robinobioside, Eupalitin-3-*O*-*β*-*D*-galactopyranoside.

Some other Chemical constituents isolated from whole plant

Alcohol-Myricyl alcohol
Fatty acid- Myristic acid, oxalic acid
Quinolone alkaloid- Punarnavine (lunamarine) etc.

YASHTIMADHU (GLYCYRRHIZA GLABRA)[13]

Saponins- The roots of *Glycyrrhiza glabra* Linn contains glycyrrhizin, which is a saponin that is 60 times sweeter than cane sugar. Glycyrrhizin (glycyrrhizic acid; glycyrrhizinate) constitutes 10–25% of licorice root extract and is considered the primary active ingredient. Glycyrrhizin is a saponin compound comprised of a triterpenoid aglycone, glycyrrhetic acid (glycyrrhetinic acid; enoxolone) conjugated to a disaccharide of glucuronic acid. Both glycyrrhizin and glycyrrhetic acid can exist in the 18α and 18β stereoisomers.

Flavonoid rich fractions include- Liquirtin, isoliquertin, liquiritigenin, rhamnoliquirilin, **Glabrene** and five new flavonoids-glucoliquiritin apioside, prenyllicoflavone A, shinflavanone, shinpterocarpin and 1-methoxyphaseolin isolated from dried roots.

Coumarin- Licopyranocoumarin, licoarylcoumarin, Licocoumarin A, glisoflavone and new coumarin-GU-12 also isolated.

Isoprenoid-substituted phenolic constituents- Licochalcone A, semilicoisoflavone B,1-methoxyficifolinol, isoangustone A, and licoriphenone isolated from roots.

Prenylated isoflavane - Glabridin, kanzonol R

Volatile components - pentanol, hexanol, linalool oxide A and B, tetramethyl pyrazine, terpinen-4-ol, α-terpineol, geraniol and others in the roots is reported.

Compounds isolated from the essential oil- Presence of propionic acid, benzoic acid, ethyl linoleate, methyl ethyl ketine, 2,3- butanediol, furfuraldehyde, furfuryl formate, 1-methyl-2-formylpyrrole, trimethylpyrazine, maltol.

The Indian roots shows- 2-methyliso - flavones, and an unusual coumarin, C liquocoumarin, 6 - acetyl- 5, hydroxy- 4 - methyl coumarin and Asparagine.

VARIOUS STUDIES ON IMMUNOMODULATORY EFFECT OF HERBS USED IN MAKING *PUNARNAVADI GHRITA*

Punarnava (Boerhaavia diffusa)

In Vitro Studies

1. A study was done to observe *In vitro* cytotoxicity of decoction (MTT Assay) of root of Punarnava in breast cell line (MCF-7). It was observed that the test sample exhibited cytotoxicity of about 65.1 ± 1.2 at 800 µg/ ml concentration (48 hrs) of incubation in MCF-7 breast cell line.[14]

2. *Mehrotra et al.* conducted research work to study immunomodulation by ethanolic extract (100 and 500 µg/ml) of *Boerhaavia diffusa* roots. It was concluded from the work that ethanolic extracts of *B. diffusa* roots inhibited human NK cell cytotoxicity *in vitro*, inhibited lipopolysaccaride induced NO (nitric Oxide) production in mouse macrophage cells RAW 264.7. At a concentration as low as 10 µg/ml it inhibited phyto haemoagglutinin stimulated IL-2 and lipopolysaccharide stimulated TNF-alpha in human PBMCs (Peripheral blood mono nuclear cells) culture and inhibited cytokine production. Intra cytoplasmic IFN-gamma and cell surface markers such as CD16, CD25, and HLA-DR did not get affected on treatment with *B. diffusa* extract. This study demonstrated immunosuppressive potential of ethanolic extract of *B. diffusa*.[15] The author suggested good immunosuppressive properties possibly because of alkaloid/lignan.

