Tino Immune®

STUDY INVESTIGATORS

Dr Poorna Prasad S & Dr Shuba Rani M

STUDY CENTRE

Sri Venkateshwara Hospital, Kuvempu Nagara, BTM 2nd Stage, BTM layout, Bangalore – 560076, Karnataka , India.

STUDY PERIOD: FEB 2020 TO AUG 2020

STUDY SPONSOR:

BOTANIC HEALTHCARE GROUP OF COMPANIES -INDIA/USA/NZ

BOTANIC HEALTHCARE PVT-LTD - INDIA
TSIIC IDA, Plot – 16/1/12 & 13
Nacharam, Uppal, Malkajgiri
Hyderabad, Telanagana – 500076, INDIA
Tel: + 04035027800 | +91 709 300 4700 | Cell: +91 709 3900 269
www.botanichealthcare.net
info@botanichealthcare.net

BOTANIC HEALTHCARE (NZ) LTD- NZ
PO Box 8522 Christchurch 8440 New Zealand
Ph: +64 21 155 1894
nz@botanichealthcare.net

BOTANIC HEALTHCARE LLC USA
55 Crestview Drive Clinton New Jersey 08809 USA
Cell: +1 908-399-5452 | Tel: + 1 908-617-9152
usa@botanichealthcare.net

BOTANIC HEALTHCARE SPECIALISES IN

Health & Nutrition | Essential Oils | Organic Extracts | Natural Colors | Personal Care

Tino Immune®

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL STUDY TO EVALUATE COMPARATIVE CLINICAL EFFICACY AND SAFETY OF

TINOIMMUNE

IN IMMUNE SUPPORT OF ADULT SUBJECTS

www.botanichealthcare.net

INTRODUCTION

Immunity is the capability of host to resist harmful microorganisms from entering it. Immunity involves both specific and nonspecific components. The nonspecific components act as barriers or eliminators of a wide range of pathogens irrespective of their antigenic make-up. Other components of the immune system adapt themselves to each new disease encountered and can generate pathogen-specific immunity.

An immune system contains innate and adaptive components. The innate system in mammalians, for example, is composed of primitive bone marrow cells that are programmed to recognize foreign substances and to react. The adaptive system is composed of more advanced lymphatic cells that are programmed to recognize self-substances and not to react. The reaction to foreign substances is etymologically described as inflammation, meaning to set on fire. The non-reaction to self-substances is described as immunity, meaning to exempt or as immunotolerance. These two components of the immune system create a dynamic biological environment where "health" can be seen as a physical state where the self is immunologically spared, and what is foreign is inflammatorily and immunologically eliminated. "Disease" can arise when what is foreign cannot be eliminated or what is self is not spared. Hence immunomodulatory therapy along with standard of care finds a unique place in treatment of many infections to boost immunity. [1, 2]

An intact immune response includes contributions from many subsets of leukocytes. The common lymphoid progenitor differentiates further into the four major populations of mature lymphocytes: B cells, T cells, natural killer (NK) cells, and NK-T cells. Cells of the granulocyte lineage that play prominent immune functions include neutrophils, monocytes, macrophages, eosinophils, basophils, and mast cells. Neutrophils produce large quantities of reactive oxygen species that are cytotoxic to bacterial pathogens. They also produce enzymes that appear to participate in tissue remodelling and repair following injury. Like neutrophils, monocytes and macrophages are also highly phagocytic for microbes and particles that have been marked for clearance by binding Ig and/or complement. They are mobilized shortly after the recruitment of neutrophils and they persist for long periods at sites of chronic inflammation and infection. In addition to participating in acute inflammatory responses, they are prominent in granulomatous processes throughout the body. They use production of nitric oxide as a major mechanism for killing microbial pathogens, and also produce large amounts of cytokines such as IL-12 and interferon (IFN)-γ giving them a regulatory role in adaptive immune responses. Classically activated macrophages produce large amounts of IFN-γ, IL-6, IL-12, and TNF and express potent pro-inflammatory and anti-bacterial activities. Phagocytic cells of the monocyte/macrophage lineage also play key roles in the adaptive immune response by taking up microbial antigens, processing them by proteolysis to peptide fragments, and presenting them in forms that can activate T responses. Thus, during infection host immune system is activated and various immune cells increase qualitatively and quantitatively. [3]

The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics. Antibiotics in a modern therapeutic system have been tremendously used in controlling the infectious diseases. Due to the extensive use of antibiotics, there has been an emergence of multidrug-resistant strains of many pathogens that are posing serious challenges to the clinicians. There is a dire need to find suitable replacement or support to the currently used antibiotics. Moreover, some antibacterial and antifungal agents exhibit serious untoward effects in the treated persons.7 Currently available therapeutic options for enhancing the immune system are very few with questionable efficacy. Hence quest for novel immune booster drug continues.

