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Analysis of Immunomodulatory Effects of Herbal Formulation by Random Clinical Trial Against Post Covid 19 Vaccination

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Abstract:- Traditional medicine has demonstrated its efficacy in treating a vast array of ailments, including infectious ones, over the years. Because of their immunomodulatory and other pharmacological actions, polyherbal formulations are suitable for COVID-19 infection prevention. After two Covaxin doses, human immunological markers were shown to diminish a few days later. This study evaluated the effect of Abayakasthaa plus (IP) capsules on the immune levels of volunteers who had two Covaxin injections. The IP is a mixture of around 11 indigenous plant extracts that have been demonstrated to possess immune-boosting properties independently. In a multispecialty hospital in central India, 30 volunteers were randomised into two groups of 15 each for a pilot randomised experiment for 28 days. Four immunological indicators (IgG) & Covaxin Dose (CD3+, CD4+, and CD8+ cell count) were evaluated on the 0th and 28th day following immunisation using blood samples. For independent samples, the t-test helped compare mean biomarker values between two groups, while the paired t-test helped compare marker values in follow-ups.At day 28, the mean (geometric) concentration of IgG antibodies in the test group was substantially greater than in the placebo group (p=0.04). Although the concentration of the marker reduced considerably in the placebo group (p = 0.021), it stayed virtually constant in the test group (p = 0.824). On day 28, other metrics were comparable and showed negligible differences between groups. The results of a preliminary study show that herbal preparations increase COVID-19 vaccination-induced immunogenicity for an extended length of time. To justify the results, more research with bigger cohorts is necessary.

Keywords*: COVID-19 vaccination, Clinical trial,Herbal formulation, Immunomodulation.*

I. INTRODUCTION

Now, a global pandemic consisting of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has significantly increased morbidity & mortality[1]. It is believed that limited preexisting immunity to this virus causedan exponential surge in cases.Virtually all SARS-CoV-2 transmission models have assumed that infection confers immunity to reinfection for one yearat least, which is crucial to theauthorities of public health implementing & supervising non-pharmaceutical therapies, sera'stherapeutic value from sick persons, & serological testing's capacity to identify immune people[2].The length of immunity to SARS-CoV-2 determines the totaltrajectory of the pandemic & post-pandemic dynamics; therefore, one must comprehend protective immunity's temporal dynamics[3].Similar to past introductions of new viruses, large global SARS-CoV-2 outbreaks may endanger persistence by limiting accessible susceptible hosts.It originally surfaced during December 2019, originating in Wuhan, China, &has spread since theninto becoming a pandemic having high morbidity and fatality rates[4].SARS-CoV transmission primarily occurred after the beginning of the disease and was linked to early respiratory virus levels that were low, peaking about 10 days after the

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onset of symptoms[5].Innate immune genes and SARS-CoV-2 factors of entry are evidenced significantly in the nasal epithelial cells.The herbal capsule(IP)used in this investigation was a mixture of 11 different herbal extracts(**Table 1**).Some of the pharmacological actions are mentioned here.[6

Table 1: Ingredients of herbal formulation

Serial No.	Herbal ingredients
1	*Withania somnifera*
2	*Terminalia chebula*
3	*Tinosporacordifolia*
4	*Emblicaofficinalis*
5	*Asparagus racemosus*
6	*Piper longum*
7	*Zingiberofficinale*
8	*Embelicaribes*
9	*Glycyrrhizaglabra*
10	*JasadBhasma*
11	*ShankhBhasma powder*

Ashwagandha [Withaniasomnifera (L.) Dunal] bioactive components have demonstrated the ability to maintain immunological homeostasis, regulate inflammation, inhibit pro-inflammatory cytokines, protect several organs, and have antiviral, anti-stress, and anti-hypertensive activities. Terminalia has multiple pharmacological and medicinal properties, including antimutagenic, anti-arthritic, anticaries, and wound healing properties. Ashwagandha [Withaniasomnifera (L.) Dunal] bioactive components have demonstrated the ability to maintain immunological homeostasis, regulate inflammation, inhibit pro-inflammatory cytokines, protect several organs, and have antiviral, anti-stress, and anti-hypertensive activities. Terminalia has multiple pharmacological and medicinal properties, including antimutagenic, anti-arthritic, anticaries, and wound healing properties. Rest all the herbal extracts are associated with various activities related to vaccine-induced immunogenicity individuals(Diagram 1).In this present investigation, various therapeutic qualities of ingredients of the capsule, such as antiviral, anti-inflammatory, analgesic, immunomodulatory, antioxidant, etc., have been used in this study for Covaxin-induced persons. The person will have 100% antibodies against COVID-19 after taking Covaxin to produce immunogenicity against COVID-19. In a few days, a reduction in antibody titre weakens immunity against COVID-19. At this time, ingestion of the proposed herbal pill would boost cell-based immunity. By acting on T-cells and B-cells that are already creating antibodies against COVID-19 as a result of Covaxintreatment, herbal capsules may increase the generation of COVID-19-specific antibodies, therefore preventing COVID-19 infection[7].