3. Pandey *et al*. worked on hexane, chloroform (50 µg/ml), and ethanol extracts (50 µg/ml) of BD leaves and found inhibition of PHA stimulated proliferation of PBMCs, two-way MLR, NK cell cytotoxicity, and LPS-induced NO production by RAW264.7 when treated with chloroform and ethanol extracts (5–500 *µ*g/mL). Eupalitin-3-*O*-*β*-*D*-galactopyranoside isolated from the ethanolic extract showed more effectiveness. It decreased the production of IL-2 and TNF-*α* in human PBMCs and repressed NF-*κ*B and AP-1, thereby depressing activation and proliferation of T cells. The author suggested specific potential of eupalitin-3-*O*-*β*-*D*-galactopyranoside for immunosuppression.[16]

4. The immunosuppressive property of eupalitin-3-*O*-*β*-*D*-galactopyranoside could be linked with antiosteoporotic activity shown by *Boerhavia diffusa* extract in various cell cultures and *in vitro* studies. *Boerhavia diffusa* is considered as one of the core ingredient in traditional and ethnopharmacological medicine in treatment of rheumatism. The evidence for presence of compounds with antiosteoporotic, immunosuppressive, and anti inflammatory activities approves the use of *Boerhavia diffusa* in rheumatic disorders.[12]

In Vivo studies

1. Immunomodulatory activity of Punarnavine alkaloid (PA) was determined in Albino mice by observing its effect on –organ weight (liver, spleen, thymus and kidney), expression of cytokines, bone marrow cellularity and alpha-esterase positive cells, Plaque Forming Assay (PFA), Delayed Type Hypersensitivity (DTH), Phagocytosis activity.PA did not exhibit any toxic effect in mice.

In DTH study, the foot pad thickness due to influx of mononuclear cells at the site of inoculation was distinctly increased in PA treated mice. PA enhanced the phagocytic activity of the polymorphonuclear cells by increasing the engulfment of the Candida cells thereby stimulating a non-specific immune response. PFA (plague forming Assay) confirmed that PA treatment could elevate the humoral immune response due to the synthesis of antibody which in turn is responsible for the enhancement of macrophages and B lymphocyte subsets. The significant increase in the number of α-esterase positive cells and bone marrow cellularity due to immunomodulatory effect indicated the proliferation of stem cells. Different organ weight was also markedly improved by PA treatment compared to SRBC sensitized group. Further, in real time PCR studies treatment of PA significantly increased IL- 7, IL- 10, IL-12a and IL-12b mRNA gene expression. From these findings it was concluded that PA could be developed as a potent immunomodulatory agent.[17]

2. Manu and Kuttan observed the effect of the purified alkaloid Punarnavine on the immune system using Balb/c mice. Intraperitoneal administration of Punarnavine (40 mg/kg body weight) was found to enhance the total WBC count on 6th day. Bone marrow cellularity and number of α-esterase positive cells were also increased by the administration of Punarnavine. Treatment of Punarnavine along with the antigen, sheep red blood cells (SRBC), produced an enhancement in the circulating antibody titer and enhancement in the number of plague forming cells (PFC) in the spleen. Maximum number of PFC was obtained on the 6th day. Punarnavine also showed enhanced proliferation of splenocytes, thymocytes and bone marrow cells both in the presence and absence of specific mitogens *in vitro* and *in vivo*. More over administration of Punarnavine significantly reduced the LPS induced elevated levels of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 in mice. These results indicated the immunomodulatory activity of Punarnavine.[18]

3.Mungantiwar *et al*. observed delayed hypersensitivity in mice immunized intraperitoneally with sheep RBC after oral administration with 25-100 mg/kg of the alkaloid fraction for 10 days around immunization. They also observed a dose related increase in humoral antibody titres in those mice. The alkaloid fraction failed to show any blastogenic effect (lymphocyte proliferation) responsiveness of murine splenocytes to

the T-cell mitogen Con A and the B-cell mitogen lipopolysaccharide (LPS).[19] The author stated that immunostimulation is due to metabolic alteration of the alkaloid to its active form.

