 doi:10.20944/preprints202205.0094.v1

Article

A Randomized, Placebo Controlled, Double Blinded, Parallel Group Clinical Study to Evaluate the Efficacy and Safety of AEV01 for Mild COVID19 in Elderly Patients at Risk of Complications

Ramesh Kannan[a], Vanitha Muralikumar[b], Vamseedhar Annam[c] , Sakthi Balan[d], Aravinda Kumar[e,*], , Sekhar MM[f] and Renu Medasani[g]

[a]Assistant Professor, Department of Pharmacology, Government Vellore Medical College, Vellore, Tamilnadu, India
[b]Professor, Department of Pathology, Raja Rajeswari Medical College & Hospital, Bengaluru, India
[c]Director, Dhanwanthralaya Ayurveda Speciality Hospital, Chennai, Tamilnadu, India.
[d]Principal Investigator, Ki3 Clinical Research Organization, Chennai, Tamilnadu, India.
[e]Associate Professor, Department of Pharmacology, Pondicherry Institute of Medical Sciences, Pondicherry, India
[f]Founder-Director, Astrel Genome Ltd, Hyderabad, India
[g]Medical Consultant, Astrel Genome Ltd, Hyderabad, India
*Corresponding author:Email: aravindkumar007@gmail.com

Abstract: Background: While several drugs are in the pipeline for treatment of various grades of COVID-19 disease, none of them have shown promise until now. Medicinal plants are crucial in developing and developed countries for their primary and basic health needs owing to better tolerability, superior compatibility with human body and having lesser side effects. AEV01 , a speciality extract of kutki (*Picrorhiza kurroa*), is one potential medicinal herb which can be effective for mild COVID19 in elderly patients at risk of complications and aging immunity. Purpose: The efficacy and safety of AEV01 for mild COVID-19 in elderly patients at risk of complications was investigated in this study. Study design: A prospective, phase 3, randomized, multicentric, placebo controlled double blinded parallel group interventional trial. Methods: This study was conducted in elderly patients diagnosed as COVID-19 with mild symptoms. 70 patients were randomized into two groups to receive AEV01 capsule (100mg) and placebo respectively thrice daily for 30 days along with standard care of treatment as per national WHO/ICMR guidelines. Clinical improvement timelines and corresponding scores using 8-point ordinal scale and NEWS were assessed for efficacy and safety of AEV01 in COVID-19 patients. Results: AEV01 group showed a significant improvement (P=0.0001) in the normalization of the SpO_2 rate and relief from cough. There was also significant difference in time to recovery, with patients in the AEV01 drug treated group recovering in 4.5 days as compared to Placebo in 9.1 days. ESR, LDH, serum ferritin, Neutrophil-Lymphocyte ratio (NLR), TNF- alpha, IL-6 and CD4 cell counts, which are considered as prognostic markers in COVID19 infected patients, showed a significant improvement in patients treated with AEV01. The AEV01 drug treated group showed significant clinical improvement in the 8-point ordinal scale severity rating and NEW Score from day 3 onwards which continued up to end of study. Conclusion: AEV01 has shown a significant improvement in clinical and laboratory parameters. There was also reduction in the progression of the COVID-19 disease in unvaccinated elderly patients from mild to moderate when treated at an earlier stage.

Keywords: COVID-19; *Picrorhiza kurroa;* AEV01; NEW Score; Immunomodulatory; unvaccinated elderly population

Abbreviations:

 doi:10.20944/preprints202205.0094.v1

WHO	World Health Organization
ICMR	Indian Council of Medical Research
SpO_2	Oxygen saturation
ESR	Erythrocyte sedimentation rate
LDH	Lactate dehydrogenase
NEWS	National Early Warning Score
CD	Cluster differentiated cell
NLR	Neutrophil lymphocyte ratio
TNF-α	Tumour necrosis factor-alpha
AST	Aspartate transaminase
ALT	Alanine aminotransferase

