Immunomodulatory Effect of Charak Immunity Tablets (CIT) on Healthy Participants: A single blind, randomized, placebo-controlled, exploratory study

Akanksha Mourya[1], Amit Yadav[1], Suresh Khadke[1], Sarika Mane[1], Asavari Joshi[1], Poonam Gupte[1], Madhavi Mahajan[2] and Supriya Bhalerao[1*]

1. [1]Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth Deemed (to be) University, Pune- 411043
2. [2] Associate Professor, Department of Kayachikitsa, College of Ayurveda, Bharati Vidyapeeth Deemed (to be) University, Pune- 411043
* Correspondence author: Dr. Supriya Bhalerao, Associate Professor, Interactive Research School for Health Affairs; (IRSHA), Bharati Vidyapeeth Deemed (to be) University, Pune, Maharashtra, Tel +91 952 758 9516, Email: supriya.bhalerao@bharatividyapeeth.edu

Abstract: Immunity has become an important aspect of concern, as the spread of corona virus, is on the rise. The strategies to boost and modulate the immunity have therefore become need of the hour. The present study was carried out to evaluate the effect of Charak immunity Tablets (CIT) on innate and adaptive immune response in healthy individuals. It was a single-blind, randomized, placebo-controlled, exploratory study. After obtaining Ethics Committee permission, 36 healthy individuals of either sex aged 18-35 years with prior consent were recruited in the study. They were randomly divided into 2 groups to receive either CIT or Placebo in 2:1 ratio. Both the interventions were given in a dose of 1 tab (500 mg) twice daily. The assessment variables were vitals [temperature, pulse, and blood pressure], respiratory health [respiratory rate, oxygen saturation and peak expiratory flow rate], questionnaire based assessment of immune status, perceived stress and quality of life along with objective assessment of immunity [CD4+, CD8+ counts, Interferon gamma (IFN γ), Tumor necrosis factor alpha (TNF-α) and Interleukin 10 (IL-10)] as well as oxidative stress; [Malondialdehyde (MDA) and Glutathione peroxidase], which were assessed at fixed time points. Of 36 recruited participants, only 18 participants completed the study. CIT treated individuals showed a statistically significant improvement in respiratory health, quality of life, perceived stress and subjective immune status. There was a decrease in the levels of serum IFN γ on day 60 compared to baseline. TNF-α and IL-10, both estimated from supernatant of Peripheral Blood Mononuclear Cells (PBMCs) stimulated with lipopolysaccharide (LPS), showed a decrease and a significant increase respectively on day 60 compared to baseline in CIT group. Further, CIT significantly decreased MDA levels.

The present study indicates that CIT is an effective and safe drug to boost immunity. However, our findings need to be confirmed in larger sample size using more specific immune parameters.

Keywords: CD4- CD8 counts; immunity; inflammation; oxidative stress; peak expiratory flow rate

Introduction

Immune system is a complex network of organs, tissues, cells, and body processes that provide resistance to infection and toxins.[1] The pressure that natural selection exerts on immune system is infinite and never-ending. Emerging infectious diseases have potential to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders.[2,3] The evolution of immune system has undergone incredible selective pressure imposed by wide array of pathogens. The two main activities on which immune system works, despite of all differences, is recognition and response towards any interacted pathogen, i.e. through innate and adaptive immune response.[4,5] These immune responses involve secretion and activation of various cells like dendritic cells, macrophages, neutrophils, natural killer cells, B-cells & T-cells, cytokines like Tumor necrosis factors, interleukins, interferons, and protein molecules.[6,7] Factors like, age, medication, infections, stress affect immune homeostasis by altering the immune regulatory network leading to decreased responsiveness by the system.[8-12] Several other studies have also validated that lack of immunological activation markers and receptors, oxidative damage (ROS production), reduced antioxidant potential can also hamper efficiency of immune system.[13-15]