4. Pre treatment with root Powder aqueous extract at a dose of 200mg/kg/day orally for 15 days prior to E.coli challenge produced significant leucocytosis with reduction in mortality in rats and also significantly increased macrophage phagocytic activity in mice. The plant extract reversed the stress induced elevations in the levels of glucose, cholesterol, SGPT, BUN and reduction in triglycerides induced by cold and forced swimming stress in rats.[20]

5.The alkaloid fraction isolated from the root was investigated for its effect on plasma cortisol, adrenal cortisol and humoral response in stressed rats. It exhibited restorative activity against stress induced changes in plasma and adrenal cortisol levels. It also significantly augmented the antibody production in stressed rats as compared to control.[21]

6.The Punarnava herb powder was given in 3 concentrations 0.5%,1%, and 1.5% in broilers. The groups were given T5, T6, T7 names respectively. T7 group (1.5%) produced higher weight of lymphoid organs. CMI response on 15th day of challenge, after 24 hrs showed lower thickness in T7 group. Hence, up to 1.0% level of punarnava powder (T6) immune system was seen more active.[22]

7. In another study, Sumanth *et al*. compared the effect of BD with ashwagandha and found comparable increase in total swimming time in mice when fed with alcoholic extract. The extract showed more potent effect on the count of total WBC, glucose level, and plasma cortisol level. The extract produced macrophage phagocytic activity comparable to the drug levamisole.[23]

Various Studies have shown immunosuppressive, immunostimulatory, immunomodulatory and anti-lymphoproliferative activity[24] of various extracts, fractions and pure compounds of *B. diffusa.*

Yashtimadhu (Glycyrrhiza glabra)

In Vitro Studies

1. Ashawat M.S. *et al*. had studied the relative reducing activity in terms of antioxidant activity of extracts by using individual extract (15mg) as well as its combination with equal amount of ascorbic acid. The extracts and ascorbic acid were dissolved separately in 1.0 ml of deionised water with phosphate buffer. The mixture was incubated at 50^0C for 20 min. Aliquot of trichloroacetate acid were added to the mixture and centrifused at 3000 rpm for 10 min. The upper layer of solution was mixed with distilled water and a freshly prepared $FeCl_3$ solution. The absorbance was measured at 700nm by making 500µg mL extract aliquot. Increased absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid.[25]

2. Visavadiya N. P. *et al*. showed antioxidant property of *Glycyrrhiza glabra Linn* root extracts using *in vitro* models. The dose dependent aqueous and ethanolic extracts demonstrated the scavenging activity against nitric oxide (concentration that caused 50% inhibition of nitric oxide radicals (IC50=72 and 62.1µg/ml, respectively), superoxide (IC50=64.2 and 38.4 µg/ml, respectively), hydroxyl (IC50=81.9 and 63µg/ml, respectively) radicals. Further, both extracts showed strong reducing power and iron-chelating capacities. In the Fe2+/ascorbate system, both extracts were found to inhibit mitochondrial fraction lipid peroxidation. In copper-catalyzed human serum and low-density lipoprotein oxidation models, both extracts significantly (P<0.05) lengthened the lag phase along with a decline in the oxidation rate, conjugated dienes, lipid hydroperoxides and thiobarbituric acid reactive substance formation. So, ethanolic extract of *G. glabra* possess considerable antioxidant activity and protective effect against the human lipoprotein oxidative system.[25]

3. Hasan *et al*., 2016 showed that in liver cancer, the compound inhibits the proliferation of HepG2 cells without affecting the normal liver cell line. In particular, 18β-glycyrrhetinic acid increases the formation of reactive oxygen species, nitric oxide production, and loss of the mitochondrial membrane potential.[26]

