ejpmr, 2021,8(9), 392-398

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A CLINICAL STUDY TO EVALUATE COMPARATIVE CLINICAL EFFICACY AND SAFETY OF BHC9633CP TINOSPORA CORDIFOLIA IN IMMUNE SUPPORT ADULT SUBJECTS

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Article Received on 26/06/2021 **Article Revised on 16/07/2021** **Article Accepted on 06/08/2021**

ABSTRACT

Background: 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. In racemes or racemose panicles, the male flowers are clustered and female are solitary. The flowering season expands over summers and winters. A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, 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. (Soham Saha et al. 2012). 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. (Sultan Alsuhaibani et al. 2017). Tinospora cordifolia extract is a potent immune booster has been suggested in the treatment of various microbial infections. Hence this clinical trial is conducted to evaluate the safety and efficacy of Tinospora cordifolia extract in healthy adult volunteers. **Objectives:** The objective of this clinical study to evaluate comparative clinical Efficacy and Safety of BHC9633CP Tinospora cordifolia in Immune Support Adult Subjects. **Conclusion**: The final results of this study showed that Tinospora cordifolia is orally safe and effective in boosting immunity in healthy subjects. This clearly indicates that Tinospora cordifolia has definitive role in the management of infections as an immune support.

KEYWORDS: NK-T cells, Investigational Product, Tinospora cordifolia, Efficacy and Safety.

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 immune tolerance. 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. (Zemskov VM et al. 2019; Lindsay B et al. 2016).

The medicinal applications of T. cordifolia in countering various disorders and usages include anti-microbial, immune booster, anti-oxidant, anti-hyperglycemic, antihyperlipidemic, hepatoprotective, cardiovascular protective, neuroprotective, osteoprotective, radioprotective, anti-anxiety, adaptogenic agent, analgesic, anti-inflammatory, antipyretic, a thrombolytic agent, anti-diarrheal, anti-ulcer, and anti-cancer agent. The plant is also a source of micronutrients viz. copper, calcium, phosphorus, iron, zinc and manganese. (Kuldeep Dhama et al. 2017; Soham Saha et al. 2012)

DESCRIPTION

Study Rationale - Tinospora cordifolia extract has been used since many years for treating various infections as immune booster in traditional medicine. Many active compounds including glycosides, alkaloids, steroids and flavonoids are derived from the Tinospora cordifolia which possesses diverse pharmacologic and biological properties. 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.

OBJECTIVES

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

METHODS

Inclusion Criteria

Healthy male and female subjects aged between 40-70 years were enrolled into study. Subjects who are willing to sign ICF was enrolled in the study. Subjects who were disposed to give consent to the study and willing for follow.

Exclusion Criteria

Subjects with genetic disorders, severe or chronic illnesses, congenital diseases, mentally challenged and those on other immune stimulant drugs were excluded.

Study was conducted by. Dr. Poorna Prasad and the Study Centre was Venkateshwara hospital, 1st floor, Room no 27, 29th main road, Rashtra Kuvempu Nagara, 2nd stage, BTM layout, Bengaluru-560076, Karnataka. Which involved in the clinical attendance of the subjects on recruitment and on follow –up. Subjects enrolled in the study received Study drug (from Baseline visit to 01 days –to EOT on day 56- The patients has taken BHC9633CP Tinospora cordifolia.

The safety and efficacy parameters were compared with baseline and follow-up data with laboratory investigations, demographics were analyzed in the study. Adverse events / side effects were noted for each follow-up visits.

Ethics Committee Approval

All study related documents Protocol, Case Report Form, Dairy card, Investigator Brochure and Informed Consent Documents (English and Kannada Versions). Written Informed Consent was obtained from the subjects before the start of the trial and after due approval from IRB. Ethics Committee notifications as per the GCP guidelines issued by Central Drugs Standard Control Organization and Ethical guidelines for biomedical research on human subjects issued by Indian council of Medical Research has been followed during the Conduct of the Study (Clinical IEC-Institutional Ethics Committee for Ethics in Research and Approved on 21/01/2020). Study was registered in CTRI (Registration Number: CTRI/2020/02/023359).

Study Outcomes

Primary Outcomes

Changes from baseline to end of the study period in.

- TLC (Total leukocyte count)
- ALC (Absolute Lymphocyte Count)
- LP (Lymphocyte percentage)

Secondary Endpoints

- Adverse events (AEs), frequency and severity.
- Changes in vital parameters and laboratory investigations.