Emergence of COVID-19 has highlighted the importance of the immune system[16] and as a result, various strategies and approaches are being implemented to improve and modulate immune system, reduce disease aggravation in patients and stimulate profound response in healthy individuals.[17] As the world turns to science in search of an effective drug or vaccine, countries, such as India and China, with heritage of using traditional medicine[18,19] have also begun to explore the role of traditional and complementary treatment. Ayurveda, the traditional Indian system of medicine has enlisted medicinal herbs like *Withania somnifera* L (Ashwagandha),[20] *Tinospora cordifolia* (Guduchi), *Asparagus racemosus* (Shatavari), *Phyllanthus emblica* (Amalaki), which have been validated for their immunomodulatory activities.[8,21] Several *in-vitro* studies are also suggestive that herbal drugs can target various cell subsets of immune system like neutrophils, macrophages, and lymphocytes and also modulate the cytokine secretion in lipopolysaccharide (LPS) stimulated cells to enhance the functioning of system.[22-25]

CIT (Charak immunity tablets), a patent and proprietary Ayurvedic formulation of Charak Pharma Pvt. Ltd. is composed of powder of *Triphala Guggul* (200mg) and extracts of *Tinospora cordifolia* (500mg), *Curcuma longa* L(500 mg), *Rubia cordifolia* L(250mg), *Plumbago zeylanica* L(150mg) and *Piper longum* L, *Piper nigrum* L, *Zingiber officinale* Roscoe, each weighing 66.66mg. A majority of these ingredients have been reported for their immunomodulatory, antioxidant and anti-inflammatory activities.[26-30] With this background, the present study was designed to assess the effect of CIT on immunity.

Material and Methods:

Ethical consideration:

The study was approved by Institutional Ethics Committee (BVDUCOA/EC/2829/2020-2021) and registered prospectively with Clinical Trial Registry of India (CTRI/2017/07/008999).

Study Design:

A single-blind, randomized, placebo controlled, exploratory study.

Sample Size:

This was an exploratory study so a sample size of 30 completed participants (20 in CIT group and 10 in Placebo group) was considered adequate. Considering a 20% attrition rate, a total of 36 individuals were recruited.

Screening and Recruitment of Healthy Individuals:

The study was conducted during the period January 2021- May 2021. Individuals in the age group of 18-35 years of either sex without any medical, surgical history, severe cardiac, renal, or liver pathologies were screened for blood investigations viz. Haemogram [Hemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR), Platelets, White blood cells (WBC count)], Fasting blood glucose (FBG), Liver function tests [LFT- serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)] and renal function test (serum creatinine). Individuals with hemoglobin levels ≤ 9 g%, LFT and RFT levels greater than 2.5 times and 1.5 times, the upper normal limit respectively, while rest of the investigations beyond normal reference ranges, were excluded from the study. Individuals with a history of consumption of herbal/ nutritional/ pre and probiotic supplements/ multivitamins/ or available marketed medications for immunity for the past 30 days were excluded. Lactating and pregnant women were also not considered for the study.

At baseline, all individuals were subjected to examination of vitals (temperature, pulse, and blood pressure), respiratory health [respiratory rate, oxygen saturation (SpO$_2$), and peak expiratory flow rate (PEFR)].

Standardized and validated questionnaires namely immune status questionnaire (ISQ), perceived stress scale (PSS) and WHOQOL-BREF (World Health Organization Quality of life) were administered to the participants to assess their immunity, perceived stress and quality of life respectively. ISQ comprises of 7 questions which gives a maximum score of 10. This score denotes good immune health of the individual, while a score of 0 indicates poor immune health. [31] PSS has 10 questions. The maximum score that can be obtained by an individual is 40 and it indicates severe stress in the person, while a score of 0 is indicative of the person being stress free.[32] The WHOQOL-BREF consists of 26 questions divided over four domains viz. physical health, physiological health, social relationship, and environment. The measure of quality of life is calculated by summing the point values for the question corresponding to each domain and then transforming the scores to 0-100-point interval. Higher the points, better the quality of life of that individual. [33]