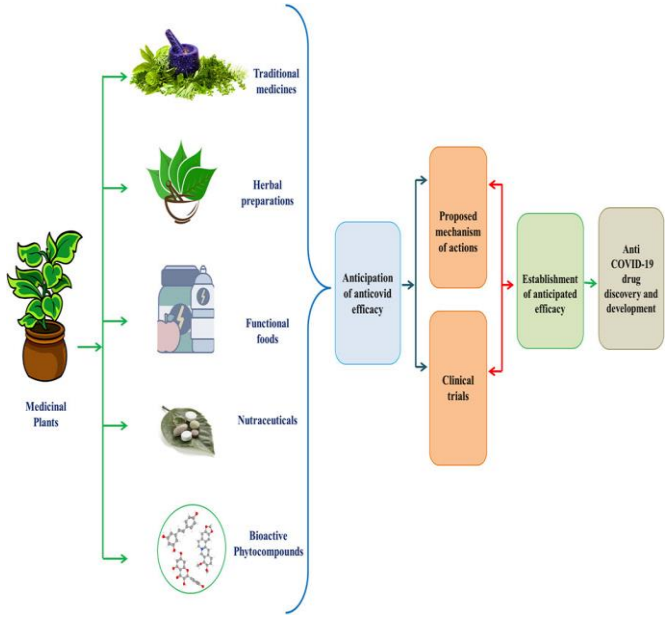

Fig 1:Bioactive compounds for therapeutic treatment of Covid-19
(*SafaetAlam et al.,Front. Pharmacol., 2021 ,Sec. Ethnopharmacology,*)

➢ *Objectives*

This study aims to evaluate the immunogenicity increase (specific cellular immune responses (CD3+, CD4+, and CD8+) of a herbal formulation in COVID-19-vaccinated subjects who received two doses of Covaxin.

II. METHODOLOGY

A. Design of the study

The trial design was a double-blind&placebo-controlled, randomised, parallel-group proof-of-concept study[Clinical Trial Registry–India (CTRI Registration Number): CTRI/2021/07/034625] to evaluate the safety & immunogenicity of a herbal preparation in healthy subjects of Indian origin who have received the COVID-19 vaccination (Covaxin). The research was done at a single-centre, multi-speciality hospital in a central India megacity.

B. Outcome measurements

Change in specific cellular immune responses (CD3+, CD4+, and CD8+) was the primary objective [Time Frame: Day 0 to Day 28], while a change in IgG antibody for RBD and spike protein COVID-19 antibody levels was the secondary endpoint [Time Frame: Day 0 to Day 28].

C. Authorizationand recruitment

The study was authorized & conducted by a board of institutional review in line with the Helsinki Declaration.Participants who provided written agreement to participate in the research were subsequently screened and recruited for the study. The institutional ethics committee assessed the protocol and granted consent for the experiment to be conducted in the multi-speciality hospital located in a megacity in central India on July 9, 2021.Also, the content of informed consent was examined, and only the authorised version of the informed consent document was presented to trial participants.

D. Inclusion Criteria

- A subject's consentfor trial participation must be documented in writing.
- Men and females aged 18 and older and 70.
- Negative test results for human immunodeficiency virus types 1 & 2
- Individuals who have received a second dosage of COVAXIN duri days are eligible for enrollment in the research.
- At the screening visit, the COVID-2019 Reverse Transcriptase Reaction (RT-PCR) test was negative (72 hours previous to Visit 1 [

E. Exclusion Criteria

- Any vaccine or immunisation administered within 30 days prior to (with the exception of hormonal contraceptives) & immune-globul blood products therapy being incomplete 30 days prior to enrollment
- Immunosuppressants were discontinued within three r enrollment.Tuberculosis and persistent systemic infections.
- The exclusion criteria consist of hypersensitivity or allergic re biological products, known allergic reactions to study product con exacerbation of allergic diseases on enrollment day.