1. Introduction:

COVID-19 has impacted several lives globally during the second wave of the pandemic. The risk of severe illness from COVID-19 increases with age. Elderly people are at high risk of infection due to their decreased immunity and associated comorbidities like hypertension, diabetes and chronic kidney disease. The course of the disease has been reported to be severe in these population resulting in higher mortality. Effectiveness of vaccines are also less in elderly due to decreased T and B-Cells with aging. Inspite of several precautionary measures after being advised by the Indian government, the spread of COVID-19 infection in these elderly population was steadily increasing.(Martins Van Jaarsveld, 2020)

The present treatment strategy for COVID-19 is supportive care supplemented by antibiotics, antivirals, immunomodulators like monoclonal antibodies, corticosteroids and convalescent plasma.(Yang et al., 2020) Scientists all over the world are working hard to develop effective treatments. The drugs being investigated range from repurposed flu treatments to failed ebola drugs, to antimalarial drugs developed decades ago, followed by vaccines.(Lythgoe and Middleton, 2020) On the other hand, the traditional medicines may serve as an alternative to support COVID-19 treatment. Combining modern and traditional therapy might prevent the progression of disease, and reduce the intensity of symptoms, death rate and side effects.

There is plenty of data supporting the effectiveness of tradition Indian/AYUSH formulations in treating viral infections. AEV01, is a root extract of *Picrorhiza kurroa*, developed *by Astrel*. *Picrorhiza kurroa* is an important alpine herb of Himalayan region, growing in an altitudinal range of 3000- 5000 meters in temperate belts. In Indian system of medicine, it is known as "Kutki" and constitutes an important drug out of 2000 drug items derived from vegetable sources. It is known to have variety of medicinal values. (Kant et al., 2013; Soni and Grover, 2019)

Kutki has been reported to have therapeutic effects on some disorders and has been shown to have anti-inflammatory, antioxidant, choleretic, hepatoprotective, immunostimulant, neuritogenic, neuroprotective, antiallergic and anti-cancer effects.(Kumar et al., 2021) It is also marketed as nutritional supplement globally. Kutki root extract contains several glycosides including three iridoid glycosides, picroside I, II and III and are used in various drug formulations as strong hepatoprotective and immunomodulatory compounds.(Sultan et al., 2016)·(Puri et al., 1992)

With the above background, this study was done with the objectives to study the efficacy and safety of AEV01 (a speciality kutki extract) for mild COVID19 in elderly patients at risk of complications.

2. Materials and Methods:

Study design and trial site:
This study is a phase 3, randomized, multicentric, placebo controlled double blinded parallel group interventional trial conducted at secondary care centres namely Dhanwanthralaya Ayurveda Speciality Hospital in Chennai (India), P.M. Medical Centre in Vellore (India) and , Raja Rajeswari Medical College and Hospital in Bengaluru (India).

 doi:10.20944/preprints202205.0094.v1

2.1 Patient enrolment and inclusion criteria

Patients who were diagnosed with COVID-19 by a standardized RT-PCR assay having mild symptoms (fever, rhinorrhoea, mild cough, sore throat, malaise, headache, muscle pain with no shortness of breath) with no signs of a more serious lower airway disease and with respiratory rate <20/min, heart rate < 90/min and oxygen saturation >94% on room air, of either sex, aged above 50 years of age and who voluntarily signed the informed consent to participate in the study were included in the study.

2.2 Exclusion criteria

The following criteria were used to exclude patients from the study: Patients with moderate and severe illness of COVID19, hepatic insufficiency (ALT or AST > 5 times the upper limit of normal), stage-4 Chronic Kidney disease patients or requiring dialysis, immunocompromised patients on immunosuppressants, participation in any other ongoing COVID-19 trial, COVID-19 vaccinated patients, uncontrolled diabetes mellitus (HbA1C >10%).

2.3 Sample size and Study groups

A sample size of 33 in each group would have 80% power to detect a probability of 0.7 that the time to clinical improvement in AEV01 group was less than the time to improvement in Placebo group using a Wilcoxon (Mann-Whitney) rank-sum test with a 0.05 two-sided significance level. Considering 5% drop outs, the sample size was fixed as 35 per group.

This was estimated based on a non-parametric test under the assumptions that there will be 2 days difference in time to clinical improvement between the two groups, where the standard deviation for both the groups are assumed to be same and approximately 2.86 days.
A total sample size of 70 participants were randomized into 2 groups with 35 in each group.