4. Y. C. Huang *et al*. 2016 studied the anticancer activity of 18α-glycyrrhetinic acid in human leukaemia, by inducing the apoptosis of HL-60 cells through the activation of extrinsic and intrinsic apoptotic pathways.[27]

5. Licochalcone E, when compared with well-known antitumour agents, licochalcone A and isoliquiritigenin, exhibited the most potent cytotoxic effect. Xiao explored the licochalcone A mechanism of action in MKN-28, AGS, and MKN-45 gastric cancer cells and human gastric epithelial immortalized cells. The results indicated that licochalcone A inhibits gastric cancer cells growth in a dose-dependent way, by blocking cell cycle progression at the G2/M transition, inducing apoptosis. In addition, licochalcone A induced apoptosis by its effects on the expression of PARP, caspase-3, Bcl-2, and Bax (X. Y. Xiao et al.,2011).[28]

6. Glycyrrhizin and glycyrrhetinic acids are effective compounds in gastric cancer treatment, glycyrrhizin suppresses thromboxane A2 in lung cancer cell with low toxicity (Deng, Wang, Zeng,Chen, & Huang, 2017).18β-glycyrrhetinic acid has antitumour activities in breast and ovarian cancer,gastric tumours, and leukaemia.[29]

7. Glabridin exhibited antitumour properties in various human cancer cells. The results revealed that glabridin

induced apoptosis in dose dependently in Huh7 cells through caspase-3, caspase-8, and caspase-9 activation and PARP cleavage (Hsieh et al., 2016).[30]

In Vivo Studies

1. Evaluation of Immunomodulatory activity of Glycyrrhiza Glabra roots in combination with Zinc has been done. Leukocyte count and phagocytic index (carbon clearance) was increased significantly with the treatment of ALE (aqueous Liquorice extract) compared to control.

Zinc(45mg/kg) in combination with ALE (0.75g/kg) showed highly significant increase of Leukocyte count and phagocytic index compared to control. Positive significant action on spleen weight was seen. In cellular immune response study an enhancement in foot pad thickness was observed when compared to control group. ALE did not show significant increase in HA titre value and antibody secreting cells of mouse spleen. However ALE (0.75g/kg) + Zn (45mg/kg) showed significant increase in HA titre and antibody secreting cells of mouse spleen.[31]

2. Antioxidant status and immune activity of Glycyrrhizin in Allergic Rhinitis mice was studied. Oxidative stress is a crucial event in Allergic rhinitis. Glycyrrhizin, a triterpene Glycoside is a major constituent of Licorice root. It has various pharmacological effects like Anti-Inflammatory, Anti - viral, Anti-oxidant activities. In AR mice Glycyrrhizin consumption decreased blood and nasal mucosa anti-oxidant enzyme activities, Lipid peroxidation and GSH levels (Glutathione), enhances IFN-y, reduces IL-4 levels, thus protecting nasal mucosa oxidative injury and increase in immunity activity. The effect increases with increasing concentration.[32]

3. Immunostimulant action of Aqueous extract of glycyrrhiza glabra was studied. The extract was found to be effective in reducing mortality by E.coli induced abdominal sepsis in wistar rats. The immunostimulant effect was observed with increased phagocytosis in CCT. (carbon clearance test) The extract was found to have significant immunostimulant activity in haemagglutination antibody titre value and delayed type hypersensitivity at dose levels of 150 and 300 mg /kg body wt.[33]

4. Shalaby, Ibrahim, Mahmoud, and Mahmoud (2004) evaluated the anti-inflammatory activity of G. glabra in male rats after 4 weeks of food intake. The authors observed a significant decrease in the total cholesterol and triglyceride levels as well as in the levels of serum liver enzymes.[34]

5. The hydromethanolic root extract of G. glabra exhibited antimutagenic potential by suppressing micronuclei formation and chromosomal aberration in bone marrow cells of albino mice (V. Sharma, Agrawal, & Shrivastava, 2014).[35]

