Review article:

The Immunomodulatory, Nitric Oxide and Cytokine activity of Septilin™

Mujeeb Hoosen

Abstract:

Objective: Herbal immunomodulatory preparations are increasing in popularity. *In vitro, in vivo* and clinical trial studies are needed to ensure safety, quality and efficacy of these herbal medicines. Septilin™, a proprietary herbal medicinal product has been reported to have immunomodulatory effects. ***Methods:*** For this narrative review the author surveyed the primary literature on Septilin™ and its ingredients with regards to immunomodulatory, nitric oxide (NO) and cytokine activity. Databases utilized included Pubmed, Science Direct and EBSCO, Google Scholar as well as a hand search through journals and bibliographies was included. English language restriction was observed. The following parameters had to be met for study inclusion: investigations on Septilin™ as a formulation (liquid or dried form) were accepted. Research on the isolated constituents of Septilin™ (single herbs) was also accepted. The accepted model types included; *in vitro* and *in vivo*, animal and human models. The following were also required; method of preparation of the Septilin™, concentration of the plant preparation and dose/exposure time. Only studies providing statistically significant results with regards to immunomodulatory, cytokine and nitric oxide activity were included. ***Results:*** Septilin™ and its ingredients had effects on at least one cytokine. The most frequently studied cytokines were IL-1, IL-2, IL-4, IL-6, TNF, and IFN. Many studies also reported on NO activities. Septilin™ and its ingredients demonstrated modulation of several cytokines with varying results on NO activity. The bulk of studies conducted on Septilin™ and its ingredients were *in vitro,* the few *in vivo* studies were mainly conducted in rats or mice models with a few studies conducted on humans. ***Conclusion:*** The *in vitro* and *in vivo* research demonstrates that Septilin™ and its ingredients modulate the secretion of multiple cytokines and NO with varying effects on cytokine and NO secretion due to divergent research methodologies. The reported therapeutic success of these herbal products by natural medicine practitioners and clinicians may be due to their effects on cytokine and NO activity.

Keywords: Septilin™, nitric oxide, immunomodulatory, cytokines

Bangladesh Journal of Medical Science Vol. 18 No. 04 October'19. Page : 675-688
DOI: https://doi.org/10.3329/bjms.v18i4.42869

Introduction

For decades there has been a significant global increase in the use of herbal medicine as curative agents.[1] These formulas modify the actions of the immune system by influencing the regulation of messenger molecules like cytokines, nitric oxide, and other peptides. These herbal medicines are often prescribed for immune-related illnesses.[2] Septilin™, a proprietary polyherbal formulation, has been reported to have immunomodulatory, antibacterial and wound healing properties in a variety of common diseases and soft tissue injury. Septilin™ is a widely used health supplement that is claimed to strengthen immunity and be effective against infections.[3,4]

Herbal medicine has distinctive characteristics that are often not well understood. For example the bio-activities are mostly unknown, with supportive evidence for safety and efficacy very rare.[5] Since these herbal medicines are easily accessible over the counter and at times cheaper than conventional drugs, many patients use self-prescribed herbal medicine without revealing this to their healthcare practitioners. Regulatory studies are crucial to provide data on the toxicology, pharmacodynamics and pharmacokinetics of these herbal medicines.[5] Due to the increased popularity of herbal medicine use, there is a growing need to verify the efficacy of herbal medicine and to establish user friendly databases for easy reference for botanical and medical information

Correspondence to: Dr Mujeeb Hoosen (Coordinator of Unani-Tibb), School of Natural Medicine, Faculty of Community and Health Sciences, the University of the Western Cape, Bellville, South Africa. **Email:** mahoosen@uwc.ac.za ; mujeebh786@gmail.com **Orchid Id:** https://orcid.org/0000-0001-9383-477

on herbal ingredients. Some studies have used such high dosages in *in vitro* and animal models, that when extrapolated has unrealistic relevance to human physiology.[2] Therefore trustworthy information on the quality, safety and efficacy of herbal medicines are of the utmost importance to reduce potential health risks to consumers.[6]

Herbal medicine and immunology

Herbal medicine with anti-inflammatory, antioxidant, antimicrobial, immunomodulatory and analgesic properties are used therapeutically to treat acute infections and inflammatory conditions, particularly in humans.[7] Various herbal medicines have been shown to exert anti-inflammatory and antioxidant effects in human and animal models based on several studies.[1,2,3,4] The bioactive constituents found in herbal medicine, like plant phenols, vitamins, carotenoids, phytoestrogens and terpernoids have been shown to exert both anti-inflammatory and antioxidant activity *in vitro and in vivo*. Modulation of various functions of inflammatory cells can be regulated by herbal constituents which can affect the immune system directly or indirectly[8]. The identification of suitable immunomodulatory preparations or drugs from natural sources to prevent and treat immunological complications are increasing in popularity[1]. Herbal immunomodulators have gained popularity amongst the public and researchers due to the emergence of drug resistant strains of micro-organisms and the high cost of synthetic drugs[9].

Recently there has been a very large growth in the field of herbal medicine leading to its popularity in both developing and developed countries.[10] Immunomodulatory medicinal plants can provide an alternative to conventional medicine for a variety of diseases. Herbal immunomodulators are reported to be valuable for regulating inflammatory pathways. *In vitro* and *in vivo* study observations have shown possible suppression and enhancement of immune functions.[1] A few immunomodualtory studies are available whilst most herbal medicines effects are unknown.[9]

Classification of Immunomodulators

The term immunomodulator refers to medicines that modify the actions of the immune system by influencing the regulation of messenger molecules like cytokines, nitric oxide, hormones, neurotransmitters, and other peptides.[2] Immunomodulators are biological or synthetic substances that may stimulate, suppress or modulate various components of the immune system including both adaptive and innate immunity. Immunoadjuvants specifically enhance the efficacy of vaccines and therefore considered to be the true modulators of the immune response. They act as selectors between cellular and humoral helper T1 (Th1) and helper T2 cells (Th2). Immunoadjuvants are believed to also influence immunoprotective and immunodestructive activity. Immunoglobulin E (IgE) versus IgG type immune responses can be determined by immunoadjuvants, which can be challenging for vaccine manufacturers.[11] Immunostimulants enhance the overall immune response against pathogens via humoral and cellular immune responses by enhancing cytokine production or stimulating B- or T-lymphocytes.[12] Immunostimulants offer prophylactic effects in healthy individuals by potentiating the basic level of the immune response. In those who have an immune impairment, immunostimulants are administered as therapeutic agents for immunity.[11] Immunosuppressants act by suppressing the immune response.[9] These drugs are commonly used to treat different types of organ transplant rejection and autoimmune diseases.[11] From a therapeutic perpective immunomodulation refers to the alteration of the immune response to a desired level which is most beneficial to the host.[9]

Despite the popularity of the use of Septilin™ there are limited *in vitro* and *in vivo* studies available.[1] Several studies report on the immunomodulatory effects of Septilin™ using different testing models, which may lead to variation in results. Many studies lack sufficient details on dosages, duration of exposure (incubation time), and method of administration, which are important factors that contribute to variation in cytokine expression. The lack of these basic parameters in articles on the topic creates doubt with respect to the findings of these studies.

This review paper aims to shed light on the immunomodulatory, nitric oxide (NO) and cytokine activity of Septilin™ and its single ingredients.