Placebo Group – Patients diagnosed as mild SARS-CoV-2 infection received Placebo+ standard of care treatment as per hospital regulation and WHO/ICMR guidelines.
AEV01 Group - Patients diagnosed as mild SARS-CoV-2 infection received AEV01+ standard of care treatment as per hospital regulation and WHO/ICMR guidelines.

2.4 Consent and ethical approval

The study was approved by the Independent Ethics Committees of all the study centres and registered prospectively at CTRI [CTRI/2021/04/032804]. Recruitment of participants was done as per the inclusion and exclusion criteria after taking informed written consent from the eligible participants.

2.5 Intervention:

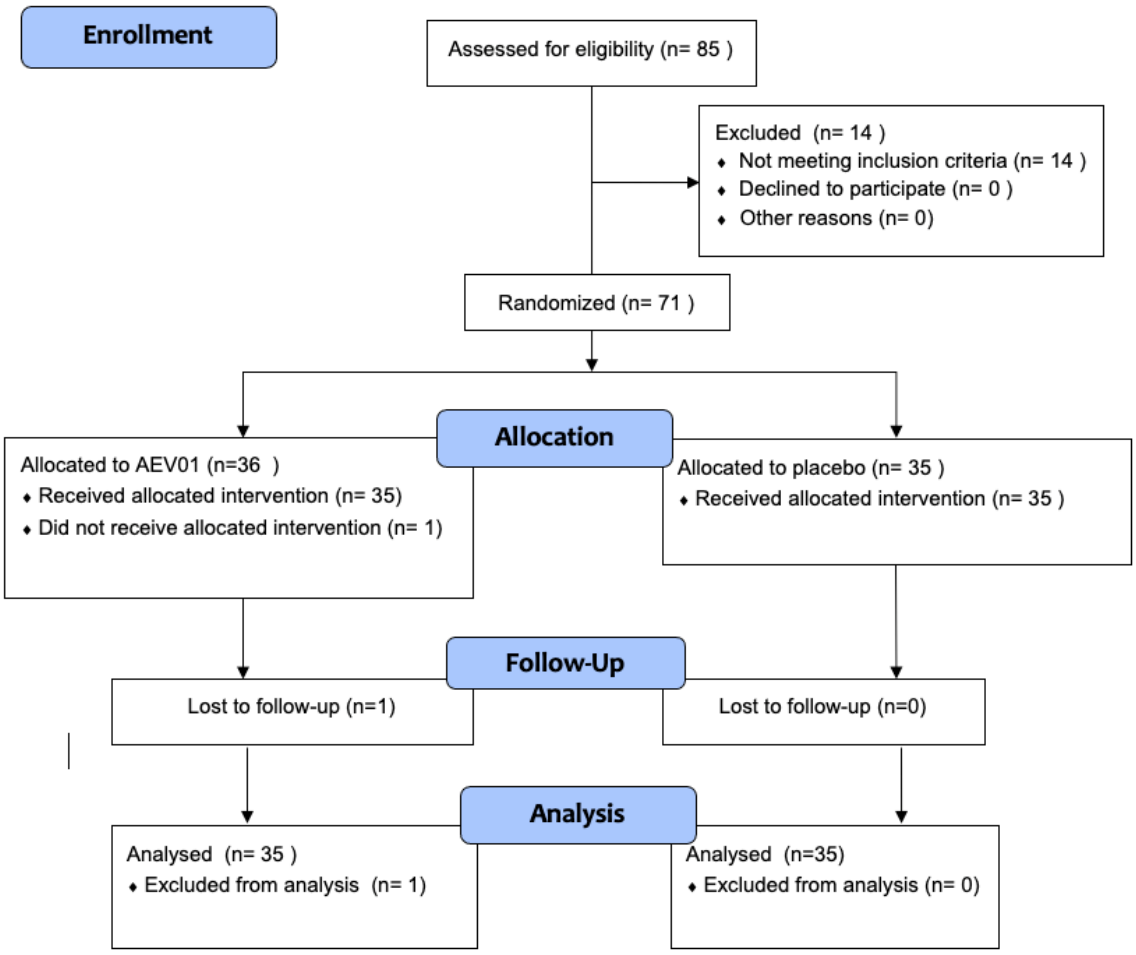
The study drug AEV01, available as capsule formulation, was sponsored by Mylan Laboratories Pvt Ltd, India. Active ingredient of one AEV01 capsule contains *Picrorhiza kurroa* speciality extract 100mg. The drug was given orally in a dose of 1 capsule (100mg) thrice daily after food for 30 days. This dosage regime was determined based on 1-3grams per day of raw herb suggested by Indian System of Medicine. All the enrolled patients also received standard of care treatment as per the hospital regulation and WHO/ICMR guidelines.
Study was conducted during the period 1st April,2021 to 30TH July, 2021 i.e. peak of Delta Variant spread in India.