Clinical study

1. A Study was done to see effects of Compound Glycyrrhizin on Serum IFN-y and IL-10 and immunological index in Patients with alopecia Areata. Alopecia is organ specific auto immune disease regulated by T-lymphocyte mediated growth and characterized by limited hair follicle damage and hair loss. Compared with control group levels of IFN-y were decreased and IL-10 were higher in study group. Level of CD3+, CD4+, CD4+/CD8+, IgA, IgG, IgM incresed, Regulated level of Th1/Th2 cytokine. Adding compound Glycyrrhizin in the reaction of alopecia areata effectively improves immune cell abnormality.[36]

DISCUSSION

Immunomodulators are the substances that can modify the activity of immune system. They can enhance or inhibit immunological responsiveness of an organism by interfering with it's regulatory mechanisms. This may be antigen independent and may directly induce production of mediators and effector molecules by the immune competent cells. This type of antigen independent immunity is distinct from one achieved by conventional immunization or by passive immunization using antibodies. Immunomodulators can regulate the cytokine production such as tumor necrosis factor, interleukins and interferons and these cytokines may in turn activate T-cells or NK cells.[37] In Ayurveda objective of immune enhancement is achieved through use of *Rasayanas.* Ayurveda prescribes usage of different medicated *ghritas* for internal administration for various uses in body like for treating mental and physical disorders, for digestion, for nourishment, for building immunity, for promoting memory, intelligence etc. Many pharmaceutical processes used in making *Ghrita* formulation are responsible for it's therapeutic properties. *Punarnavadi Ghrita* is one such formulation of the *Rasayana* catogary. It has four constituents-*Punarnava* (*Boerhaavia diffusa*), *Yashtimadhu(Glycyrrhiza glabrra), Godughda* (cows milk) and *Goghrita* (cows ghee). In *Bhavprakash nigantu,*[38] *Madanpal nigantu*[39] and *Ashtanga Hridaya,*[40] *Punarnava* has been mentioned as a *Rasayana* that rejuvenates the body cells. *Acharya Charaka* has kept this drug under *Vayasthapana varga*.[41] It has *Madhur rasa* and *Madhur vipaka* similar to the quality of *Oja*. It is reported to possess antiaging, disease prevention, life strengthening activities. It has adaptogenic effects. It increases the adaptability of an organism against any type of stress, namely, physical, chemical, or biological. It possesses various pharmacological and biological activities such as immunomodulatory, anti-metastatic, anti-oxidant, anti-estrogenic, Nitric oxide scavenging, anti-inflammatory, hepatoprotective, anti-proliferative, anti-bacterial, anti-viral, chemopreventive etc. The roots are the source of two documented immunostimulants, syringaresinol mono-β-D glucoside (eleutherosideE1 and acanthoside