Materials and methods

Search strategy

Titles were screened for all hits to the terms "Septilin™ and cytokines," "Septilin™ and nitric oxide," "Septilin™ and immunomodulation," "Septilin™ and immunity," as well as for the single herbs found in Septilin™ as seen below:

Table of search terms for single herbs found in Septilin™

Septilin™ - single herbs	Search terms
Balsamodendron mukul / Commiphora mukul	"*Balsamodendron mukul / Commiphora mukul* and cytokines," "*Balsamodendron mukul / Commiphora mukul* and nitric oxide," "*Balsamodendron mukul / Commiphora mukul* and immunomodulation," "*Balsamodendron mukul / Commiphora mukul* and immunity,"
Maharasnadi quath	"Maharasnadi quath and cytokines," "Maharasnadi quath and nitric oxide," "Maharasnadi quath and immunomodulation" "Maharasnadi quath and immunity,"
Tinospora cordifolia	"*Tinospora cordifolia* and cytokines," "*Tinospora cordifolia* and nitric oxide," "*Tinospora cordifolia* and immunomodulation," "*Tinospora cordifolia* and immunity,"
Rubia cordifolia	"*Rubia cordifolia* and cytokines," "*Rubia cordifolia* and nitric oxide," "*Rubia cordifolia* and immunomodulation," "*Rubia cordifolia* and immunity,"
Emblica officinalis	"*Emblica officinalis* and cytokines," "*Emblica officinalis* and nitric oxide," "*Emblica officinalis* and immunomodulation" "*Emblica officinalis* and immunity,"
Moringa pterygosperma	"*Moringa pterygosperma* and cytokines," "*Moringa pterygosperma* and nitric oxide," "*Moringa pterygosperma* and immunomodulation," "*Moringa pterygosperma* and immunity,"
Glycyrrhiza glabra	"*Glycyrrhiza glabra* and cytokines," "*Glycyrrhiza glabra* and nitric oxide," "*Glycyrrhiza glabra* and immunomodulation" "*Glycyrrhiza glabra* and immunity,"
Shankha / *Shankh bhasma*	"Shankha / *Shankh bhasma* and cytokines," "Shankha / *Shankh bhasma* and nitric oxide," "Shankha / *Shankh bhasma* and immunomodulation," and "Shankha / *Shankh bhasma* and immunity,"

Data collection began on the 4th January 2017 and ended on June 30th, 2017. Databases used included Pubmed, Science Direct and EBSCO, Google Scholar as well as a hand search through journals and bibliographies was also conducted. English language restriction was observed.

Criteria for inclusion

The following parameters had to be met for study inclusion: investigations on Septilin™ as a formulation (liquid or dried form) were accepted. Research on the isolated constituents of Septilin™ (single herbs) was also accepted. The accepted model types included; *in vitro* and *in vivo*, animal and human models. The following were also required; method of preparation of the Septilin™, concentration of the plant preparation and dose/exposure time. Only studies providing statistically significant results with regards to immunomodulatory, cytokine and nitric oxide activity were included. 82 titles and abstracts were reviewed for inclusion criteria. 34 studies were eliminated due to failing inclusion criteria and 48 papers met the criteria.

Ethical clearance: Prior to submission, this study was approved by ethics committee of the University of the Western Cape, Bellville, South Africa.

Results and Discussion

The chemical composition of Septilin™

A study on the evaluation of the phytochemicals present in Septilin™ drops was conducted using thin layer chromatography. This study reported the presence of sugars, tannins, alkaloids, saponins, flavonoids, proteins and glycosides.[13]

The medicinal value of Septilin™

According to the Therapeutic Index of the Himalayan herbal drug company, Septilin™ is a phytopharmaceutical formulation which is recommended for the treatment and management of various infections.[14] It has immunomodulatory action which potentiates the body's immune response. Septilin™ is considered to be a valuable adjuvant in infection management as it builds the defence mechanism of the body and ensures a faster recovery rate when co-prescribed with antibiotics. It has shown to have stimulatory effects on the humoral immunity by increasing antibody forming cells. Septilin™ is an immunomodulator in upper respiratory tract infections, lower respiratory tract infections, allergic disorders of the upper respiratory tract, skin and soft tissue infections, ocular infections, bone and joint infections, urinary tract infections, early recovery in postoperative conditions, the reduction of infection in those who are prone to recurrence, as an adjuvant to anti-infective therapy and for those patients who are resistant to antibiotic therapy.[14]

The herbal ingredients of Septilin™

Septilin™ contains the following herbal ingredients (452mg);

Guggulu / Indian bedellium (*Balsamodendron mukul / Commiphora mukul*) 0.324gm

Maharasnadi quath 130mg

Guduchi / Gulancha tinospora (*Tinospora cordifolia*) 98mg

Manjishtha / Indian madder (*Rubia cordifolia*) 64mg

Amalaki / Indian gooseberry (*Emblica officinalis*) 32mg

Shigru / Horse-radish tree (*Moringa pterygosperma*) 32mg

Yashti-Madhu / Licorice (*Glycyrrhiza glabra*) 12mg

Conch shell calx (Shankha / *Shankh bhasma*) 64mg.[14,15]

The immunological activity of *Commiphora mukul*

Guggulsterone is an anti-inflammatory phytochemical, a plant sterol derived from the oleo gum resin from the Guggul tree known as *Commiphora mukul*, which has been used for centuries to treat various diseases, including atherosclerosis, rheumatism, and obesity, and its biological activity and anti-inflammatory activities were first demonstrated in 1960.[16] Guggulsterone is known to suppress nuclear factor- κβ (NF- κβ) activation induced by inflammatory agents which has led to a considerable amount of interest on this phytochemical. An *in vitro* study on cultured human middle ear epithelial cells focused on the molecular mechanisms underlying the anti-inflammatory activities of guggulsterone in relationship to otitis media. The results showed that guggulsterone (2.5-50μM) has an inhibitory effect on TNFα expression and COX-2 production (LPS induced) which may be mediated through its inhibition of NF-κβ activation.[16] Guggulsterone, the plant sterol, has been extensively used in Indian medicine to treat inflammatory disorders such as hyperlipidemia, obesity, and arthritis for many years. Pregnane plant sterols cis-guggulsterone (E-GS) and trans-guggulsterone (Z-GS) are the active substances in *Commiphora mukul*.[16]

Guggulsterone has been shown to reduce colonic inflammation in two mice colitis models. Guggulsterone-fed mice (15 and 30mg/kg) were shown to be protected against the development of signs and symptoms of colon inflammation (based on macroscopic and microscopic damage scores). The *in vitro* (mechanistic study) that employed CD4+ cells isolated from the intestinal lamina propria of these colitis induced mice demonstrated that guggulsterone can effectively regulate the function of effector T cells by modulating the cell signalling activation pathway caused by CD3/CD28. Guggulsterone attenuated the generation of IL-2, IL-4 and IFNγ as well as T cell proliferation (*in vitro and in vivo*) in the above mentioned study.[16]

Table 1: Studies on the effects of *Commiphora mukul* on cytokine and NO activity.

Preparation	Dose	Model	Cytokine and/or NO effect	Reference
Methanolic extract of the gum resin	13μg/ml	*in vitro,* RAW 264.7 macrophages	Decrease IL-12, IL-1B, TNFα, IFNγ and NO	18
Guggulsterone	2.5-50μM	*in vitro,* cultured human middle ear epithelial cells	Inhibitory effect on TNFα	16
Guggulsterone in100 mM DMSO, diluted in methylcellulose 1%,administrated intraperitoneally, final volume of 200 μl/mouse	15 and 30mg/kg	*in vivo* mice colitis models *in vitro,* isolated CD4+ cells (intestinal lamina propria) from mice colitis model	Decrease IL-2, IL-4, IFNγ and TNFα Decrease IL-2, IL-4 and IFNγ secretion	17
Guggulsterone, isolated phytosterol (Commiphora mukul)	0-25μM	*in vitro,* RAW 264.7 macrophages	Decrease IL-1β, TNFα and NO secretion	19

The immunological activity Maharasnadi quath

Today suitable preparations from natural sources are the centre of attention in the field for preventing immunological complications of various organs. Herbal constituents such as Maharasnadi quath of immunomodulatory drugs have been found to be promising in managing inflammatory disorders. Maharasnadi quath has been reported to possess antibacterial, anti-inflammatory, antiexudative, and immunomodulation properties.[15] Maharasnadi quath is one of the main components of Indian polyherbal formulations for its analgesic, antiphlogistic and anti-pyretic properties. It has also been used for centuries in the treatment of rheumatism and arthritis in traditional Indian medicine.[20] There are several studies on polyherbal immunomodulatory products containing Maharasnadi quath as a constituent. However there are no studies available that focus on Maharasnadi quath on its own.