Tinospora cordifolia (Guduchi/ Giloy) extract has been used since many years for treating various infections as immune booster in traditional medicine. It is having pharmacological functions and medicinal values due to its several constituents such as terpenes, glycosides, alkaloids, steroids and flavonoids. Thus, it has been rightly mentioned in old texts as "Amrita". The medicinal applications of T. cordifolia in countering various disorders and usages include anti-microbial, immune booster, anti-oxidant, anti-hyperglycaemic, antihyperlipidemic, hepatoprotective, cardiovascular protective, neuroprotective, anti-anxiety, adaptogenic agent, analgesic, anti-inflammatory, antipyretic, anti-ulcer, and anti-cancer agent. The plant is also a source of micronutrients viz. copper, calcium, phosphorus, iron, zinc and manganese. [4, 5] Tinospora cordifolia has been known for immune stimulant property from many years which is beneficial in treating various bacterial and viral infectious conditions as an immune support drug.

BACKGROUND INFORMATION

TINOSPORA CORDIFOLIA

Tinospora cordifolia commonly named as “Guduchi” in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatic, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant. Recently, the plant is of great interest to researchers across the globe because of its medicinal properties like antimicrobial, immune booster, anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. [5]

The aqueous extract of T. cordifolia has been shown to protect against Escherichia coli and Staphylococcus aureus infections. T. cordifolia enhances the phagocytic and intracellular bactericidal activities of macrophages and neutrophils against E. coli-induced peritonitis. It has been shown to control the drug-resistance Mycobacterium tuberculosis infection by inducing Th1 immune responses.

The stem of T. cordifolia has been used as a constituent in many Ayurvedic and Unani preparations for the treatment of general debility, dyspepsia, fever, and urinary diseases. The stem is used as diuretic, stimulates bile secretion, and cures jaundice. The extract of the stem is also useful in skin diseases and in combination with other drugs act as an antidote to snakebite. The dry bark of T. cordifolia has been shown to possess antipyretic, antiallergic, anti-inflammatory, and antileprotic properties. Moreover, T. cordifolia has been shown to be effective against diabetes mellitus. [6]

IMMUNOMODULATORY PROPERTY OF TINOSPORA CORDIFOLIA

T. cordifolia and its constituents have been shown to possess the immune-stimulating properties. T. cordifolia and its constituent α-D-glucan stimulate NK cells, B cells, and T cells with simultaneous production of various immune-stimulatory cytokines. A polysaccharide from T. cordifolia, G1-4A, has been shown to inhibit the intracellular growth of Mycobacterium tuberculosis through toll-like receptor 4- (TLR4-) dependent signalling. Salmonella spp. use multiple strategies to evade the immune system to establish itself in the host. Macrophages are an important part of the innate immunity and play a critical role in defending the host against the microbial invasion. Classically activated M1 are characterized by the increased secretion of cytokines like TNF-α, IL-1β, IFN-γ, IL-12, and IL-6 and show strong microbicidal activities, whereas alternatively activated macrophages or M2 are characterized by increased secretion of IL-4, IL-10, and TGF-β and are considered poorly microbicidal. [6]

Active compounds 11-hydroxymustakone, N-methyl-2-pyrrolidone, N-formylannonain, cordifolioside A, magnoflorine, tinocordiside and syringing has been reported to have potential immunomodulatory and cytotoxic effects.

They have been reported to function by boosting the phagocytic activity of macrophages, production of reactive oxygen species (ROS) in human neutrophil cells enhancement in nitric oxide (NO) production by stimulation of splenocytes and macrophages indicative of anti-tumor effects.

Aqueous Tinospora extracts has been also reported to influence the cytokine production, mitogenicity, stimulation and activation of immune effector cells. The (1,4)-alpha-d-glucan (alpha-d-glucan), derived Tinospora cordifolia have been shown to activate human lymphocytes with downstream synthesis of the pro- and anti-inflammatory cytokines, in vitro. [5, 8]

The synergistic immunomodulatory activity of Tinospora cordifolia reported from various literatures and studies makes it evident that Tinospora cordifolia has immune boosting and antimicrobial properties. Hence Tinospora cordifolia is suggested in the treatment of various microbial infections.