2.6 Outcome measure/efficacy evaluation:

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 May 2022 doi:10.20944/preprints202205.0094.v1

The primary outcome measure was to assess the efficacy of the drug measured as "time for clinical improvement" defined by normalization of pyrexia and body pain, respiratory rate (RR) less than 24/minute, oxygen saturation (spO_2) greater than 94% and relief from cough, maintaining all together for more than 72 hours.

The secondary outcome measures included percentage of subjects reporting each severity rating on a 8-point ordinal scale, change from baseline in biochemical, immunological, haematological parameters and National Early Warning Score (NEWS), cumulative incidence of adverse events, time to recovery and incidence/duration of hospitalization/oxygen use.



2.8 Statistical analysis

The data on discrete variables has been represented as n (%). The data on continuous variables has been represented as mean (SD). The continuous data has been analysed by using One-way Repeated Measure ANOVA with Bonferroni correction in post-hoc analysis. A p-value of <0.05 has been considered significant. The data was analysed using SPSS Version 21.0.

 doi:10.20944/preprints202205.0094.v1

Figure 1: Consort 2010 flow diagram

3. Results:

This study enrolled 70 patients diagnosed with COVID-19 by a standardized RT-PCR assay having mild symptoms with no signs of a more serious lower airway disease. The baseline demographic characteristics as shown in table 1 had no difference between the AEV01 and placebo group. Both the groups were comparable with respect to age, sex and BMI.

Table 1: Baseline characteristics of the study participants

Parameters	**Placebo (mean±SD)**	**AEV01 (mean±SD)**	**p value**
Age (years)	62.14 ± 8.12	63.25 ± 11.43	
Gender Male (n) Female (n)	 19 16	 19 16	P >0.05
BMI (Kg/m²)	24.1 ± 3.7	24.2 ± 3.4	
Blood glucose (g/dL)	205.3 ± 127.3	193.6 ± 100.2	
Liver function tests Bilirubin SGOT SGPT	 1.16 ±4.49 45 ± 35 49 ± 42.3	 0.53 ± 0.19 38.1 ± 30.1 48.1 ± 59.8	 0.48 0.38 0.94
Renal function test Urea Creatinine	 32.2 ± 14.4 1 ± 0.3	 29.9 ± 14.9 0.9 ± 0.3	 0.51 0.14
Serum electrolytes Sodium Potassium Chloride	 139.4 ± 3.6 4.26 ± 0.63 105.5 ± 25.4	 138.7 ± 3.2 4 ± 0.52 100.5 ± 1.9	 0.42 0.06 0.25

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 May 2022 doi:10.20944/preprints202205.0094.v1

Time to clinical improvement was assessed based on four outcome parameters namely normalization of pyrexia and body pain, respiratory rate less than 24/minute, SpO_2 rate greater than 94% and relief from cough. Time to recovery was recorded as the time taken for clinical improvement.