The immunological activity of *Tinospora cordifolia*

Tinospora cordifolia is a vital component of many Indian polyherbal preparations. The many therapeutic properties exhibited by the extracts of this plant includes its use as a general tonic, an anti-inflammatory, anti-arthritic, antimalarial, aphrodisiac, anti-allergic, antidiabetic, antihepatotoxic, antipyretic and nephro-protective.[21] The immune activity of *Tinospora cordifolia* was assessed in one experimental animal study (*Leishmania donovani* infected BALB/c mice). Mice were fed the dried pure herb (100mg/kg body weight for 15 days daily), *Tinospora cordifolia* displayed stimulated differentiation of T cells into Th1 sub population, and an up-regulation in the release of cytokines such as IFNγ and IL-2 by these cells were also noted.[21] Seven immunomodulatory active compounds belonging to different classes have been isolated and characterized from the extracts of *Tinospora cordifolia*. These groups of compounds may interact synergistically.[22] Increase in ROS is directly related to inflammation via intracellular signalling.[23]

The protective efficacy of the extracts of *Tinospora cordifolia* were assessed in asthma induced mice. Protective efficacy was observed against oxidative stress and inflammation during asthma, based on modulation of glutathione homeostatsis, as well as increased total anti-oxidant capacity of serum and decrease in lipid peroxidation.[23] The extract also showed increased levels of IkB down-regulate translocation of NF-κβ to nucleus resulting in down-regulation of pro-inflammatory genes iNOS, COX-2 and ICAM-1, TNFα, IL-4, NO and IgE leading to prevention of inflammation in the mice models.[23] On the other hand, one study that looked at 8 immune enhancing botanicals, which included *Tinospora cordifolia,* reported that the *in vitro* macrophage activation exhibited by extracts of these plants were due the presence of bacterial lipoproteins and lipopolysaccharides. Trace amounts of bacterial lipoproteins was shown to contribute significantly to the activity exhibited by the melanin fraction derived from these immunomodulatory herbal products. This study pointed out that careful consideration should be taken when monitoring the activity of "purified" plant constituents such as a polysaccharide or other large molecular weight plant constituent in order to rule out the influence of bacterial lipoproteins. Contaminating bacterial lipoproteins present at 10ng/ml (0.10% to 0.01% contaminants) within polysaccharide preparations contributes substantially to the activity of these botanicals when monitored at concentrations of 10 to 100µg/ml, *in vitro*.[24]

Table 2: Studies on the effects of *Tinospora cordifolia* on cytokine and NO activity

Preparation	Dose	Model	Cytokine and/or NO effect	Reference
Isolated polysaccharide, 0.1% yield of the total dry material (powdered) extraction as a puffy solid that dissolved in water	0-100µg/ml	*in vitro,* human lymphocytes	Increased IL-1B, IL-6 IL-12, IL-18, IFNγ and TNF-B No effect on IL-2, IL-4, IL-10, IFN-α, TNFα and NO.	25
Isolated polysaccharide (methanol extract) from dried powdered stem	2.5, 5.0, 12.5, 25.0mg/ kg body weight, intraperitoneally	*in vivo,* mice model	Increase IL-1B, IL-6 and IFNγ Decrease TNFα and IL-10 Increase NO	26
Aqueous extract from fine dried stem powder (60gm) soaked in 600ml of water.	25-625µg/ml	*in vitro,* B16F10 mouse melanoma cells.	Increased IL-6 and NO production	27
Protein quantitation of the dry stem powder(capsule), dried powered plant and aqueous extract (20%w/v)	0-10µg/ml	*in vitro,* RAW 264.7 macrophages	Increase NO secretion	28
Ethyl acetate, water fractions and hot water extract of air dried powered stem	0.1- 2.5µg/ml	*in vitro,* human isolated PMNs	Increase NO secretion	22
Pure dried herb (tablet form) dissolved in distilled water	100 mg/kg (daily) for 15 days	*in vivo,* mice model	Decrease IL-10 and IL-4 Increase IL-2 and IFNγ	21
Powdered stem (20g) ethanol extract (100ml, 50%)	Oral dose of 100 mg/kg from days 15 to 23	*in vivo,* asthma mice model	Decreased IL-4 and TNFα and increased IFNγ Decreased NO secretion	23

The immunological activity of *Rubies cordifolia*

Rubia cordifolia is a perennial, herbaceous climbing plant known for its traditional therapeutic uses in Indian medicine for the treatment of various immune-related diseases[29]. Free radical-induced oxidative stress has been shown to impair the cellular and humoral components of the immune system. Chronic inflammation also contributes to free radical formation.[30] The aqueous extract of *Rubia cordifolia* showed anti-inflammatory activity in rats with carrageenan paw oedema at a dose of 10 and 20ml/kg which was comparable to that of phenylbutazone (100mg/kg).[30] The administration of the ethanol extracts of *Rubia cordifolia* (100mg/kg, body weight orally for 21 days) in cyclophosphamide induced immunosuppressed albino rats significantly increased ($P<0.05$) total white blood and red blood cell count indicating stimulation of the haemopoetic system.[29] An *in vivo* evaluation study was conducted on the antioxidant effects of the ethanol extracts of *Rubia cordifolia* in ethanol-induced immuno-suppressed rats.[30] The treatment group (100mg/kg of herbal extract) showed significantly decreased ($P<0.05$) oxidative stress parameters when compared to the control group.[30] A comparative study was conducted on the anti-inflammatory compounds from *Ventilago madraspatana, Rubia cordifolia* and *Lantana camara*. The NO scavenging activity of these plants were determined on LPS/IFNγ activated murine peritoneal macrophage cultures and Western blot analysis evaluated iNOS and COX-2 expression. The isolated compound, 1-hydroxytectoquinone from *Rubia cordifolia* displayed dose dependent inhibition of NO via suppression of iNOS protein. *Rubia cordifolia* had no effect on macrophage viability [31] Studies on the effect of *Rubia cordifolia* on cytokine and NO activity are extremely rare.

The immunological activity of *Emblica officinalis*

Emblica officinalis belongs to the genus *Emblica* (Family *Euphorbiaceae*), well known in Indian medicine as amla or as the Indian gooseberry. The fruits of this species have been reported to have anti-inflammatory effects. However there are very few *in vitro* or *in vivo* studies to support this.[32] An *in vitro* study on the anti-inflammatory effects of the whole plant ethanolic extracts of *Emblica officinalis* was conducted on Iβ3-1 cystic fibrosis airway bronchial epithelial cells. IL-6 and the PAO1-dependent expression of the neutrophil chemokines, IL-8, GRO-a, GRO-y and of the adhesion molecule ICAM-1 were strongly inhibited by *Emblica officinalis*[32]. Another *in vitro* study looked at the antioxidant and immunomodulating properties of *Emblica officinalis* on chromium (immunosuppressive agent) treated lymphocytes. This study reported that the fruit extracts of *Emblica officinalis* (10µg/ml-1mg/ml) significantly inhibited chromium-induced free radical production in lymphocytes.[32] An *in vitro* study was performed on the synergistic anti-*Staphylococcus aureus* activity of amoxicillin in combination with the stamen extracts of *Emblica officinalis*. This study noted increased efficacy of amoxicillin when combined with *Emblica officinalis*.[34]

Table 3: Studies on the effects of *Emblica officinalis* on cytokine and NO activity

Preparation	Dose	Model	Cytokine affected	Reference
Ethyl acetate extract (polyphenol-rich fraction)	40 or 10mg/kg body weight per day for 100 days	*in vivo,* rat model	Decrease NO secretion	35
Dried fruit ethanolic extracts (9.33% yield)	500µg/ml of the herbal extract for 24 hrs	*in vitro,* IB3-1 cystic fibrosis airway bronchial epithelial cells	Decrease IL-6 and IL-8	32
Extraction of the powder (2g) was carried out with 200 ml of 90% ethanol in Soxhlet apparatus for 5h.	10µg/ml-mg/ml	*in vitro,* isolated splenocytes from Sprague-Dawley rats	Decreased IL-2 and IFNγ	33

The immunological activity of *Moringa pterygosperma*

Moringa oleifera also known *Moringa pterygosperma* belongs to the *Moringaceae* family. This is a popular Indian medicinal herb known commonly as drumstick tree, sajiwan or sajna. *Moringa oleifera* has been reported to have antipyretic, anti-inflammatory, anti-ulcer, antioxidant, renal and hepatoprotective activities amongst others.[36] An *in vivo* animal study on the antioxidant potential of *Moringa oleifera* pods and its isolated saponin was conducted in mice treated with renal carcinogens and with the hydroethanolic extract of the herb (200 and 400mg/kg body weight). The results showed significant ($p<0.001$) suppressed

renal oxidative stress and toxicity in the treatment group when compared to the control group.[36] A study on the anti-inflammatory effects of the aqueous extract of the root of *Moringa oleifera* was conducted on rats using indomethacin (10mg/kg) as the standard drug. Inhibition in the development of oedema was observed in the *Moringa oleifera* treatment group (dose of 750mg/kg).[37] Studies on the effect of *Moringa* species on NO activity are extremely rare.