STUDY OBJECTIVE

The objective of this clinical study to evaluate comparative clinical Efficacy and Safety of BHC9633CP Tinospora cordifolia in Immune Support Adult Subjects

STUDY DESIGN

A randomized, double-blind, placebo-controlled clinical study

OVERALL STUDY PLAN

Healthy volunteer Subjects were enrolled into study along with other inclusion/ exclusion criteria. After obtaining a written informed consent, duly approved by institutional ethics committee, subjects were randomized into one of the two treatment arms, placebo arm and active arm (Tinospora cordifolia extract). All 30 subjects received either placebo or active product in the ratio of 1:1 for a period of 56 days, as one capsule thrice daily after meals. The efficacy of daily doses of Tinospora cordifolia extract was evaluated with that of a placebo in improving TLC, ALC and lymphocyte percentage after 56 days.

DOSE AND METHOD OF ADMINISTRATION

Two 500mg soft gel capsule daily in the morning after breakfast and in night post dinner

STANDARDISATION

Each capsule of 500 mg Tinoimmune (Tinospora cordifolia extract) contains Total Bitters NLT 5% w/w and Alkaloids NLT 2% w/w by Gravimetry and Berberine NLT 0.25% w/w by HPLC.

SELECTION & WITHDRAWAL OF SUBJECTS

SUBJECT SCREENING

Medical history and demographic data including sex, age, body weight (kg), and height (cm), and habits were recorded during general screening of volunteers that was organized 1 day prior to study start. Each subject underwent a complete general physical examination and laboratory tests.

INCLUSION CRITERIA

- Subjects aged 40-70 years of age and of either sex
- Subjects who were disposed to give consent to the study and willing for follow-
- Subjects who are willing to sign ICF

EXCLUSION CRITERIA

- Children with genetic disorders, severe or chronic illnesses, congenital diseases, mentally challenged and those on other immunostimulant drugs were excluded

STATISTICAL ANALYSIS

The data generated in the clinical study will be analysed by applying appropriate statistical method. Unless otherwise stated, all hypotheses will be tested at a significance level of 0.05 and 95% confidence interval. The Statistical analysis plan will also contain the rules and data handling conventions used to perform the analyses, and the procedure used for accounting for missing data.

RESULTS

DEMOGRAPHICS AND BASELINE CHARACTERISTICS

VAverage age of subjects enrolled into the study was 51 years, approximately the same between both the groups at the time of screening. Total 14 male (46.7%) and 16 females (53.3%) participated in the study. Average BMI was 25.9 kg/m2, on the baseline visit.

SAFETY RESULTS:

Vitals monitored and recorded at all the visits. There was no clinically significant abnormality observed in test and control group subjects inferring the active product is safe for administration. The safety

EFFICACY RESULTS

Immune markers TLC, ALC and Lymphocyte percentage are evaluated in this study.

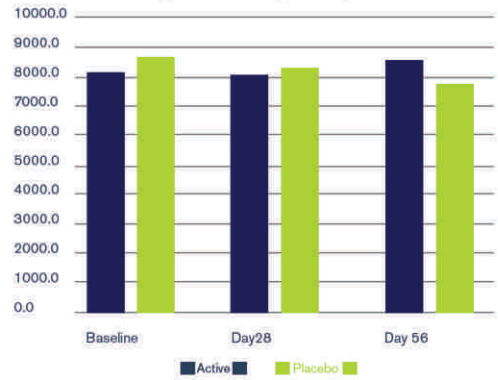
TOTAL LEUKOCYTE COUNT (TLC)

Total leucocyte count includes all the various white blood cell count including Neutrophils, Basophils, eosinophils, lymphocytes and monocytes. Neutrophils are first line defence mechanism for acute bacterial infections whereas lymphocytes, monocytes are mainly involved in fight against bacterial and viral infections. The increase TLC is an important parameter of active immune function and raised in various infections. (Chaplin DD. 2010). Normal Total leukocyte count in the blood is 4,500 to 11,000 WBCs per microliter (4.5 to 11.0 × 109/L). In present study in active group TLC on baseline is 8046/ ml and increased to 8571/ml on Day 56. In placebo group TLC in baseline is 8713/ml and 7842/ml on day 56. There is increase in TLC from Baseline to End of study (Day 56) in active group compared to placebo group.