B) and punarnavine. Punarnavine is a potent immunomodulatory alkaloid which stimulates immune system by enhancing stem cell proliferation, stem cell differentiation, antibody and proinflammatory cytokine production.It also shows enhancement of NK cell activity. It has anti-metastatic potential. If given prophylactically (40mg/kg) for 10 days after tumor inoculation, it can restrain lung melanoma metastasis upto 93.9% - 95.25%. Two rotenoids (Boeravinones G and H) from *punarnava* roots are potential efflux inhibitors for breast cancer resistance protein (ABCG2). β Ecdysone an ecdysteroid found in roots has been reported to have immunomodulatory properties. Liriodendrin, quercetin and kaempferol extracted from roots and leaves have anti-inflammatory property. Quercetin increases the bioavailability of Tamoxifen (used to treat breast cancer) upon coadministration. Eupalitin 3-O-β-D galactopyranoside isolated from ethanolic extract shows immunosuppressive property. It decreases production of IL-2 and TNF-α in human PBMCs. It represses NF-kB and AP-1 and depresses activation and proliferation of T cells.[12] *Yashtimadhu* (*Glycyrrhiza glabra*) has been described as *medhya rasayana* by *Acharya Charaka.*[42] It not only acts on rejuvenating the nervous system but also helps prolonging life, capable of curing several diseases, improving overall immunity, digestion, complexion and quality of voice. It has been kept under *Jivaniya varga* by *Acharya Charaka.*[43] It has *Madhur rasa*, *Madhur vipaka*, *Sheeta virya* and *guru, snighda gunas.* Glycyrrhizin is the principally effective ingredient of Licorice extract. It is a Triterpene Saponin which has Aglycone component known as Glycyrrhetinic Acid. Both Glycyrrhizin And Glycyrrhetinic Acid have been demonstrated to possess Anti-Oxidant properties as well as Anti-Inflammatory, AntiViral, Anti-Tumor, Immunoregulatory Properties. Glycyrrhizin is seen capable of triggering the blockade of Receptor-Mediated endocytosis resulting in inhibition of Viral infilteration into the cells. Glycyrrhizin Triggers biological activities at cellular level via novel (gbps) which are responsible for anti-inflammatory and anti-viral effects. Glycyrrhizin produces interferons (IFNs), accelerated activity of NK cells, Regulated Growth response of lymphocytes via the acceleration of interleukins (IL-2) production. Modulates immune response at initial stage of disease process via dendritic cells.[44] Glycyrrhizin is broken down in intestine and exhibits anti-inflammatory effect comparable with that of corticosteroid hormone including hydrocortisone.[45] It also has neuroprotective action by inhibiting ROS generation, cytotoxicity, and glutathione downregulation (GSH). The decreased GSH levels are the main cause in increased oxidative stress in dementia.[46] It also exhibits aphrodisiac potential.[47] It enhances the integrity of red blood cell membranes against proteolytic and oxidative injury by inhibiting any changes caused by diamide and n-ethylmaleimide treatment, thus preventing proteolytic injury.[48] Glycyrrhetic acid promotes pro apoptotic pathway by enhancing mitochondrial permeability transition which stimulates tumor cell apopotosis.[49] Glycyrrhiza Glabra flavanoids have antioxidant effects. Glabridin an isoflavin isolated from glycyrrhiza glabra root shows antioxidant effect and also inhibits LDL oxidation induced by copper ions or mediated by Macrophages.[50] This effect resides mainly in the 2' hydroxyl moiety of isoflavan. Isoflavan- hispaglabridin A, Hispaglbridin B, 4'- o methyl Glabridin, 2 Chal cones, Isoprenyl Chal cone Derivative, Isoliquiritigenin, Isoflavone, Formononetin, all these components isolated from *glycyrrhiza glabra* have anti-oxidant property. Among these Glabridin – 11.6% w/w constitute the major amount in crude extract.. Anti-oxidant capacities were tested against β carotene destruction and LDL oxidation. Glabridin being most abundant and potent antioxidant. LDL oxidation is key event in formation of early Atherosclerotic lesion. The use of these natural antioxidants may prove beneficial to attenuate atherosclerosis.[51] Glycyrrhiza glabra shoot and root polysaccharide fraction stimulates macrophages. All crude Polysaccharides have been shown to induce nitric oxide production by murine peritoneal macrophages *in vitro*.[52] In addition to oxygen intermediates macrophages also produce reactive nitrogen intermediates (RNS) esp. NO (nitric oxide) as effector molecule. NO is labile, highly reactive gas, cytotoxic, cytostatic effector molecule against tumour cells and various intracellular and extra cellular pathogens. It can be one of the possible therapeutic strategies against tumours and infections. These plant derived immunomodulators can be used in conjugation with anti-tumour or anti biotic drugs for synergistic action.[53]Glycyrrhizin, liquiritigenin and 18 β-glycyrrhetinic acid are the main components of licorice responsible for anti-allergic effects. They act by inhibiting immunoglobulin E (IgE) Liquiritin, Isoliquiritigenin, glycycoumarin also show neuroprotective property. Glycycoumarin neuroprotective effect can be due to its capacity to suppress the caspase-3 proapoptotic activity and isoliquiritigenin inhibits N-methyl-D-aspartate receptors. Licochalcones B and D have potential anti-oxidant efficacy by preventing microsomal lipid peroxidation and thus inhibits red blood cells from oxidative hemolytic effects. It also shows strong scavenging activity on DPPH radical.5 new flavanoid compounds are extracted from air dried roots of *Glycyrrhiza glabra*. These are –Glucoliquiritin apioside, Prenylucoflavone A, Shinflavone, Shinpterocarpin, 1-methoxy phaseollin. These are all potent Antioxidants.[38] Dehydro stilbene derivatives act as free radical scavengers.[54] N-acetyl muromoyl peptide is a glycyrrhizin isotope that shows *in vitro* activity of ceasing the influenza virus's reproduction.[55] Isoliquiritigenin and Licochalcone-A show anti-neoplastic activity. Methanolic licorice extract and it's isolated compound licocoumarone stimulates the phosphorylation of BCl_2 and halt the G2/M cycle in cancer cell lines and also induces apoptosis in human monoblastic leukemia U937 cell line. Hydromethanolic root extract demonstrated anti-mutagenic activity by