Table 4: Studies on the effects of *Moringa pterygosperma* on cytokine activity

Preparation	Dose	Model	Cytokine affected	Reference
Coarse powder (500g) of the dried seeds was defatted using petroleum ether (60°C-80°C) and then extracted using 95 % ethanol (2L) in a Soxhlet extractor at 55 C for 6 hours	100mg/kg orally	*in vivo,* guinea pig model	Decrease IL-6, IL-4 and TNFα	37
Extraction of the powder (2g) was carried out with 200 ml of 90% ethanol in Soxhlet apparatus for 5h.	10µg/ml-mg/ml	*in vitro,* isolated splenocytes from Sprague-Dawley rats	Decreased IL-2 and IFNγ	33

The immunological activity of *Glycyrrhiza glabra*

Glycyrrhiza glabra Linn. (Leguminosae), also known as licorice has been used for centruries for medicinal purposes. The roots and stolons of licorice are considered to contain its medicinal active constituents.[39] Numerous triterpenes such as glycyrrhizin and glycyrrhetic acid, alongside flavones, isoflavones, chalcones and numerous related compounds are suggested to contribute to the many therapeutic properties ascribed to *Glycyrrhiza glabra.* These include anti-inflammatory, antioxidant, antimicrobial and immunomodulatory properties.[40] The immune and antioxidant activities of *Glycyrrhiza glabra* were investigated in four groups of growing Kunming mice on a high fat diet. Mice that were fed a high fat diet had reduced levels of antioxidant enzyme activity (SOD, CAT, GSH-Px and total antioxidant capacity). Significant ($P<0.01$) dose-dependent increased antioxidant enzyme activity (SOD, CAT, GSH-Px and total antioxidant capacity) were observed in the treatment group.[41]

Table 5: Studies on the effects of *Glycyrrhiza glabra* on cytokine and NO activity

Preparation	Dose	Model	Cytokine affected	Reference
Dried roots (2 kg) from *Glycyrrhiza glabra* grinded into fine powder in liquid nitrogen, with a mortar and pestle. Afterwards, two consecutive extraction with 4L of boiling distilled water for 2.5 h	50 µM for 24 hours.	*in vitro,* human myelomonocytic leukaemia cells	Decreased IFNγ and NO secretion	42
Dried roots were extracted thrice using acetone in 1:4 proportions at room temperature. The resultant liquid extract obtained after every extraction was mixed and filtered under vacuum at temperature 55ºC	1-40µg/ml	*in vitro,* J774A.1 murine macrophages.	Inhibition of IL-6, IL-1 and NO	43
Glycyrrhizin (93%) was extracted from the dried roots of licorice	10, 20 and 30 mg/kg orally for 20 days once a day	*in vivo,* allergic rhinitis mice model	Decrease IL-4, IL-5, IL-6, and TNFα Increase IL-2, IL-12 and NO secretion	44

The anecdotal claims of Shankh bhasma

Conch refers to the empty shell of *Turbinella rapa/ Xanchus pyrum*, a marine gastropod. The shell is well known in Indian medicine as Shankh bhasma and recognized as a source of calcium salts.[45] Shankh bhasma is obtained by incinerating the conch shell and is indicated for the treatment of dyspepsia, digestive impairment, malabsorption syndrome, enlargement of liver-hepatomegaly, hyperacidity and duodenal ulcer hyperpyrexia. The Conch shell ash is used both internally and externally to treat various illnesses including: ophthalmic and other ocular infections, earache, ulcers, dyspepsia, gonorrhoea, colic, dysentery, jaundice, tympanitis and flatulence.[45] There are no immunomodulatory studies on Shankh bhasma.

Immunomodulatory studies on Septilin™

An animal study was undertaken by Sharma and Ray (1997) to investigate the effect of Septilin™ as an immunomodulator in immunosuppressed mice

(primary and secondary immunization with sheep red blood cells) specifically focused on humoral (antibody titre of IgM and IgG) and cellular immunity (footpad thickness as an indicator of localised delayed hypersensitivity). The oral administration of Septilin™ (500mg/kg) alone or alongside the immuno-suppressive drug, prednisolone (4mg/kg) showed stimulation of both the primary and secondary immune response.[46] The Septilin™ treated group demonstrated a significant increase in cell mediated immunity ($p<0.01$) while the Septilin™ with prednisolone treated group demonstrated an insignificant difference in cell-mediated immunity ($p>0.05$). Results of secondary antibody titre showed significant increases ($p<0.001$) in both IgM and IgG concentrations in the Septilin™ treated group. This suggests that there is a significant potentiating action of Septilin™ on humoral immunity.[46]

Immunomodulatory studies on male albino rats and mice were conducted to assess various arms of the immune response to Septilin™ at varying doses (1-3g/kg).[3] The parameters monitored included that of weight gain, resistance against *E. coli* sepsis, haemogram, phagocytic activity of polymorphonuclear (PMN) cells and reticulo-endothelial system, delayed hypersensitivity to oxazolone and the plaque-forming cell response of splenic lymphocytes to sheep erythrocytes. The results showed that high doses of Septilin™ reduced phagocytic activity of the PMN cells/reticulo-endothelial system whilst both high and low doses increased the percentage and absolute quantity of circulating neutrophils. These results showed stimulation of humoral immunity (B-lymphocyte function was determined by the plaque-forming cell response of rat splenic lymphocytes to sheep erythroytes) and suppression of cellular immunity (delayed hypersensitivity to oxazolone). [3] The humoral stimulatory findings agreed with previous results of Sharma and Ray (1997). This study reported dual effects of Septilin™, where high doses showed suppressive effects on cellular immunity and low doses yielded greater stimulatory effects on humoral immunity.[3]

In a similar animal model study conducted in the Pharmacology Department of the University College of Medical Sciences in Dehli in India, researchers looked at the anti-inflammatory and analgesic effects of Septilin™ (Khanna and Sharma, 2003). Acute, sub-acute and chronic models of inflammation were used to assess inflammatory activity based on four study groups consisting of a control, a Septilin™ treated group (500mg/kg), a prednisolone treatment group (4mg/kg) and a combination treatment group of Septilin™ (500mg/kg) and prednisolone (4mg/kg). The results confirmed consistent anti-inflammatory effects of Septilin™ when compared to the reference drug, prednisolone[4]. The acute and sub-acute models demonstrated that Septilin™ was less effective as an anti-inflammatory than prednisolone. Septilin™ as an adjuvant over a long period proved to be superior to prednisolone. Prednisolone demonstrated potent anti-inflammatory effects but suppressed host immune response over the long term while Septilin™ potentiated non-specific defence mechanisms.[4]

Studies on the effects of Septilin™ on irradiated mice, with a focus on mortality and symptoms of radiation sickness, found significant differences between the treatment and control groups.[47] Pre-treatment of mice to the herbal product four days before exposure to radiation compared to the untreated group resulted in a dose-dependent reduction in animal mortality up to 100mg/kg, while higher dosages led to decreased survival rates. The group pre-treated with Septilin™ were protected against radiation sickness and death due to gastrointestinal complications when compared to the untreated group. This study considered Septilin™ to be an effective radio protective agent at specific doses of 40, 60, 80 and 100mg/kg.[47] Another study noted an increase in phagocytosis, leukocyte counts, percentage of polymorphs in blood, proliferation of bone marrow cells and protection against mylosuppression and leukopenia in cyclophosphamide induced mice.[48]

In a review on the cytokine expression by herbal immunomodulators, Spelman *et al*, suggests that the inconsistencies noted in previous studies on the effects of several herbal products on cytokine expressions (immune activity) is due to a biphasic dose response. The biphasic effect explains that exogenous and endogenous compounds may have opposing, dose-dependent physiological effects. Paradoxical responses in cytokine activity by herbal products in both *in vitro* and *in vivo* studies is not an uncommon finding.[49] Contradictions in results of Septilin™ and its ingredients (single herbs) in similar models in the above mentioned studies may be due to a biphasic dose response. Divergent models, dosages, duration of exposure (incubation time), and method of administration of herbal products are factors which also contribute to variation in cytokine expression.