Disposition of Subjects

Total of 30 subjects

Group 1: BHC9633CP Tinospora cordifolia (15 subjects)

Group 2: Placebo **(**15 subjects)

Description	No. of Patients
Screened	38
Enrolled	30
Screen failures	08
Completed end of study	30
Included in the safety analysis	30
Included in efficacy analysis	30

Visit Details

The patients were screened and enrolled. All 30 subjects were randomized into 2 groups and received Investigational product/Placebo. Efficacy and safety parameters as per study end points were monitored on each visit of the study. At the end of the day 56 complete data analysis was done.

Statistical Analysis

The data generated in the clinical study will be analyzed 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

In the study 30 patients were enrolled after meeting the inclusion Criteria and they were randomized randomly into Test and Placebo. The enrolled subjects consisted of 14 male (46.7%) and 16 female (53.3%) participated in the study. Average BMI was 25.9 kg/m2, on the baseline visit.

Data sets analyzed

Efficacy Evaluation

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

I. 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.

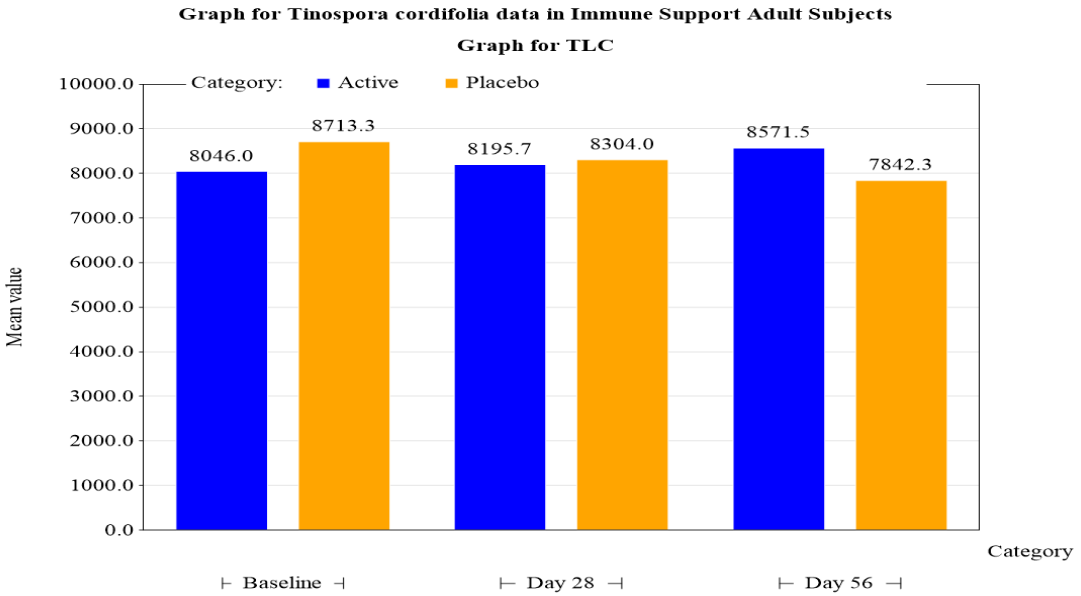
Descriptive statistics for Tinospora cordifolia data

Parameter: TLC

Visit	Statistics	Active	Placebo
Baseline	n	15	15
	Mean (SD)	8046.0(1935.19)	8713.3(2185.47)
	Median	8460.0	9150.0
	Min, Max	4950,12080	4110,12660
Day 28	n	14	15
	Mean (SD)	8195.7(1942.50)	8304.0(978.90)
	Median	8115.0	8360.0
	Min, Max	4970,11900	6500,10320
Day 56	n	13	13
	Mean (SD)	8571.5(1968.57)	7842.3(1390.32)
	Median	8680.0	8390.0
	Min, Max	5300,11910	5880,9780

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

In present study in active group TLC on baseline is 8046/ ml and increased to 8713/ml on Day 56. In placebo group TLC in baseline is 8669/ml and 8390/ml on day 56. There is increase in TLC from Baseline to End of study (Day 56) in active group compared to placebo group.