F. Randomization and Evaluation Schedule

The trial participants were given 450 mg capsules of the active or twice daily. The randomization into two groups was accomplished biostatistician using computer-generated codes in a 1:1 ratio and was inc staff.

G. Monitoring Clinical Trial

Throughout the length of the clinical study, an independent cl visited the clinical site to assess the individuals' source papers and case monitoring visit was conducted to ensure that the site team had enroll trial in accordance with the protocol's eligibility criteria, maintained accountability of investigational products, obtained written informed consent from study subjects, and minimized errors in site documentation.

H. Adverse Reactions & Co-Medication

The blood samples were obtained before and after the treatment period, on day 0 and day 28, to determine whether there are any aberrant haematology and serum chemistry parameters.Participants were contacted by telephone to enquire about fever or other vaccine-related adverse effects, if any, and to remind them to take the study medicine as prescribed. In addition, the participants were asked if they had used concomitant drugs throughout their stay at home, and the information was documented on the applicable case record forms.

I. Statistical Calculation

Considering there was no previous data and this was proof-of-concept research, a 5% change in the immunological markers between the two treatment groups was deemed sufficient to validate the effectiveness of the test product with 80% power.Consideration was given to a modest sample size of 30 participants, with 15 subjects in each group. The t-test for independent samples helped compare mean biomarker values between the two groups, whilst paired t-test helped compare marker values among visits 1 & 4 in every group**(Table 2).** A p-value lesser than 0.05 is statistically significant, &analyses were conducted usingSPSS version 26.0 software (IBM Corp. USA).

III. RESULTS

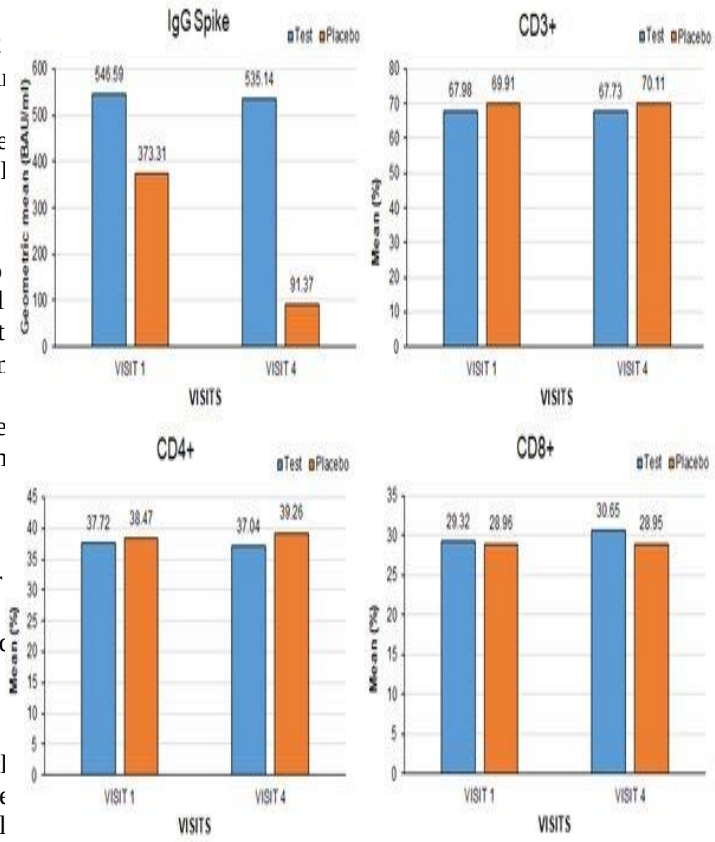

Fig 2: The mean values for immunological parameters of subjects in two groups at visit 1 and visit 4.