Parameter	Hypothesis Type	DF	Type III SS	Mean Square	F Value	Pr > F
TLC	3	1	6433588.8867	6433588.8867	2.95	0.0991

Visit	Statistics	Active	Placebo
Baseline	n Mean (SD) Median Min, Max	15 8046.0(1935.19) 8460.0 4950,12080	15 8713.3(185.47) 9150.0 4110,12660
Day 28	n Mean (SD) Median Min, Max	14 8195.7(1942.50) 8115.0 4970,11900	15 8304.0(978.90) 8360.0 6500,10320
Day 56	n Mean (SD) Median Min, Max	13 8571.5(1968.57) 8680.0 5300,11910	13 7842.3(1390.32) 8390.0 5880,9780

Graph For Tinospora Cordifolia data in Immune Support Adult Subject Graph for TLC



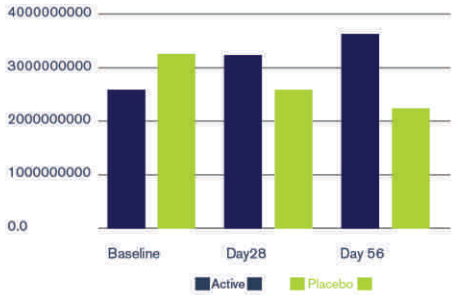
ABSOLUTE LYMPHOCYTE COUNT (ALC)

Absolute lymphocyte count is an important marker of active immune system. The normal lymphocyte range in adults is between 1 and 4.8 X 10 9/L of blood. In many viral infections including EBV, CMV and other common viruses like Influenza, hepatitis, mumps, measles, rubella, human T Lymphocytic virus type 1 (HTLV-1), adenovirus, some bacterial infections including mycobacterium tuberculosis and parasitic infestations (malaria, toxoplasma gondii) lymphocyte count (lymphocytosis)is increased as a response of immune system. (Brigden ML et al.1999; Rosenberg ES et al.1999; Fiala M et al.1977). Absolute lymphocyte count in active group on baseline is 2.6X 10 9/L and 3.6X 10 9 /L on day 56. In placebo group on baseline is 3.1 X 10 9/L and decreased to 2.1X 10 9 /L on day 56. There is statistically highly significant increase in ALC in active group from baseline to day 56 compared to placebo group. (P value < 0.0001)

Parameter	Hypothesis Type	DF	Type III SS	Mean Square	F Value	Pr > F
ALC	3	1	1.6477826E17	1.647782617	28.49	<.0001

Visit	Statistics	Active	Placebo
Baseline	n Mean (SD) Median Min, Max	15 269797333.3(86382605.51) 245340000.0 133650000,434880000	15 319664666.7(88697969.93) 335520000.0 167700000,458500000
Day 28	n Mean (SD) Median Min, Max	14 316630714.3(103860313.84) 299405000.0 168980000,523600000	15 2646800000.0(59921079.64) 248200000.0 195000000,412800000
Day 56	n Mean (SD) Median Min, Max	13 362466153.8(115902774.67) 373920000.0 196100000,559770000	13 128835384.6(54599560.68) 227850000.0 105840000,323340000

Graph For Tinospora Cordifolia data in Immune Support Adult Subject Graph for ALC



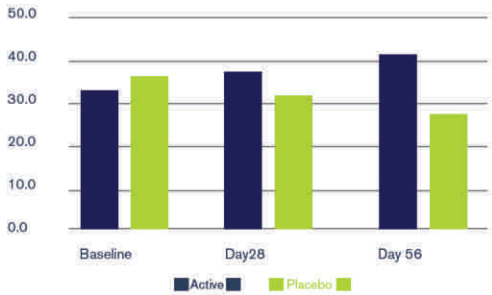
LYMPHOCYTE PERCENTAGE (LP)

Lymphocyte percentage (LYM%), an independently measured value to reflect peripheral lymphocyte count and good marker of immunity, anti-inflammaotry marker and clinical outcome. Normal lymphocyte percentage is 20% to 40%. Low LYM% is associated with significant increase in mortality. (N Kuwae et al. 2005) LP is simple and new powerful prognostic factor for patients as a bedside marker of immune status (Weiwei Zhao et al. 2017). In present study in test group LP on baseline is 33.3% and increased to 41.7 % on day 56. In placebo group LP on baseline is 37.3% and 27.8 % on day 56. There is statistically significant increase in LP in active group from baseline to day 56 compared to placebo group. (P value < 0.0001)