II. 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).

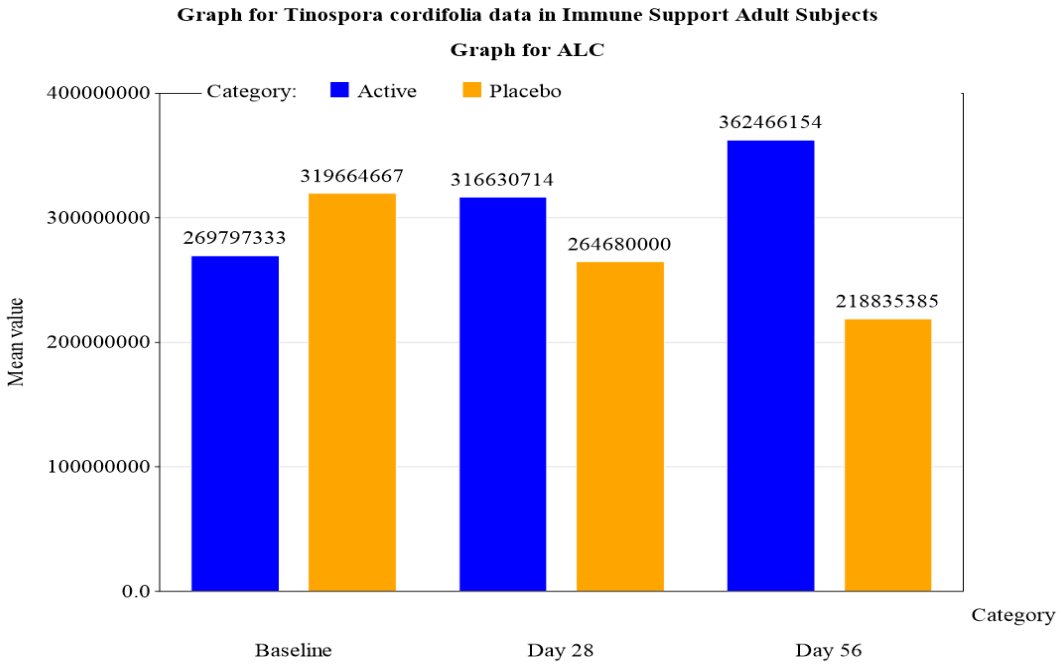
Descriptive statistics for Tinospora cordifolia data

Parameter: ALC

Visit	Statistics	Active	Placebo
Baseline	n	15	15
	Mean (SD)	269797333.3(86382605.51)	319664666.7(88697969.93)
	Median	245340000.0	335520000.0
	Min, Max	133650000,434880000	167700000,458500000
Day 28	n	14	15
	Mean (SD)	316630714.3(103860313.84)	264680000.0(59921079.64)
	Median	299405000.0	248200000.0
	Min, Max	168980000,523600000	195000000,412800000
Day 56	n	13	13
	Mean (SD)	362466153.8(115902774.67)	218835384.6(54599560.68)
	Median	373920000.0	227850000.0
	Min, Max	196100000,559770000	105840000,323340000

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

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)