Patients' descriptive statistics for demographic and anthropometric featuresare divided into 2 groups(Test and placebo)described in **figure2**. In addition, the gender distribution in the two mentioned groups was statistically insignificant (p=0.690), with males predominating in both(Statistical results summarized in Table 2).At the first visit, the geometric mean of the IgG spike between the two groups did not vary substantially (p=0.619). At visit 4, however, the geometric mean IgG spike in the test group (535.14 SD: 7.70 BAU/ml) was considerably larger than in the placebo group (91.37 SD: 13.36) with a p-value of

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0.047.The decrease in IgG concentration between visits 1 and 4 happens to be statistically insignificant in the test group (p=0.824), whereas it does happen to be significant in the placebo group (p=0.021). Mean change on log scale from visit 1 to visit 4 was substantially different between the test group (0.98 SD: 1.44 BAU/ml) and placebo group (0.24 SD: 8.19 BAU/ml) with a p-value of 0.024.Several immunological measures, such as CD3+, CD4+, and CD8+, demonstrated statistically insignificant changes between the two groups at visits 1 and 4. Both groups' mean values fell within the usual range.

Table 2:Statistical Results of Laboratory Parameters

Parameters		P-value	
Clinical		VISIT 1	VISIT 4
	Pulse rate (beats/min)	0.268	0.222
	Respiratory rate (/min)	0.999	0.115
	Systolic BP (mmHg)	0.344	0.761
	Diastolic BP (mmHg)	0.514	0.818
Haematological	RBC (million/mm3)	0.256	0.075
	Haemoglobin (gm/dl)	0.68	0.836
	MCV (fl)	0.506	0.157
	Haematocrit	0.514	0.348
	Neutrophils (%)	0.423	0.67
	Eosinophils (%)	0.955	0.677
Biochemical	ALT (U/L)	0.928	0.380
	Serum creatinine (mg/dl)	0.702	0.657
	Bilirubin (mg/dl)	0.148	0.421

IV. DISCUSSION

The polyherbal adjuvants may complement numerous antigenic epitopes of infections, such as SARS Cov-2, and might also be capable of mitigating viral alterations.Using bioactive herbal components as therapeutic agents & adjuvants can bepotent immune stimulants.SARS-CoV-2 is a positive-strand RNA virus that encodes for envelope protein (E), spike protein (S), nucleocapsid protein (N) & a number of open reading frames (ORFs).The RBD (Receptor Binding Domain) of the S protein binds to the human ACE2 (Angiotensin-converting enzyme 2) receptor, followed by its priming & cleavage with cellular serine protease.The interaction among the SARS-CoV-2 virus & the human immune system may result in the clinical manifestation of COVID-19(**Figure 3**). While adaptive and innate immune systems are engaged to combat COVID-19, adaptive immune responses are essential for SARS-CoV-2 viral clearance, which includes T and B cells. Bcells generate neutralising antibodies, & IgG is the most effective antibody against COVID-19 infections. Peripheral T cells express CD4 or CD8 on their surfaces in conjunction with CD3 as part of the T-cell-receptor (TCR) complex.Herbal remedies are renowned for their possible antioxidant, antibacterial, antitumor, and antiviral qualities. It is a well-known fact that the antioxidant activity of herbs is the key to increasing adaptive and innate immune responses via inter-cell communication and regulation of antigen-presenting cells.From this study, it was revealed that the number of B cells increases during the first month of infection and then stabilizes thereafter. SARS-CoV-2-specific CD4+ T cells grow then fall having a half-life of around 2-3 months; CD8+ T cell assessments varied, with at least one research showing [8]almost no reduction in the first three months after infection.Reduced vaccination efficacy may result from a combination of declining antibody titres and diminished neutralising power in the context of extensive circulation of variants with partial immunological escape [9],[10].Those who got two doses of Covaxin vaccination in the current trial were examined for the maintenance of immunological markers as a result of their synergistic effects in light of this decline in immune levels. The herbal formulation contains substances that have been independently proven as immune boosters.During visits 1 and 4, the mean values for CD3+, CD4+, and CD8+ in the present investigation were comparable to those of normal persons. IgG COVID-19 antibodies (IgG antibody for RBD and Spike protein) results were likewise favourable for the test group.

Importance of herbal intervention enhances the immunity (antibodies)following vaccine is a very important finding in this proof of concept study. Otherwise in vaccinated person the immunity reduces significantly over a period of time, which necessities booster dose at certain intervals. Combination of vaccination and herbal formulation will help to prolong the immunity offered and may reduce the requirement of booster dose. So it is concluded that that the adaptability and immune response potential of herbal formulation (blended by 11 extracts) may create a balance between human life and health by maintaining his or her immunity.