Table 2: Time to recovery in placebo and AEV01 group COVID19 patients

	Placebo Mean (SD)	**AEV01** Mean (SD)	P-value
Time to Recovery (days)	9.12 (4.36)	4.51 (1.96)	0.0001
Duration of hospitalization (days)	3.68 (4.13)	2.23 (2.08)	0.069
Duration of Oxygen use (days)	1.5 (2.73)	0.69 (1.20)	0.112

Time to clinical recovery time is defined as the time from initiation of study treatment until normalization of fever, respirator rate, oxygen saturation and alleviation of cough, sustained for at least 72 hours, whichever comes first. There is a significant improvement in the time to recovery in AEV01 drug treated group compared to Placebo. Patients in the AEV01 drug treated group recovered in 4.5 days when compared to Placebo in 9.1 days as shown in table 2.

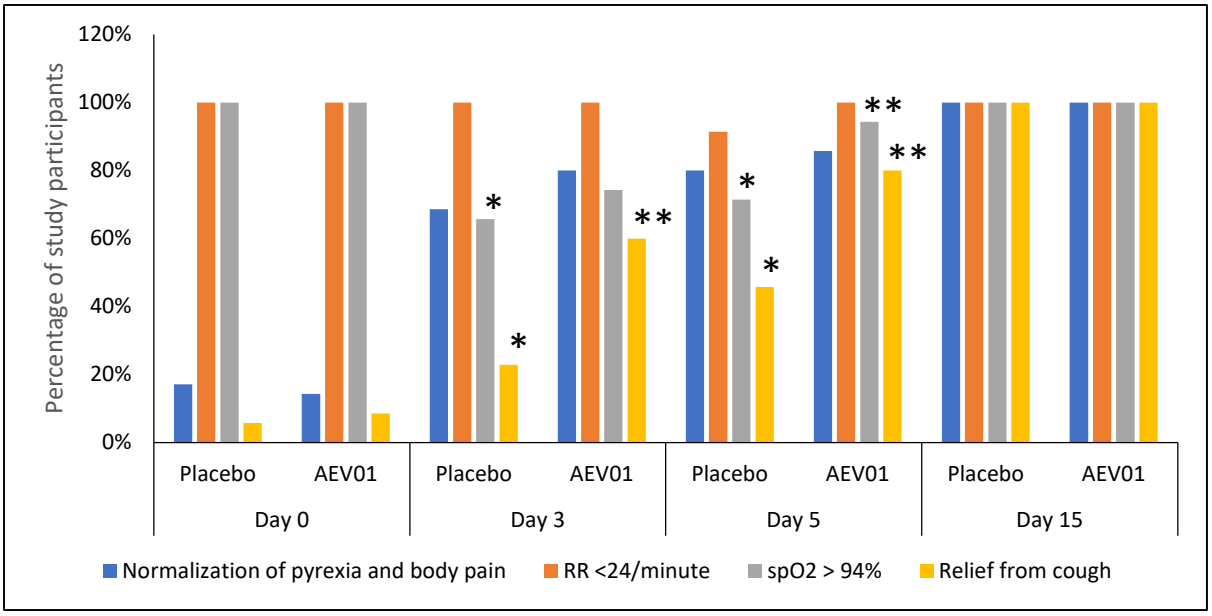


Figure:2 Time to clinical improvement during the course of COVID-19 disease in the elderly population

There is a significant improvement (P=0.001) in the normalization of the SpO_2 rate and relief from cough on day 3 and day 5 in AEV01 group when compared to the placebo group. All patients in the study maintained respiratory rate <24. Though normalization of pyrexia and body pain is numerically higher in AEV01 group as compared to placebo on day 3 and day 5, this was not statistically significant. (figure 2)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 May 2022 doi:10.20944/preprints202205.0094.v1