This was followed by blood collection (12 ml) for the estimation of immune and oxidative stress parameters. Subsequently, the participants were randomized to two groups viz. CIT and Placebo in the ratio of 2:1. Initially the study interventions were provided for a period of first 30 days. On day 30, the participants were followed up and examined for vitals, respiratory health along with subjective assessment of immunity, stress and quality of life using the above mentioned questionnaires. The participants were provided with medicines for further 30 days and called on day 60. Above mentioned clinical as well as questionnaire based parameters were also done on day 60. Participants were

telephonically followed up on 15th and 45th day to enquire about their health and to ensure compliance.

Study Intervention and Dosage:

Both interventional drugs, CIT & Placebo were supplied by Charak Pharma Pvt. Ltd. The size, color, shape and packaging of both CIT and Placebo were identical to avoid identification by participants.

Placebo was composed of maize starch (205mg) as the main ingredient along with potassium sorbate (1.3mg), sorbic acid (0.15mg), sodium benzoate (0.49mg), calcium stearate (1mg), colloidal silicon dioxide (10mg), sodium crosscarmellose (20 mg), crospovidone (40mg) and coating mix (28.8mg).

Both the tablets, CIT & Placebo were administered in the dose, 1 tablet (500mg each) twice a day after meals with water for a total duration of 2 months.

Blood Sampling and Processing:

Blood collection was done through closed collection procedure to minimize contamination. Of the 12 ml blood collected at baseline, 1.5 ml blood was processed for flow cytometry based estimation of immunological surface markers (CD4+ and CD8+). Nine ml blood was processed to isolate PBMCs and RBCs. Remaining 1.5 ml blood was centrifuged to obtain serum for estimating serum IFN-γ. On day 30, only 1.5 ml blood was taken for assessment of serum IFN-γ. On day 60, in addition to 12 ml blood collected at baseline, 3 ml blood (total 15 ml) was collected for estimation of parameters carried out during screening except fasting glucose.

Cytokine estimation from stimulated PBMCs:

Briefly, blood (9 ml) was subjected to density gradient centrifugation using histopaque [(1:2 in phosphate buffered saline (PBS)] to separate PBMCs and RBCs. Isolated PBMCs in the density 5×10^5 were cultured in each well and seeded in RPMI 1640 medium (HiMedia) for 2 h. Later, the cells were stimulated with *Escherichia coli* derived lipopolysaccharide (LPS- Sigma Aldrich) at the concentration of 0.5 μg/ml and incubated for 4 h, at 37oC in 5% CO_2 incubator. The culture supernatant was harvested for the estimation of cytokines viz. TNF-α, IL-10. The cytokines were estimated using commercially available ELISA kits [Biolegend].

Oxidative stress estimation from RBCs:

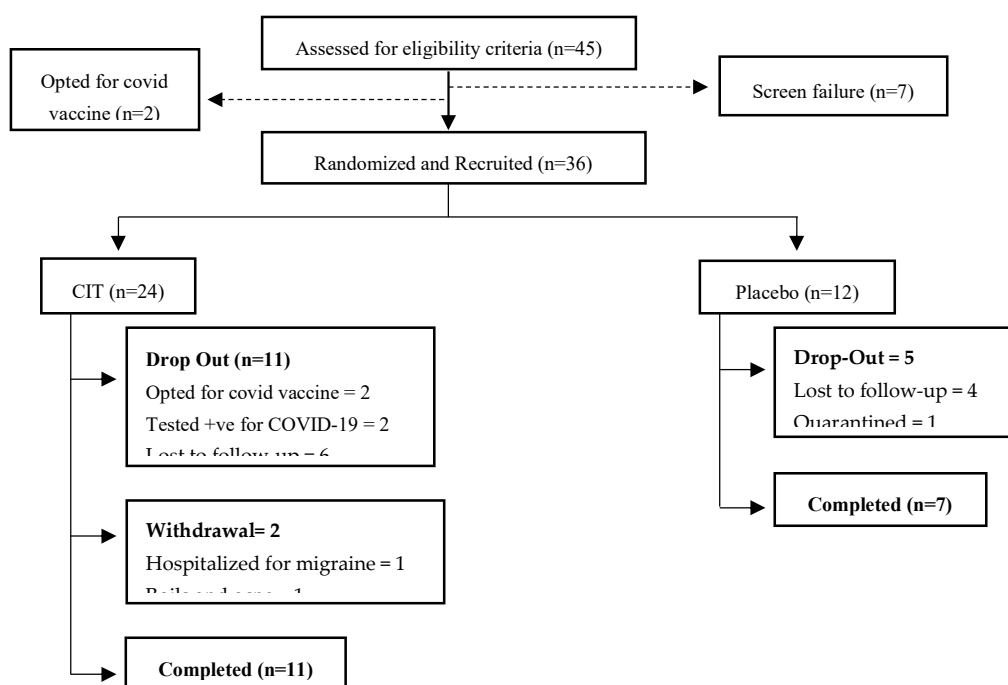
Malondialdehyde (MDA) was quantified from RBCs using Thiobarbituric acid reactive substances (TBARS) method described as per Kanias et al.[34] The method is based on the acidic reaction of MDA with thiobarbituric acid, which forms a (TBARS) chromophore which is absorbed at 532 nm. Glutathione peroxidase (GPx) levels from the RBCs lysate (1:10 diluted in phosphate buffer) were measured using commercially available colorimetric assay kit (Cayman, US). The method is based on coupled reaction with glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) which is accompanied by decreased absorbance at 340nm. This rate of decrease is directly proportional to the amount of GPx present inside the cell.

Statistical Analysis:

The parametric data is presented as Mean ± SD and analyzed using paired or unpaired t test and repeated measures ANOVA test. The non-parametric data is presented as Median (Range) and analyzed using Wilcoxan Rank sum test /Mann Whitney test and Friedman's test. Tukey's post hoc test was applied where applicable. GraphPad InStat software, version 9 was used to perform the analysis. $p < 0.05$ was considered as level of statistical significance.

Results:

A total of 45 individuals were screened, of which 36 healthy individuals were recruited. Only 18 individuals completed the study. This attrition was mainly because of rise in COVID-19 pandemic throughout the city and implementation of subsequent lockdown, which occurred during the course of the study. The detailed participant flow is given in Figure 1.

Figure 1: Flowchart of study participants

The male to female ratio of completed participants in the CIT group was 6:5 while that in the placebo group was 4:3. The mean age of the participants in CIT group was 22.9 ± 3.7 while that in placebo group was 23.3 ± 5.9. The distribution of participants as well as their age in both groups was comparable.

The vitals and parameters indicating respiratory health were in normal range post the study. There was a gradual increase noted in PEFR in CIT group, which was statistically significant (p<0.05) on day 60 compared to baseline. No such increase was seen in placebo group (Table 1).

Table 1: Effect of CIT on Vitals and Respiratory Health

Parameters	CIT group (n=11)			Placebo group (n=7)		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Temperature (^{0}C)	36.2 ± 0.1	36.3 ± 0.1	36.2 ± 0.2	36.4 ± 0.2	36.4 ± 0.1	36.3 ± 0.1
Pulse (/ min)	77 ± 4.9	79.5 ± 7.9	78.5 ± 4.3	82.3 ± 7.1	80.3 ± 9.1	79.3 ±7.5
BP- Systolic (mm Hg)	115.8 ± 14.2	115.6 ± 12.6	117.5 ± 5.1	110.1 ± 13.1	109 ± 15.3	112.3 ± 12.4
BP-Diastolic (mm Hg)	83.9 ± 15.5	78.9 ± 10.4	83.7 ± 4	71 ± 10.2	71.4 ± 9.2	76.9± 10.9
Respiratory rate (/min)	19.5 ± 1.4	19.9 ± 2	19.8 ± 1.1	20.5 ± 1.8	20.1 ± 2.2	21.5 ± 2.2
SpO2 (%)	97.2 ± 1.4	96.9 ± 1.3	97.9 ± 0.7	96 ± 1.6	97.3 ± 1	97.1 ± 0.9
PEFR (lit/min)	298.2 ± 78.7	310.9 ± 69.9	337 ± 50*	290.9 ± 115.9	284.8 ± 110.9	297.7 ± 131.2