In one *in vitro* whole blood culture (WBC) study[53] on Septilin™ no effect on IL-6 production was seen in LPS stimulated cells which was contrary to other studies. However up regulation of IL-6 was seen

in unstimulated WBC. In this experimental design blood was first diluted in LPS enriched medium before Septilin™ was added, which differs from the approach used in other *in vitro* studies of this nature. This may have mounted an immune response indicated by IL-6 release before the addition of Septilin™. No effect on IL-6 release in stimulated WBC were seen which suggests that Septilin™ may not be potent enough to serve as a therapeutic intervention during or after infection. However, this does not rule out the possibility that Septilin™ may be effective as a preventative treatment. Septilin™, as with many similar herbal products, is prescribed as a daily health supplement used for preventative treatment. This *in vitro* experimental design only assessed Septilin™ as a therapeutic intervention and not as a preventative treatment. Addition of Septilin™ to unstimulated WBC resulted in a significant higher release of IL-6 across all concentrations (16.125µg/ml-258µg/ml) of Septilin™ when compared to the control. This suggests that Septilin™ has a stimulatory effect on IL-6 production in the absence of a stimulus. Activation of the immune system may be valuable in preventative treatment. On the other hand an overactive immune system is implicated in many pathologies including autoimmunity, chronic inflammatory diseases, systemic vasodilatation, carcinogenesis sepsis and anaphylactic shock. Septilin™ may have triggered an immune response against itself as cells may have recognised Septilin™ as a threat.

Residual bacterial endotoxins are known to be highly potent pro-inflammatory agents, which have been reported in several previous studies.[49] A few molecules may induce cytokine expression. Plant extracts and herbal preparations have been reported to contain endotoxin contaminants, which is an important consideration, especially in patients with chronic inflammatory conditions. IL-6 secretion in the absence of a stimulus has been noted in previous studies on other herbal products. However, very few studies have reported this on Septilin™ using an *invitro* WBC model.

Most studies have reported on the safety of Septilin™ within *in vitro* and *in vivo* studies. In one study[53] on the metabolic activity of unstimulated and stimulated RAW 264.7 cells that were exposed to Septilin™ it was found that Septilin™ induced significant increases in metabolic activity ($P<0.001$) across the concentrations of 63-1000µg/ml in unstimulated RAW 264.7 cells. However, Septilin™ significantly decreased metabolic activity ($P<0.001$) at the highest concentration tested (1000µg/ml) in LPS stimulated RAW 264.7 cells. These findings are contrary to the results in unstimulated RAW 264.7. [53] Septilin™ may be cytotoxic at high doses, however, further investigation is necessary. This highlights the importance of dosage optimisation when prescribing this medication for infectious conditions. Septilin™ is indicated for acute infectious conditions as was mentioned in previous studies. Most patients believe that herbal medicines are safe, which improves compliance but this misconception regarding the safety of herbal medicines may cause patients to misuse them. This is the first study to note decreased metabolic activity at the above mentioned dose (1000 µg/ml) in LPS stimulated RAW 264.7 cells. No toxicological studies have been done on this herbal preparation and the current literature lacks sufficient evidence regarding its safety and toxicity effects, thus highlighting the need for further studies. In this study, Septilin™ had no effect on NO secretion in unstimulated RAW 264.7 Septilin™ had no significant anti-inflammatory effects (NO inhibition) in unstimulated RAW 264.7 cells. This is the second known study[53] which followed a similar model to that of Varma *et al.,* 2011 by assessing anti-inflammatory effects (NO inhibition) of Septilin™ and hence its importance since this herbal preparation is widely used as an anti-inflammatory agent.

Septilin™ showed no effect on NO activity on stimulated RAW 264.7 cells. These findings are contrary to that of Varma *et al* who reported significant inhibition ($P<0.001$) of NO in LPS stimulated macrophages by Septilin™[1]. The findings of Varma *et al* were tested at concentrations of 2.5% and 5% of Septilin™ which are 25 to 50 times higher than the concentrations of Septilin™ (31-1000ug/ml) used in this study. Such high concentrations of the herbal product could be unrealistic and problematic if these concentrations were to be extrapolated for *in vivo* application. Mansour *et al*., reported on the reduction of NO secretion in an *in vivo,* radiation-induced rat model. In this study liquid preparation of Septilin™ was injected intraperitonially (100 mg/kg b.wt.) for five consecutive days[46]. Sharma and Ray(1997) conducted a study using an oral dose of 500mg/kg of Septilin™ in rodents, which is equivalent to an intake of 25-50g in humans. These dosages are too high which is a common problem found in *in vitro* and *in vivo* studies on herbal medicines.

Pre-clinical evaluation of herbal products should begin with *in-vitro* models, by testing cytotoxicity, mutagenicity, and acute and sub-chronic safety. These

safety studies should be followed up by *in-vivo* models at appropriate doses of the herbal products according to internationally accepted standards. Extrapolating doses of the herbal products for *in vivo* application proves to be challenging. Dose-finding studies before formal animal studies are crucial in the preliminary phase to establish the efficacy of herbal products.[46]

In a comparative study on the NO scavenging activities of traditional polyherbal drugs, Septilin™ was tested at the same concentrations (31-1000ug/ml). It was reported that Septilin™ inhibited the production of NO in a dose dependent manner up to 125 µg/ml (69.66%), which was then followed by a gradual increase of NO production at the higher doses.[46] The results of Jagetia *et al* showed far less efficacy of NO inhibition by Septilin™ to that of Varma *et al*. This could be due to the differences in the concentrations tested.

Bhattacharya and Deepa, 2011, conducted three clinical trials on the efficacy of Septilin™ (tablets and syrup) on children and infants with upper respiratory tract infections (RTIs).[50] The first clinical trial assessed hemoglobin, total and differential white cell counts, and throat swab for culture and sensitivity in patients who had been diagnosed with upper respiratory tract infections. Younger patients were given one tablet twice a day while older children were given one tablet three times a day for 12 weeks. Results showed that 80% of patients showed no recurrence of infection and 20% showed a poor response in relation to the disappearance of previous documented signs and symptoms.[50]

The second clinical trial assessed routine blood counts, throat cultures, and chest x-rays in infants and children (aged 9 months to 5 years old) with upper RTIs. Infants were given one teaspoon of Septilin™ while children were given two teaspoons a day for 12-16 weeks. Significant decreases in symptoms were noted in most patients, associated with a good appetite with concomitant weight gain in all patients.[50] The third clinical trial included children (aged 6 months to 5 years) with repeated attacks of acute tonsillopharyngitis. Dosages of half a tablet to one tablet 2-4 times per day were administered according to age for 6-7 weeks after antibiotic therapy (7 days). These patients were followed up after 6-9 months and results showed a reduction of recurrent infections in 80% of patients compared to previous history of recurrent infection without the use of Septilin™ as an intervention.[50] This clinical trial does not clearly explain the impact that antibiotic therapy could have on the results reported. This study also did not include a control group. However, the results could be valuable for future follow up clinical trials.