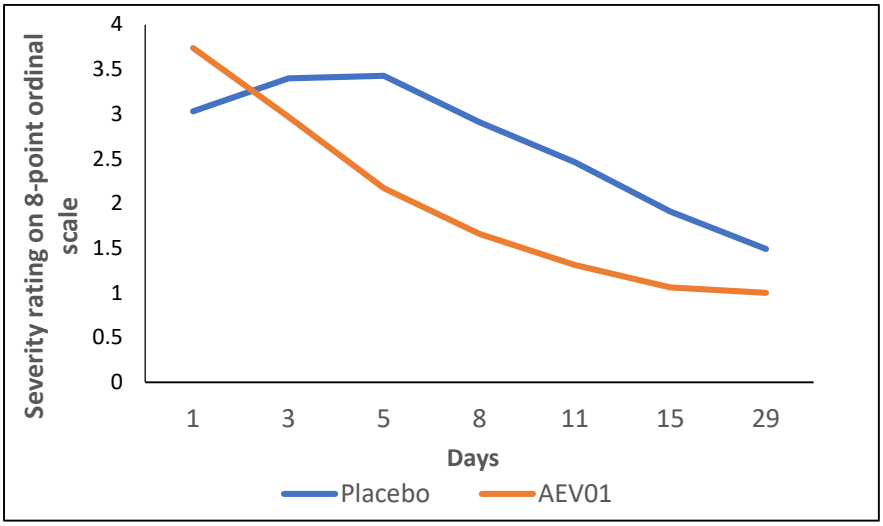


Figure 3: Severity rating score based on 8-point ordinal scale between placebo and AEV01 group patients.

From figure 3, it is evident that the AEV01 drug treated group showed significant improvement in the 8 point ordinal scale severity rating from day 3 onwards which continued up to day 29. But in Placebo treated group, the severity rating score deteriorated on day 3 and day 5, following which an improvement is shown, which is also evident in the above graph.

Table 3: COVID severity progression in COVID-19 patients of placebo and AEV01 group

COVID severity		Baseline (day 0)	Day 3	Day 5	Day 15	P value
AEV01	Mild	35 (100%)	26(74%)	33	35(100%)	0.0001
	Moderate	0	9 (25.7%)	2(5.7%)	0	
	Severe	0	0	0	0	
Placebo	Mild	35 (100%)	23 (66%)	25(71%)	35(100%)	0.0001
	Moderate	0	12 (34.3%)	10 (28.6%)	0	
	Severe	0	0	0	0	

Progression of patients from mild to moderate COVID19, with oxygen requirement was more in Placebo group when compared to AEV01 group [12(34.3%) in placebo group vs 9 (25.7 %) in AEV01 group on day 3 and 10 (28.6 %) in placebo group vs 2 (5.7%) in AEV01 group on day 5] as shown in table 3.

Table 4: Hematological, biochemical and immunological parameters of study participants at baseline compared to end of study

Parameter	Placebo			AEV01		
	Day 0	Day 29	P value	Day 0	Day 29	p value
Total leucocyte count	7961.71 (3054.93)	7581.06 (1871.07)	0.01	8360.86 (4711.61)	7527.77 (1400.43)	0.07
Neutrophil	79.71(11.65)	72.91(14.02)	0.06	73.14(14.79)	56.69(8.77)	0.4
Lymphocyte	15.49(9.93)	18.62(12.87	0.053	20.48(12.21)	36.73(7.67)	0.515
Neutrophil to Lymphocyte ratio	5.18 (1.17)	3.92(1.06)	0.0001*	3.57(1.2)	1.54(1.78)	0.0001*
ESR	18.03(20.098)	14.29(34.9)	0.54	25.45 (24.80)	11.86 (13.90)	0.001*
CRP (0 - 6 mg/L)	34.42(49.18)	8.8(8.91)	0.001*	56.91(14.8)	3.62(6.69)	0.164

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 May 2022 doi:10.20944/preprints202205.0094.v1