*p<0.05 as compared to day 0 using repeated measures ANOVA followed by Tukey's post hoc test

In CIT group, the immune status was found significantly improved on day 60 as compared to its baseline as well as placebo group. There has been a gradual decrease in levels of stress in CIT Group, with a significant reduction on day 60 (p = 0.007) as compared to baseline; whereas in the placebo group, an increase in stress levels was observed. There was an increase in scores of all four domains of quality of life in CIT group on day 60 with a significant increase in physical (p = 0.0015) and environmental (p = 0.0157) domain. In placebo group, the increase in scores was seen only in 2 domains, which was not significant statistically. (Table 2).

Table 2: Effect of CIT on clinical assessments

Parameters	CIT group (n=11)			Placebo group (n=7)		
	Day 0	Day 30	Day 60	Day 0	Day 30	Day 60
Immune status	8 (6-9)	9 (6-10)	10*#(7-10)	7 (6-10)	8 (6-10)	8 (6-10)
Perceived stress	18 (7-34)	15 (9-30)	14*(7-26)	13 (7-32)	18 (12-30)	16 (10-29)
Quality of life						
Physical health	70 (63-100)	81 (70-94)	85** (72-99)	75 (69-86)	81 (69-100)	85 (69-10)
Physiological health	75 (56-96)	75 (56-94)	80 (58-94)	81 (56-89)	81 (56-88)	84 (75-86)
Social relationship	70 (31-94)	79 (35-94)	83 (50-86)	81 (50-100)	75 (31-94)	65 (40-96)
Environment	75 (63-89)	81 (63-94)	90* (75-96)	84 (69-94)	81 (63-94)	81 (75-90)

*p < 0.05, **p<0.01 compared to day 0 using Friedman test
#p < 0.05 compared to placebo on day 60 using Mann Whitney test

Irrespective of the study groups, hematological parameters as well as SGOT, SGPT and serum creatinine remained in the normal range at the end of the study and did not show any statistically or clinically significant change.

It was observed that on day 60 the CD4+ counts did not show much change compared to baseline, while CD8+ counts reduced irrespective of the group. This decrease was not statistically significant. Owing to the drop in CD8 counts, CD4:CD8 ratio was found to be significantly (p< 0.01) increased in both the groups (Table 3).

Table 3: Effect of CIT on CD4 & CD8 Cells

Parameters	CIT group (n=11)		Placebo group (n=7)	
	Day 0	Day 60	Day 0	Day 60
CD4 (cells/µl)	879.4 ± 262.4	879.2 ± 293.5	992.7 ± 165.2	943 ± 319.3
CD8 (cells/µl)	887.9 ± 417.3	704.8 ± 277.3	752.6 ± 226.3	583.9 ± 276.6
CD4:CD8	1.1 ± 0.36	1.3 ± 0.39**	1.45 ± 0.49	1.73 ± 0.57**

**p< 0.01 compared to day 0 using Paired t test

Median levels of serum IFN-γ in the CIT group on day 30 were observed to be increased compared to baseline which later got reduced on day 60 with no statistically significant difference, whereas in Placebo group on day 30, the levels decreased followed by increase on day 60 (Figure 2).