Shetty *et al.,* conducted a double blinded randomized controlled clinical trial on 96 healthy patients with chronic periodontitis following scaling and root planning. Group 1 was administered Septilin™ tablets twice daily for two weeks while group 2 was given probiotic tablets twice daily for two weeks, and group 3 was not given any intervention. Gingival index, gingival bleeding index, pocket depth and IL-6 levels in gingival crevicular fluid and saliva were assessed. Results showed statistically significant reduction ($p<0.001$) in clinical parameters and IL-6 levels in group one (Septilin™) when compared to group 2 and 3.[51]

Divergent models produce varying results due to variations in dosages, duration of exposure (incubation time) and the method of administration. These factors contribute to the variation in cytokine expression[51-53].

Contradictions in studies could be attributed to variations that exist in different batches of herbal products. The chemical composition of herbs differ depending on various factors which includes the botanical species, the anatomical part of the plant used, storage methods, sun, humidity, type of soil, time of harvest, and geographic location, amongst others. Batch to batch variations can be found within the same manufacturing company, which can result in significant variations in pharmacological activities influenced by pharmacodynamics and/or pharmacokinetic factors[54]. Several *in vitro* and *in vivo* studies on the individual ingredients of Septilin™ were conducted on various models with varying effects on NO activity. *Commiphora mukul*, *Rubia cordifolia*, *Emblica officinalis* and *Moringa pterygosperma* has shown decreased NO secretion in previous studies[55,56]. Most studies of *Tinospora cordifolia* reported increased NO production. *Glycyrrhiza glabra* studies reported either increased NO production or decreased NO production. Many studies on the molecular modes of activities of individual herbs have little relevance to its practical application as most herbal medicines are formulations (combinations of several herbs)[2]. These formulas introduce extremely complex mixtures of compounds that may act synergistically to produce therapeutic effects. The overall effect of the formulation may be different to the sum of the individual effects of each herb which makes the study on herbal medicines extremely challenging due to its complex chemistry. A study by Raveendran Nair and Chanda, on the efficacy of medicinal plants

Table 6: Studies on the effects of Septilin™ on cytokine and NO activity

Preparation	Dose	Model	Cytokine affected	Reference
100grams of Septilin™ powder in 50% ethanol (1 l) at 50 to 60°C in a Soxhlet apparatus for 120 h. The cooled hydroalcoholic liquid extracts were concentrated by evaporating its liquid contents	0-1000µg/ml	*in vitro,* sodium nitroprusside treated	Inhibition of NO secretion	47
Tablet form as commercially available	1 tablet twice a day for two weeks	*in vivo,* human chronic periodontal disease model	Reduction in TNFα	50
Liquid preparation of Septilin™ was received from Himalaya Drug Company,	2.5-5% Septilin™ incubation for 24hrs	*in vitro,* RAW 264.7 macrophages	Decrease IL-6, IL-8, TNFα and NO secretion	1
Liquid preparation of Septilin™ was received from the Himalaya Drug Company	Septilin™ injected intraperitoneally (100 mg/kg b.wt.) for five consecutive days	*in vivo,* radiation induced rat model	Decreased TNFα and NO secretion	52
Tablet form as commercially available	Septilin™ tablets twice daily for 2 weeks	*in vivo*, human double blinded randomized controlled clinical trial	Decrease IL-6	51
Liquid preparation - the tablet was crushed by means of a sonicator then diluted in 35ml of distilled water. The sample was incubated on a shaker for 1 hour at ambient temperature. The sample was then centrifuged at 40000 rpm for 10mins. After that it was sterile filtered using 0.50nm sterilized filters and stored in 1ml aliquots at -80 ºC.	Extracts of the Septilin™ were diluted in normal medium to give a concentration range from 0-2000µg/ml. Three replicates of the diluted extracts were transferred to a 96 well plate at 100µl/well. This was followed by the addition of 100µl/well of diluted blood in LPS stimulated medium to each of the diluted extract replicates. Overnight incubation at 37ºC and 5% CO_2 .	*in vitro,* whole blood cultures (WBC)	Decrease IL-6 in unstimulated WBC No effect in LPS stimulated cells Decrease IL-10 in LPS stimulated WBC No effect in unstimulated WBC Inconclusive results on interferon gamma in LPS stimulated and unstimulated WBC	53
Liquid preparation - the tablet was crushed by means of a sonicator then diluted in 35ml of distilled water. The sample was incubated on a shaker for 1 hour at ambient temperature. The sample was then centrifuged at 40000 rpm for 10mins. After that it was sterile filtered using 0.50nm sterilized filters and stored in 1ml aliquots at -80 degrees Celsius.	Extracts of the Septilin™ were diluted in normal medium to give a concentration range from 0-2000µg/ml. At confluence half the plate received normal medium (unstimulated cultures), while the other half plate received LPS containing medium (stimulated cultures) at 100µl/well. This was followed by the addition of a further 100µl/well of the medium containing various extract concentrations. Final concentration ranges of the extracts were between 0-1000µg/ml. After overnight incubation at 37ºC and 5% CO_2	*in vitro,* RAW 264.7 macrophages	No effect on NO secretion in unstimulated and LPS stimulated RAW 264.7 macrophages No effect on IL-6 secretion in unstimulated and LPS stimulated RAW 264.7 macrophages	53

against pathogenic bacterial strains reported greater effects by the ethanol extract of the samples than the aqueous extract which is another contributing factor to variations that exist in previously mentioned studies[57-59].

Future *in vitro* studies on Septilin™ should be standardised and regulated in terms of the dosages used. Concentrations used *in vitro* should be calculated based on anecdotal dosages with the objective of extrapolating *in vitro* data for *in vivo* application. This could contribute to an efficient system of researching this product which could be cost effective and less time consuming. Future studies should consider that different batches of the product may produce varying results due to the complex chemistry. Also, there are vast pharmacological differences between aqueous and ethanol extracts of herbal products therefore studies should include both forms. Divergent *in vitro* cell models produce varying results regarding cytokine and NO activity by herbal products.

Septilin™ has shown some immunomodulatory effects in several *in vitro* and a few *in vivo* (animal and human) models, however further studies are needed to confirm these findings due to the variations in methodologies used in these studies.

Acknowledgement:

Competing, financial interests:

The author declares no competing financial interests.

Conflict of Interest:

The author declares that he has no conflict of interest.

Author's contribution:

Data gathering and idea owner of this study: Mujeeb Hoosen

Study design: Mujeeb Hoosen

Data gathering: Mujeeb Hoosen

Writing and submitting manuscript: Mujeeb Hoosen

Editing and approval of final draft: Mujeeb Hoosen

References:

1. Varma, R., Ashok, G., Vidyashankar, S., Patki, P., Nandakumar, K. S. (2011) Anti-inflammatory properties of Septilin in lipopolysaccharide activated monocytes and macrophage. *Journal of immunopharmacology and immunotoxicology*, **33**(1): 55-63. https://doi.org/10.3109/08923971003739236
2. Burns, J. J., Zhao, L., Taylor, E. W., Spelman, K. (2010) *The influence of traditional herbal formulas on cytokine activity. Toxicology*, **278**: 140-159. https://doi.org/10.1016/j.tox.2009.09.020
3. Daswani, B. R., Yegnanarayan, R. (2002) *Immunomodulatory activity of Septilin, a polyherbal preparation. Phytotherapy research*, **16**: 162-165. https://doi.org/10.1002/ptr.996
4. Khanna, N., Sharma S. B. (2003) Anti-inflammatory and analgesic effect of herbal preparation: Septilin. *Indian journal of medical sciences*, **55**: 195-202.
5. Adewunmi, C. O., Ojewole, J. A. O. (2004) Safety of traditional medicines, complementary and alternative medicines in Africa. *African journal of traditional, complementary and alternative medicines*, **1** (1): 1-3. https://doi.org/10.4314/ajtcam.v1i1.31090
6. Ndhlala, A. R., Ncube, B., Van Stade, J. (2012) Ensuring quality in herbal medicines: toxic phthalates in plastic-packaged commercial herbal products. *South african journal of botany*, **82**: 60-66. https://doi.org/10.1016/j.sajb.2012.07.004
7. Hart, B. L. (2005) The evolution of herbal medicine: behavioral perspectives. *Animal behavior*, **70**: 975-989. https://doi.org/10.1016/j.anbehav.2005.03.005
8. Iwalewa, E. O., McGaw, L. J. Naidoo, V., Eloff, J. N. (2007) Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African journal of biotechnology*, **6** (25): 2868-2885. https://doi.org/10.5897/AJB2007.000-2457
9. Mahima, Rahal. A., Deb, R., Latheef, S. K., Abdul Samad, H., Tiwari, R., Verma, A. K., Dharma, A. K., Dharma, K. (2012) Immunomodulatory and therapeutic potentials of herbal, traditional/ indigenous and ethnoveterinary medicines. *Pakistan journal of biological sciences*, **15** (16): 754-74. https://doi.org/10.3923/pjbs.2012.754.774
10. Ganju, L., Karan, D., Chanda, S., Srivastava, K. K., Sawhney, R. C., Selvamurthy, W. (2003) Immunomodulatory effects of agents of plant origin. *Journal of biomedicine & pharmacotherapy*, **57**: 296-300. https://doi.org/10.1016/S0753-3322(03)00095-7
11. Kumar, D., Arya, V., Kaur, R. Z., Bhat, A., Gupta V. K., Kumar, V. (2012) A review of immunomodulators in the Indian traditional health care system. *Journal of microbiology, immunology and infection*, **45**: 165-184. https://doi.org/10.1016/j.jmii.2011.09.030
12. Tan, B. K. H., Vanitha, J. (2004) Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. *Current Medicinal Chemistry*, **11**: 1423-1430. https://doi.org/10.2174/092986704336516113.