Parameter	Hypothesis Type	DF	Type III SS	Mean Square	F Value	Pr > F
LP	3	1	1281.3385466	1281.3385466	43.25	<.0001

Visit	Statistics	Active	Placebo
Baseline	n Mean (SD) Median Min, Max	15 33.3(5.85) 32.0 26,46	15 37.3(7.82) 36.0 22,50
Day 28	n Mean (SD) Median Min, Max	14 38.2(6.24) 37.0 30,50	15 32.1(7.39) 30.0 23,48
Day 56	n Mean (SD) Median Min, Max	13 41.7(6.13) 41.0 33,52	13 27.8(4.59) 28.0 18,35

Graph For Tinospora Cordifolia data in Immune Support Adult Subject Graph for LP



CONCLUSION

Strong immune system is vital for fighting against microbial infections and boosting immune system during infections is an important measure of management. BHC9633CP Tinospora cordifolia extract when administered orally for a period of 56 days in healthy adult subjects demonstrated significant improvement in laboratory immune markers Total leukocyte count (TLC), Absolute lymphocyte count (ALC) and Lymphocyte percentage (LP). It demonstrated an excellent safety and efficacy profile in healthy adult subjects in comparison to placebo. None of the patients experienced serious adverse effects and all patients completed study period. The final results of this study showed that BHC9633CP Tinospora cordifolia extract is orally safe and effective in boosting immunity in healthy subjects, which indicate its definitive role in the management of infections as an immune support.

REFERENCES

- Zemskov VM, Pronko KN, Zemskov AM, and Zemskova VA. Contradictions of clinical immunology: Nonspecific and specific mechanisms in immunogenesis. Clinical Practice. 2019; 16 (3).
- Lindsay B et al. The immune system. Essays Biochem. 2016 Oct 31; 60(3): 275–301.
- Chaplin DD. Overview of the Immune Response. J Allergy Clin Immunol. 2010 Feb; 125(2): S3–23.
- Kuldeep Dhama et al. Medicinal and Beneficial Health Applications of Tinospora cordifolia (Guduchi): A Miraculous Herb Countering Various Diseases/Disorders and its Immunomodulatory Effects. Recent Pat Endocr Metab Immune Drug Discov. 2017; 10(2):96-111.
- Soham Saha et al. Tinospora cordifolia: One plant, many roles. Anc Sci Life. 2012 Apr-Jun; 31(4): 151–159.
- Sultan Alsuhaibani et al. Immune-Stimulatory and Therapeutic Activity of Tinospora cordifolia: Double-Edged Sword against Salmonellosis. J Immunol Res. 2017; 2017: 1787803.
- Ventola CL. The Antibiotic Resistance Crisis Part 1: Causes and Threats. P T. 2015 Apr; 40(4): 277–283.
- P.K. Raveendran Nair et al. Immune stimulating properties of a novel polysaccharide from the medicinal plant Tinospora cardifolia. Int Immunopharmacol. 2004; 4(13): 1645–1659.
- Brigden ML, Au S, Thompson S, Brigden S, Doyle P, Tsaparas Y. Infectious mononucleosis in an outpatient population: diagnostic utility of 2 automated hematology analyzers and the sensitivity and specificity of Hoagland's criteria in heterophile-positive patients. Arch. Pathol. Lab. Med. 1999 Oct;123(10):875-81.
- Rosenberg ES, Caliendo AM, Walker BD. Acute HIV infection among patients tested for mononucleosis. N. Engl. J. Med. 1999 Mar 25;340(12):969.
- Fiala M, Heiner DC, Turner JA, Rosenbloom B, Guze LB. Infectious mononucleosis and mononucleosis syndromes. West. J. Med. 1977 Jun;126(6):445-59.
- N Kuwae et al. A low lymphocyte percentage is a predictor of mortality and hospitalization in hemodialysis patients. Clin Nephrol. 2005 Jan;63(1):22-34.
- Weiwei Zhao et al. Lymphocyte count or percentage: which can better predict the prognosis of advanced cancer patients following palliative care? BMC Cancer. 2017 Aug 2;17(1):514. doi: 10.1186/s12885-017-3498-8

ACCREDITATION