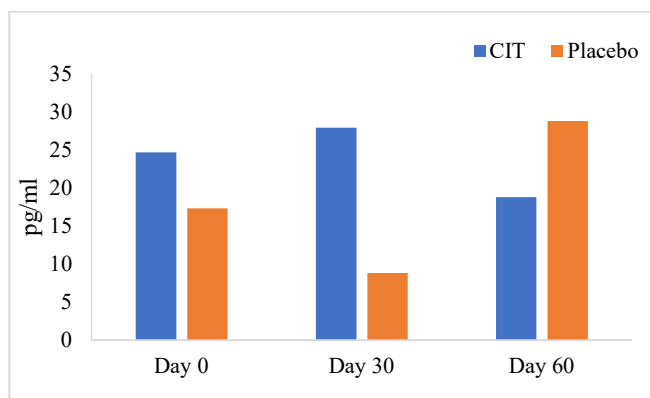
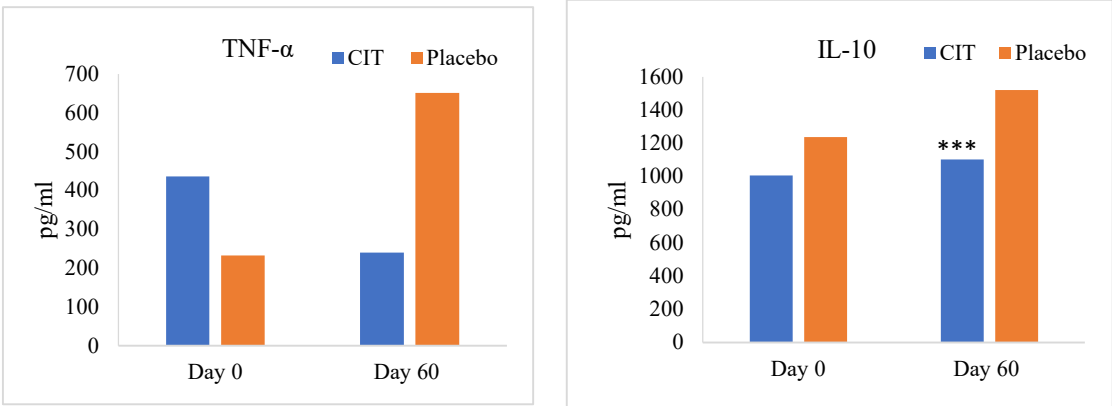
Figure 2: Effect of CIT on IFN-γ

CIT group showed reduction in TNF-α levels in LPS stimulated PBMCs while in placebo group the levels increased on day 60 compared to day 0. There was no statistical difference observed in the levels of TNF-α within or between groups. IL-10 levels in LPS stimulated PBMCs were found to be increased in both groups on day 60 as compared to day 0 with a statistical significance (p = 0.0007) observed in CIT group. (Figure 3).

Figure 3: Effect of CIT on TNF-α & IL-10 in LPS stimulated PBMCs

***p< 0.001 compared to day 0 in CIT group using Wilcoxan Rank- Sum test

The MDA levels in CIT group significantly (p = 0.0189) decreased on day 60 compared to baseline; while in placebo group, the MDA levels increased on day 60. The median GPx levels in both the groups exhibited increase on day 60 as compared to day 0 (Table 4).

Table 4: Effect of CIT on MDA and GPx

Parameters	CIT group (n=11)		Placebo group (n=7)	
	Day 0	Day 60	Day 0	Day 60
MDA (mmol/ml)	5.01 ± 2.24	3.48 ± 1.49*	2.85 ± 1.42	3.20 ± 1.21
GPx (nmol/min/ml)	1339.7 (264.9 – 2760.9)	1589.3 (387.1 –4604.9)	1151.2 (860.9- 1619.8)	1377.9 (407.5-1818.5)

*p< 0.05 compared to day 0 using Paired t test

Discussion:

The present clinical study aimed to evaluate the immunomodulatory effects of Charak immunity tablets (CIT). We observed a significant increase in Peak expiratory flow rate compared to baseline in CIT group. CIT significantly improved questionnaire based assessment parameters viz. immune status, quality of life and perceived stress. There was an increase in the serum IFN γ levels on day 30 followed by a decrease on day 60. The levels of TNF-α (pro-inflammatory cytokine) reduced in CIT group along with a significant increase in the levels of IL-10 (anti-inflammatory cytokine) at the end of the study. The oxidative stress (MDA) levels too showed a significant decrease in CIT group compared to baseline. The parameters haemogram, SGOT, SGPT and serum creatinine remained within their normal reference ranges at the end of the study indicative of the safety of drug CIT.

CIT is a polyherbal formulation that consists of herbs with known immunomodulatory activities. In the present study, we evaluated its effect using a wide array of parameters. Peak Expiratory Flow Rate (PEFR) is a simple indicator of lung capacity. [35] We carried out the study during the COVID-19 pandemic. The decreased lung capacity in individuals infected with SARS-CoV-2 is well reported. [36,37] On this background, a significant increase in the PEFR after CIT administration seen in our study participants is important.

CIT also showed significant improvement in parameters assessed by different questionnaires. We assessed immune status of the study participants using Immune Status Questionnaire, which gives an idea

about self-perception of the health status. Our study showed a significant increase in immune status after CIT treatment compared to baseline as well as placebo suggestive of a positive effect of CIT. Further, we measured the perceived stress levels of the study participants pre- and-post intervention. The profound effect of physiological factors like stress on immune system is well known.[12] A moderate level of stress impacting mental health of general population during the quarantine period of COVID has been reported. [38,39] Through the concept of psycho neuro endocrine immune (PNEI) axis the link between psychological and physical health is well established.[40] The association of PNEI axis with negative impacts of SARS-CoV-2 infection has also been reported lately.[41] We observed a significant decrease in stress levels in CIT group on day 60. Thus, the decreased stress levels are suggestive of improved immunity. Further, it is seen that many social, environmental, lifestyle factors promote pathology of systemic chronic inflammation in our body leading to disorders which result in reduced immune functioning and subsequently have a negative impact on wellbeing and quality of life.[42] A handful of studies done during this pandemic situation have shown reduction of scores in different domains pertaining to quality of life.[43-45] In our study, CIT improved scores in all the 4 domains of QOL, suggestive of better quality of life despite a harsh social crisis such as COVID.

Apart from the questionnaires, we studied the effect of CIT on different cells involved in immune responses. CD4+ T cells (T helper cells) are involved in elimination of pathogens as part of adaptive immune response along with regulation of the cytokine secretion.[46] Monitoring the levels of CD4+ is thereby essential to understand the extent of immune status and its role in development of immune response.[47] These cells are also critical for the generation of high-affinity memory B cells, long-lived plasma cells, and memory CD8+ T cells.[48] On the other hand CD8+ cells are surface markers for cytotoxic T lymphocytes which are critically important in cell mediated immunity to confront virally infected cells through endogenous antigen presentation.[3] This makes them essential for maintaining protective immunity to many classes of infectious pathogens.[48] Further, the effects of psychological stress on immune functions have also been observed, which include decreased percentages of CD4 helper T cells and CD8 cytotoxic T cells along with other factors.[49] CD4/CD8 ratio is also considered as one of the important factors for description of overall immune dysfunction and can be used as a better biomarker for disease progression.[50]

In the present study, CD4+ count was maintained in both the groups at the end of the study, while CD8+ counts showed a decrease, when compared to baseline. There was a significant increase in this ratio in both the groups in comparison to baseline levels. An important point to highlight here is that the major ingredient of the placebo used in our study was starch. Prior study on starch has suggested its immune boosting effect in management of COVID 19 infection.[51] This supports the positive effects of placebo observed by us in case of few parameters.