Pushpa, K. J., Azizahmed Arbar, Savita, S. B. (2014) Phytochemical screening of Septilin drops- a polyherbal formulation. *Journal of ayurveda and holistic medicine*, **1**(2): 40-46.

14. Manal, M. (2014) Therapeutic index. Bangalore. Himalaya global holdings ltd.
15. Deore, G. D., Gurav, A. N., Patil, R., Shete, A. R., Naiktari, R. S., Inamdar, S. P. (2014) Herbal anti-inflammatory immunomodulators as host modulators in chronic periodontitis patients: a randomized, double-blind, placebo-controlled, clinical trial. *Journal of periodontal and implant science*, **44**: 71-78. https://doi.org/10.5051/jpis.2014.44.2.71
16. Song, J. J., Kwon, S. K., Chang, G. C., Park, S. W., Chae, S. W. (2010) Guggulsterone suppresses LPS induced inflammation of human middle ear epithelial cells (HMEEC). *International journal of pediatric otorhinolaryngology*, **74**: 1384-1387. https://doi.org/10.1016/j.ijporl.2010.09.012
17. Mencarelli, A., Renga, A., Palladin, G., Distrutti, E., Fiorucci, S. (2009) The plant sterol guggulsterone attenuates inflammation and immune dysfunction in murine models of inflammatory bowel disease. *Biochemical pharmacology*, **78**: 1214-1223. https://doi.org/10.1016/j.bcp.2009.06.026
18. Matsuda, H., Morikawa, T., Ando, S., Oominami, H., Murakami, T., Kimura, I., Yoshikawa, M. (2004) Absolute stereostructures of polypodane- and octanordammarane-type triterpenes with nitric oxide production inhibitory activity from guggul-gum resins. *Bioorg med chem*, **12**: 3037-3046. https://doi.org/10.1016/j.bmc.2004.03.020
19. Zhang, J. H., Shangguan, Z. S., Chen, C., Zhang, H. J., Lin, Y. (2016) Anti-inflammatory effects of guggulsterone on murine macrophage by inhibiting LPS induced inflammatory cytokines in NF-κB signaling pathway. *Drug design development therapy*, **10**: 1829-1835. https://doi.org/10.2147/DDDT.S104602
20. Bhalerao, A. R., Desai, S. K., Serathia, B. R., Vartak, K. M., Doshi, G. M. (2011) Antiarthritic studies on Nyctanthes arbor tristis and Maharasnadi ghan. *Scholars research library*, **3**(4): 101-110.
21. Sachdeva, H., Sehgal, R., Kaur, S. (2014) Tinospora cordifolia as a protective and immunomodulatory agent in combination with cisplatin against murine visceral leishmaniasis. *Experimental parasitology*, **137**: 53-65. https://doi.org/10.1016/j.exppara.2013.12.006
22. Sharma, U., Bala, M., Kumar, N., Singh, B., Munshib, R. K., Bhalerao, S. (2012) Immunomodulatory active compounds from Tinospora cordifolia. *Journal of ethnopharmacology*, **141**: 918-926. https://doi.org/10.1016/j.jep.2012.03.027
23. Tiwaria, M., Dwivedi, U. N., Kakkar, P. (2014) Tinospora cordifolia extract modulates COX-2, iNOS, ICAM-1, pro-inflammatory cytokines and redox status in murine model of asthma. *Journal of Ethnopharmacology*, **153**: 326-337. https://doi.org/10.1016/j.jep.2014.01.031
24. Pugh, N. D., Tamta, H., Balachandran, P., Wu, X., Howell, J. L., Dayan, F. E., Pasco, D. S. David, S. (2008) The majority of in vitro macrophage activation exhibited by extracts of some immune enhancing botanicals is due to bacterial lipoproteins and lipopolysaccharides. *International immunopharmacology*, **8**(7): 1023-1032. https://doi.org/10.1016/j.intimp.2008.03.007
25. Raveendran Nair, P. K., Rodriguez, S., Ramachandran, R., Alamo, A., Melnick, S. J., Escalon, E., Garcia, Jr. P. I., Wnuk, S. F., Ramachandran, C. (2004) Immune stimulating properties of a novel polysaccharide from the medicinal plant Tinospora cordifolia. *International immunopharmacology*, **4**: 1645-1659. https://doi.org/10.1016/j.intimp.2004.07.024
26. Desai, V. R., Ramkrishnan, R., Chintalwar, G. J., Sainis, K. B. (2007) G1-4A, an immunomodulatory polysaccharide from Tinospora cordifolia, modulates macrophage responses and protects mice against lipopolysaccharide induced endotoxic shock. *International immunopharmacology*, **7**: 1375-1386. https://doi.org/10.1016/j.intimp.2007.06.004
27. Upadhyaya, R., Pandey R. P., Sharma V., Verma Anita K. (2011) Assessment of the multifaceted immunomodulatory potential of the aqueous extract of Tinospora cordifolia. *Research journal of chemical sciences*, **1**(6): 71-79.
28. Aranha, I., Clement, F., Venkatesh, Y. P. (2012) Immunostimulatory properties of the major protein from the stem of the ayurvedic medicinal herb, guduchi (Tinospora cordifolia). *Journal of Ethnopharmacology*, **139**: 366-372. https://doi.org/10.1016/j.jep.2011.11.013
29. Kannan, M., Ranjit Singh, A. J. A., Narayanan, M. (2009) Phytochemistry and ethanopharmacological studies on Rubia cordifolia Linn. (Rubiaceae). *Ethnobotanical leaflets*, **13**: 338-342.
30. Joharapurkar, A. A., Zambad, S. P., Wanjari, M. M., Uma, S. N. (2003) The in vivo evaluation of antioxidant activity of alcoholic extract of Rubia cordifolia Linn. and its influence on ethanol-induced immunosuppression. *Indian journal of pharmacology*, **35**: 232-236.
31. Ghosh, S., Das, S. M., Patra, A., Hazra, B. (2010) Anti-inflammatory and anticancer compounds isolated from Ventilago madraspatana Gaertn., Rubia cordifolia Linn. and Lantana camara Linn. *Journal of pharmceutical pharmacology*, **62** (9): 1158-66. https://doi.org/10.1111/j.2042-7158.2010.01151.x
32. Nicolis, E., Lampronti, I., Dechecchi, M. C., Borgatti, M., Tamanini, A., Bianchi, N., Bezzerri, V., Mancini, I., Giri, M. G., Rizzotti, P., Gambari, R., Cabrini, G. (2008) Pyrogallol, an active compound from the medicinal plant Emblica officinalis, regulates expression of pro-inflammatory genes in bronchial epithelial cells. *International immunopharmacology*, **8**: 1672-1680. https://doi.org/10.1016/j.intimp.2008.08.001
33. Sai Ram, M., Neetu, D., Yogesh, B., Anju, B., Dipti, P., Pauline, T., Sharma, S. K., Sarada, S. K. S., Ilavazhagan, G., Kumar, D., Selvamurthy, W. (2002) Cyto-protective and immunomodulating properties of amla (Emblica officinalis) on lymphocytes: an in-vitro study. *Journal of ethnopharmacology*, **8**: 5-10. https://doi.org/10.1016/S0378-8741(01)00421-4
34. Mandal, S., Mandal, M. D., Pal, N. K., Saha, K. (2010) Synergistic anti-Staphylococcus aureus activity of amoxicillin in combination with Emblica officinalis and Nymphae odorata extracts. *Asian pacific journal of tropical medicine*, 711-714.