suppressing the formation of micronucleus and chromosomal abnormalities in bone marrow cells of albino.[56] Recent studies have examined the anti-viral effectiveness of glycyrrhizin towards corona virus in SARS infected patients. It restrains viral reproduction and shows a prophylactic effect. *Acharya Charaka* has described 10 qualities of cow's milk i.e sweet, cold, soft, unctuous, dense, smooth, slimy, heavy, slow, calming.[57] These are also the properties of "*ojas*". So milk having identical properties is conducive to the promotion of *ojas*. Thus milk acts as a *Rasayana*. Milk is also a rich source of vitamins like B2, B3 and vitamin A, Vitamin-C, carotenes, flavones, sterols, phenols which help increasing immunity and delaying process of aging. It is a good source of zinc, which is required for synthesis of insulin by the pancreas and for immunity function. The substances in milk which have an antimicrobial effect are immunoglobulins, lactoferrin, lysozyme, lactoperoxidase and vitamin B12-binding protein. The immunoglobulins, mainly IgA are not broken down by the digestive enzymes. Thus, they not only act against the microorganisms in the intestine but also prevent the absorption of foreign proteins. Lactoferrin is an iron binding glycoprotein that occurs in cow milk at a level of 0.2 mg/ml. A number of milk enzymes like lactoperoxidase, xanthin oxidase and lysozyme are involved in antibacterial mechanisms. Certain peptides from casein stimulate the production of immunoglobulins. Immune-stimulatory peptides from milk can stimulate the phagocytic activities of murine and human macrophages and enhance resistance against certain bacteria. A specific fatty acid (a cis-trans isomer of linoleic acid) has been identified in milk fat, which appears to be an inhibitor of cancerous growth. Conjugated linoleic acid (CLA) in cow milk prevents the uncontrolled spread of cancer-affected cells.[58] Similarly *Acharya Charaka* has described Ghee as a potent ingredient capable of promoting memory, intellect, power of digestion, semen levels and *Ojas*.[59] Various studies have proved immunostimulant activity of Cow's ghee.[60]

CONCLUSION

The constituents of *punarnavadi ghrita* has shown positive immunomodulatory effect in various studies. Because of it's potential therapeutic application, low cost and easy availability of constituents and no toxicity, this herbal supplement may be helpful in obtaining better protective immune response against bacterial,viral and other diseases and hence prove to be a potent immunostimulant. Further experimental studies and clinical trials are needed to validate it's efficacy and safety.

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