Serum IFN-γ levels increased on day 30 compared to baseline, but the levels went down on day 60. The placebo group in contrast, showed a decrease on day 30 followed by an increase on day 60. However, these levels were within normal range as per the studies reported earlier. [52,53] IFN-γ is primarily released on the activation of CD4+ cells and is chief

mediator of innate and adaptive immune response [54]. IFN-γ up regulates a variety of pro-inflammatory parameters such as interleukin IL-12, IL-15 and tumor necrosis factor- α (TNF-α).[55] It has also been reported that IFN-γ has the potential to direct inflammatory responses by inhibiting production of pro-inflammatory cytokines, IL-1 and IL-8, by upregulating the production of cytokine antagonists such as IL-1Ra and IL18BP.[56]

TNF-α is a pro-inflammatory cytokine which is released in pathogenic conditions. Increased level of TNF-α is indicative of high inflammation in the cell.[57] On the contrary IL-10 is an anti-inflammatory cytokine which acts as an antagonist and thereby inhibits the production of pro-inflammatory cytokines, IFN-γ and TNF-α. [58-61] Our results have shown decreased level of TNF-α from LPS stimulated PBMCs in CIT group on day 60 suggesting less amount of inflammation in cells, while in placebo group, increased levels of TNF-α were seen on day 60 indicative of higher degree of inflammation after stimulation. Though IL-10 levels increased in both the groups, the increase was more evident in CIT group compared to placebo. Thus, by its immune regulatory action, IL 10 did not allow elevation of levels of TNF α in the CIT group compared to placebo on day 60.

Generation of Reactive oxygen species (ROS) can lead to peroxidation of lipids present inside cells and form products like MDA, which can be quantified to determine the level of oxidative damage to the cell.[62] Studies have suggested that compromised immune functioning can increase ROS production (Reactive Oxygen Species) in RBCs and oxidative stress inside cell[13,14] which can cause an effect on cell integrity, membrane damage and hemolysis of RBSs.[63] Thus, MDA level of biological membranes becomes one of the most studied indicators of oxidative stress.[64]A decreased level of MDA observed after 60 days of CIT treatment indicates its efficacy to reduce oxidative stress in RBCs. No such trend was observed in placebo group. On the other hand, free radicals deteriorate the immune system, leading to various diseases and early ageing, therefore antioxidants play an important part in cell defence mechanism. Glutathione peroxidase (GPx), is an enzyme which is an integral part of antioxidant system to protect cells from oxidative damage that can be caused by accumulation of hydrogen peroxide.[64] It has been studied for its effect in modulating processes like normal cellular growth and proliferative responses and apoptosis. It is also involved in modulating pro-inflammatory responses.[65] In this study, both CIT and placebo groups showed an increase in levels of GPx on day 60 indicating its antioxidant potential. As mentioned earlier, starch used as placebo has been reported to possess antioxidant properties.[66] Besides, another study by Li. et al on fish fed with starch has shown significantly higher antioxidant potential compared to fish fed with glucose. [67]

It is well known that starch as a component is widely used in formulation of placebo drugs in clinical studies.[68] However, as per our study findings, the placebo drug containing starch was seen to possess an important role in immune function along with antioxidant properties and thus its use as placebo in trials on immunity should be considered with better understanding.

Limitations:

The up rise of COVID pandemic during the month of March, resulting in subsequent lockdown, interfered with the study conduct. Almost 50% of study participants could not report to the study site for the follow up visits thereby leading to high attrition.

Conclusion:

It can be inferred from the results of the study that administration of CIT drug for two months improved immune status and quality of life with a decrease in stress. It was found to be effective in reduction of oxidative stress in the RBCs and enhanced its antioxidant capacity. It thus proved to possess immunomodulatory effect on healthy individuals. However, it still needs a larger sample size and detailed scientific investigations towards T cell activation and secretory cytokine levels.

Conflicts of interest- The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

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