https://doi.org/10.1016/S1995-7645(10)60171-X
35. Yokozawa, T., Kim, H. Y., Kim, H. J., Okubo, T., Chu, D., Juneja, L. R. Raj, L. Amla (Emblica officinalis Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. *British journal of nutrition* 2007;**97**: 1187-1195. https://doi.org/10.1017/S0007114507691971
36. Sharma, V., Paliwal, R. (2014) Potential chemoprevention of 7,12 dimethylbenz[a] anthracene induced renal carcinogenesis by Moringa oleifera pods and its isolated saponin. *Indian journal of clinical biochemistry*, **29** (2): 202-209. https://doi.org/10.1007/s12291-013-0335-y
37 Ndiaye, M., Dieye, A. M., Mariko, F., Tall, A., Sall Diallo, A., Faye, B. (2002) Contribution to the study of the anti-inflammatory activity of Moringa oleifera (moringaceae). *Dakar medicine*, **47**(2): 210-212.
38. Mahajan, S. G., Banerjee, A., Chauhan, B. F., Padh, H., Nivsarkar, M., Mehta, A. A. (2009) Inhibitory effect of n-butanol fraction of Moringa oleifera Lam. seeds on ovalbumin-Induced airway inflammation in a guinea pig model of asthma. *International journal of toxicology*, **28**(6): 519-527. https://doi.org/10.1177/1091581809345165
39. Asha, M. K., Debraj, D., Prashanth, D., Edwin, J. R., Srikanth, H. S., Muruganantham, N., Dethe, S. M., Anirban, B., Jaya, B., Deepak, M., Agarwal, A. (2013) In vitro anti-Helicobacter pylori activity of a flavonoid rich extract of Glycyrrhiza glabra and its probable mechanisms of action. *Journal of ethnopharmacology*, **145**: 581-586. https://doi.org/10.1016/j.jep.2012.11.033
40. Siracusa, L., Saija, A., Cristani, M., Cimino, F., D'Arrigo, M., Trombetta, D., Rao , F., Ruberto, G. (2011) Phytocomplexes from liquorice (Glycyrrhiza glabra L.) leave-chemical characterization and evaluation of their antioxidant, anti-genotoxic and anti-inflammatory activity. *Fitoterapia*, **82**: 546-556. https://doi.org/10.1016/j.fitote.2011.01.009
41. Hong, Y. K., Wu, H. T., Ma, T., Liu, W. J., He, X. J. (2009) Effects of Glycyrrhiza glabra polysaccharides on immune and antioxidant activities in high-fat mice. *International journal of biological macromolecules*, **45**: 61-64. https://doi.org/10.1016/j.ijbiomac.2009.04.001
42. Franceschelli, S., Pesce, M., Vinciguerra, I., Ferrone, A., Riccioni, G., Patruno, A., Grilli, A., Felaco, M., Speranza, L. (2011) Licocalchone-C extracted from Glycyrrhiza Glabra inhibits lipopolysaccharide-interferon-g inflammation by improving antioxidant conditions and regulating inducible nitric oxide synthase expression. *Molecules*, **16**: 5720-5734. https://doi.org/10.3390/molecules16075720
43. Thiyagarajan, P., Chandrasekaran, C. V., Deepak, H. B., Agarwal, Amit. (2011) Modulation of lipopolysaccharide-induced pro-inflammatory mediators by an extract of Glycyrrhiza glabra and its phytoconstituents. *Inflammopharmacol*, **19**: 235-241. https://doi.org/10.1007/s10787-011-0080-x
44. Li, X. L., Zhou, A. G. (2011) Evaluation of the immunity activity of Glycyrrhizin in AR mice. *Molecules*, **17**: 716-727. https://doi.org/10.3390/molecules17010716
45. Gopal, R., Vijayakumaran, M., Venkatesan, R., Kathiroli, S. (2008) Marine organisms in Indian medicine and their future prospects. *Natural product radiance*, **7** (2): 139-145.
46. Sharma, S. B., Ray, S. (1997) Effect of herbal preparation on immune response of immunosuppressed mice. *Indian journal of physiology pharmacology*, **41** (3): 293-296.
47. Jagetia, G. C., Balinga, M. S. (2004) Polyherbal extract of Septilin protects mice against whole body lethal dose of gamma radiation. *Phytotherapy research*, **18**: 619-623. https://doi.org/10.1002/ptr.1508
48. Kumar, P. V., Kuttan, R., Kuttan, G. (1997) Immunopotentiating activity of Septilin. *Indian journal experimental biology*, **35**: 1319-1323.
49. Spelman, K., Burns, J. J., Nichols, D., Winters, N., Ottersberg, S., Tenborg, M. (2006) Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. *Alternative medicine review*, **11**(2): 128-150.
50. Bhattacharya, S., Deepa, H. S. (2011) Current concepts in the management of recurrent respiratory tract infections. *Perinatology*, **12**(2): 66-71.
51. Shetty, S., Bose, A., Thoudam, B. (2015) A comparative evaluation of clinical efficacy and salivary and gingival crevicular fluid interleukin - 6 levels with herbal and probiotic host modulation therapy in chronic periodontal disease. *International journal of dentistry and oral health*, **1** (2). https://doi.org/10.16966/2378-7090.111
52. Mansour, H. B., Rifaat Ismael, N. E., Hafez, H. F. (2014) Ameliorative effect of Septilin, an ayurvedic preparation against γ-irradiation-induced oxidative stress and tissue injury in rats. *Indian journal of biochemistry & biophysics*, **51**: 135-141.
53. Hoosen, M., Pool, E. J. (2017) The use of whole blood cell cultures as a model for assessing the effects of SeptilinTM on the immune system. *Western Cape: UWC. (M.Sc. Immunology)* **3**: 68-104.
54. Gericke, N. (2011) Muthi to medicine. *South African journal of botany*, **77**: 850-856. https://doi.org/10.1016/j.sajb.2011.08.007
55. Mujeeb Hoosen (2019). The Effects of Aspalathus linearis (Rooibos Tea) on Nitric Oxide (NO) and Cytokine Activity. International Journal of Human and Health Sciences (IJHHS). 03(03): 150-157. DOI: http://dx.doi.org/10.31344/ijhhs.v3i3.93
56. Mujeeb Hoosen, Edmund John Pool (2019). An In Vitro Study to elucidate the Effects of Artemisia afra, Aspalathus linearis (rooibos) and SeptilinTM on Immune Pathways. International Journal of Human and Health Sciences (IJHHS). 03(03): 134-145. DOI: http://dx.doi.org/10.31344/ijhhs.v3i3.91
57. Raveendran Nair, P. K., Rodriguez, S., Ramachandran, R., Alamo, A., Melnick, S. J., Escalon, E., Garcia, Jr. P. I., Wnuk, S. F., Ramachandran, C.
58. Varma, R., Ashok, G., Vidyashankar, S., Patki, P., Nandakumar, K. S. (2011) Anti-inflammatory properties of Septilin in lipopolysaccharide activated monocytes and macrophage. *Journal of immunopharmacology and immunotoxicology,* 33(1): 55-63.
59. Burns, J. J., Zhao, L., Taylor, E. W., Spelman, K. (2010) The influence of traditional herbal formulas on cytokine activity. *Toxicology,* 278: 140-159.