III. 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).

Descriptive statistics for Tinospora cordifolia data

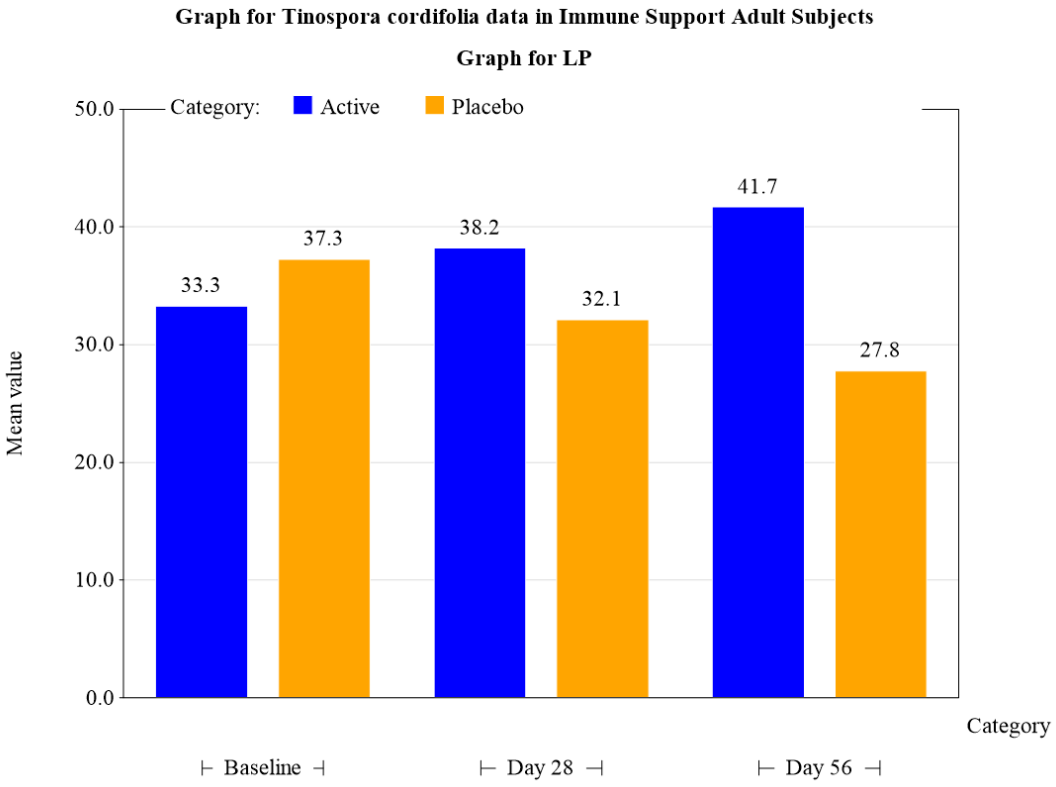
Parameter: LP

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

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

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).

Graph for Tinospora cordifolia data in Immune Support Adult Subjects



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 parameters including ECG

and laboratory tests (CBC, RFT and LFT) were within normal limits on screening and on day 56.

DISCUSSION AND CONCLUSION

The trial was conducted in Venkateshwara hospital, 1st floor, Room no 27, 29th main road, Rashtra Kuvempu Nagara, 2nd stage, BTM layout, Bengaluru-560076, KARNATAKA with Dr Dr. Poorna prasad as Principal investigator, post its Institutional Ethics Committee approval /favorable opinion on the trial proposal.

The study was initiated on 13/02/2020 and first subject was enrolled on 17/02/2020. There were a total of 08 screen failures and the last subject's last visit completed on 17/08/2020. All the 30 subjects were distributed equally between the two groups. A detailed general examination and vitals examination at screening visit was done. LFT, RFT, HEMATOLOGY and ECG were measured at baseline and end of study visit of all the enrolled subjects to check if there were any significant or clinically abnormal changes observed during the course of the treatment. Average age of subjects enrolled into the study was 51 years, approximately the same between both the groups at the time of screening. Total 14 males (46.7%) and 16 females (53.3%) participated in the study. Average BMI was 25.9 kg/m^2, on the baseline visit.

Detailed physical examination was performed on all the 30 enrolled subjects throughout all the 4 visits and none found to be abnormal. 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. There were 4 adverse events (3 nausea, 1 head ache) observed for four different subjects which were categorized as mild to moderate in severity in active group and in placebo group 3 adverse events observed (2 gastritis, 1 nausea) with none of the events were judged to be related to study product in the Investigator's opinion. All the adverse events were managed by routine clinical measures and none of the participant discontinued the study due to adverse events. The safety parameters including ECG and laboratory tests (CBC, RFT and LFT) were within normal limits on screening and on day 56. This infers that subjects who received active product did not show any clinically significant abnormality when compared to that of placebo group subjects. Therefore, it can be concluded that the active product in soft gel capsule formulation is completely safe for human oral consumption.

TOTAL LEUKOCYTE COUNT (TLC) - In the present study there is increase in TLC from Baseline to End of study (Day 56) in active group compared to placebo group.

ABSOLUTE LYMPHOCYTE COUNT (ALC)- In present study there is statistically highly significant increase in ALC in active group from baseline to day 56 compared to placebo group. (P value < 0.0001).

LYMPHOCYTE PERCENTAGE (LP**)-** In present study there is statistically significant increase in LP in active group from baseline to day 56 compared to placebo group. (P value < 0.0001).

Thus, in the present study Immune markers TLC, ALC and lymphocyte percentage were significantly improved in Active group treated with BHC9633CP Tinospora cordifolia extract when compared to placebo group. The therapy was well tolerated and all the participants completed the study duration. There were no significant adverse effects observed in the study.

CONCLUSIONS

Strong immune system is vital for fighting against microbial infections and boosting immune system during infections is an important measure of management. The aim of the present study was to evaluate safety and efficacy of BHC9633CP Tinospora cordifolia extract by assessing and immune markers. Tinospora cordifolia has demonstrated an excellent safety and efficacy profile in healthy adult subjects in comparison to placebo. Tinospora cordifolia 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). None of the patients experienced serious adverse effects and all patients completed study period. The final results of this study showed that Tinospora cordifolia is orally safe and effective in boosting immunity in healthy subjects. This clearly indicates that Tinospora cordifolia has definitive role in the management of infections as an immune support.

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