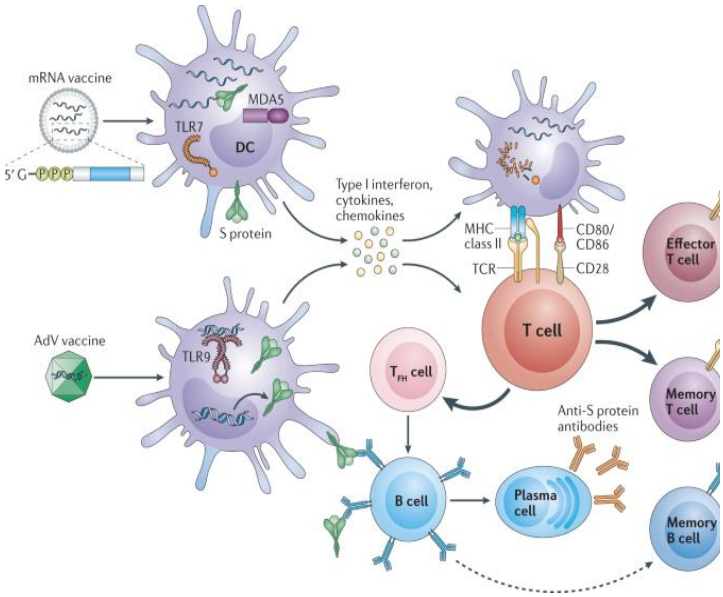

Fig 3: SARS-CoV-2 immunity induced by mRNA and adenovirus vector vaccines
(Source: Teijaro, J.R.and Farber, D.L. 2021, *Nat Rev Immunol* **)**

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V. CONCLUSION

The present clinical investigation concludes that the dynamics of decreasing immune indicators following vaccination with Covaxin may be addressed using herbal test items, which effectively exhibited persistent immune markers even one month after therapy. Several researchers have already described the mechanism of action of the separate components of the exploratory test product, as well as the projected synergistic effect of the combination of these medicinally enriched items.In a prior clinical trial with the same medication done on patients with COVID-19 without vaccination, there was a substantial decrease in viral load and an improvement in clinical condition.The new study is an extension of the previous experiment and again verified our results in volunteers who received two doses of Covaxin. This of course needs study in larger population to confirm our results. Secondly such kind of study has not been carried out earlier as per our literature search. No adverse reactions were seen in this study which confirm safety of formulation and combination herbal preparation vaccine.

REFERENCES

[1]. Chinazzi, M, Davis JT, Ajelli M, Gioannini C, Livinova M, Merler S. et al. The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. *Science*. 2020; 368:395-400.
[2]. Read JM, Bridgen JRE, Cummings DAT, Ho A, Jewell CP. Novel coronavirus 2019-nCoV: early estimation of epidemiological parameters and epidemic predictions. *medRxiv*. 2020*;*
[3]. Kucharski AJ, Russell TW, Diamond C, Liu Y, Edmunds J, Funk S, et al. Early dynamics of transmission and control of COVID-19: a mathematical modelling study. *Lancet Infect Dis*.2020; 20:553-558.
[4]. Casadevall A, Pirofski LA. The convalescent sera option for containing COVID-19. *J Clin Invest*. 2020; 130:1545-1548.
[5]. Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. *Science*. 2020;368:860-868.
[6]. Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med*. 2020; 26:681-687.
[7]. Hossan MS, Fatima A, Rahmatullah M, Khoo TJ, Nissapatorn V, Galochkina AV et al. Antiviral activity of EmbeliaribesBurm. f. against influenza virus in vitro. *Arch Virol*. 2019; 163:2121-2131.
[8]. Naaber P, Tserel L, Kangro K, Sepp E, Jurjenson V, Adamson A et al. Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. *Lancet RegHealth Eur*. 2021; 10:100208
[9]. Rosenberg, ES, Holtgrave DR, Dorabawila V, Conroy M, Greene D, Lutterlo E. et al. New COVID-19 Cases and Hospitalizations Among Adults, by Vaccination Status – New York, May 3-July 25,2021. *MMWR Morb Mortal Wkly Rep*. 2021. 70: 1150-1155.
[10]. Chemaitelly H, Tang P, Hasan MR, Almukdad S, Yassine HM, Benslimane FM et al. Waning of BNT162b2 Vaccine Protection against SARS-CoV-2 Infection in Qatar. N *Engl J Med*. 2021;385:e83.