S ferritin	438.109(422.10)	90.59(69)	0.0001*	282.95(221.09)	94.85(88.95)	0.0001*
LDH (230 - 450u/L)	361.55(176.53)	146.01(79.9)	0.001*	353.1(163.39)	156.06(88.38)	0.001*
D-dimer (0 - 500ng/mL)	922.74(175.07)	261.34(30.2)	0.009	690.53(69.5)	152.96(24.06)	0.015
TNF-α	12.65(17.6)	24.7(9.7)	0.451	22.85(2.71)	6.5(1.84)	0.008*
IL-6 (0 - 7pg/mL)	25.10(55.17)	14.37(39.1)	0.06	36.24(9.63)	3.07(0.8)	0.043*
CD4	515.02 (300.70)	952.28 (452.56)	0.039*	698.5 (417.14)	1099.62 (447.70)	0.175
CD8	297.25 (254.75)	535.37 (313.39)	0.128	400.5 (300.81)	605.25 (377.55)	0.404
CD4/CD8 ratio	2.76(2.59)	2.38(1.83)	0.478	2.37(1.85)	2.73(2.49)	0.5
CD4 naïve cell count	159.3571 (124.93)	254.28(11.0 69)	0.043*	231.57(164.77)	442.21(278.46)	0.021*
CD8 naïve cell count	84(83.67)	100.14(76.37)	0.586	64.21(66.80)	153.07(21.8)	0.134
Glucose	205.3 (127.3)	141.1 (41.1)	0.67	193.6 (100.2)	142.3 (40.0)	0.89
AST	45 (35)	27.9(10.2)	0.38	38.1 (30.1)	28.7 (11.9)	0.75
ALT	49 (42.3)	35.5(15.1)	0.94	48.1 (59.8)	35.5 (14.4)	0.99
S creatinine	1.0 (0.3)	0.9 (0.2)	0.14	0.9 (0.3)	0.9 (0.1)	0.53

The hematological, immunological and biochemical parameters of all study participants were measured at various time points during the study period and they were analyzed by comparing the baseline values with the values obtained at the end of study as shown in table 4. A number of parameters namely ESR, LDH, serum ferritin, neutrophil lymphocyte ratio, TNF- α, IL-6 and CD4 naïve cell counts, which are considered as prognostic indicators in COVID19 infected patients, showed a significant improvement in patients treated with AEV01 compared to the placebo group.

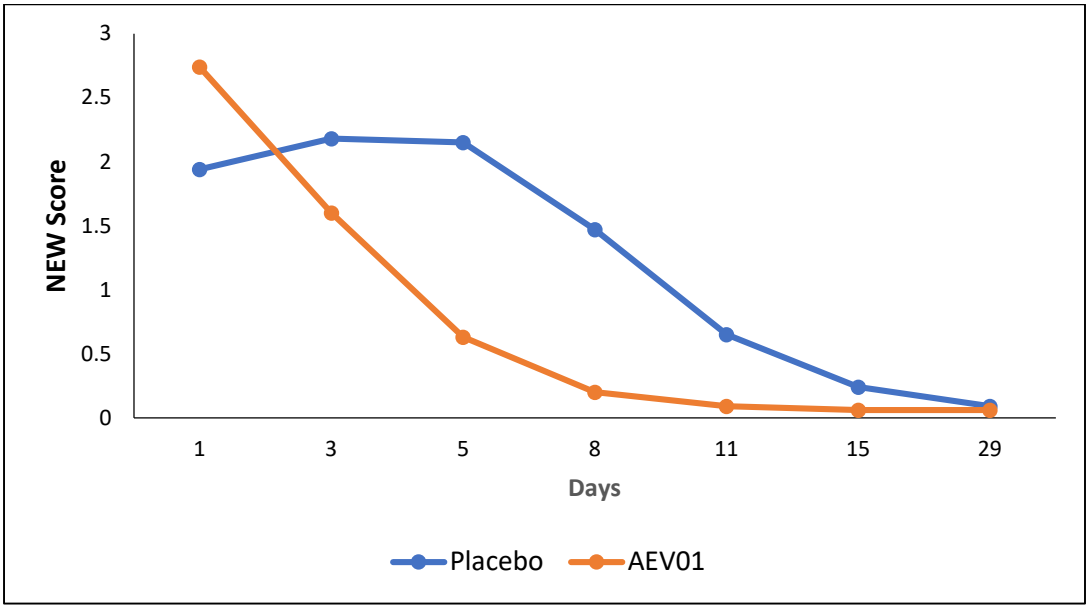


Figure 4: National early warning score (NEWS)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 May 2022 doi:10.20944/preprints202205.0094.v1

Figure 4 shows the pattern of NEW Score in both groups. In AEV01 group, there is improvement in the NEW Score severity rating from day 3 onwards which continued up to day 29. But in Placebo treated group, the NEW Score deteriorated on day 3 and day 5, following which it has shown an improvement, which is also evident in the above graph.

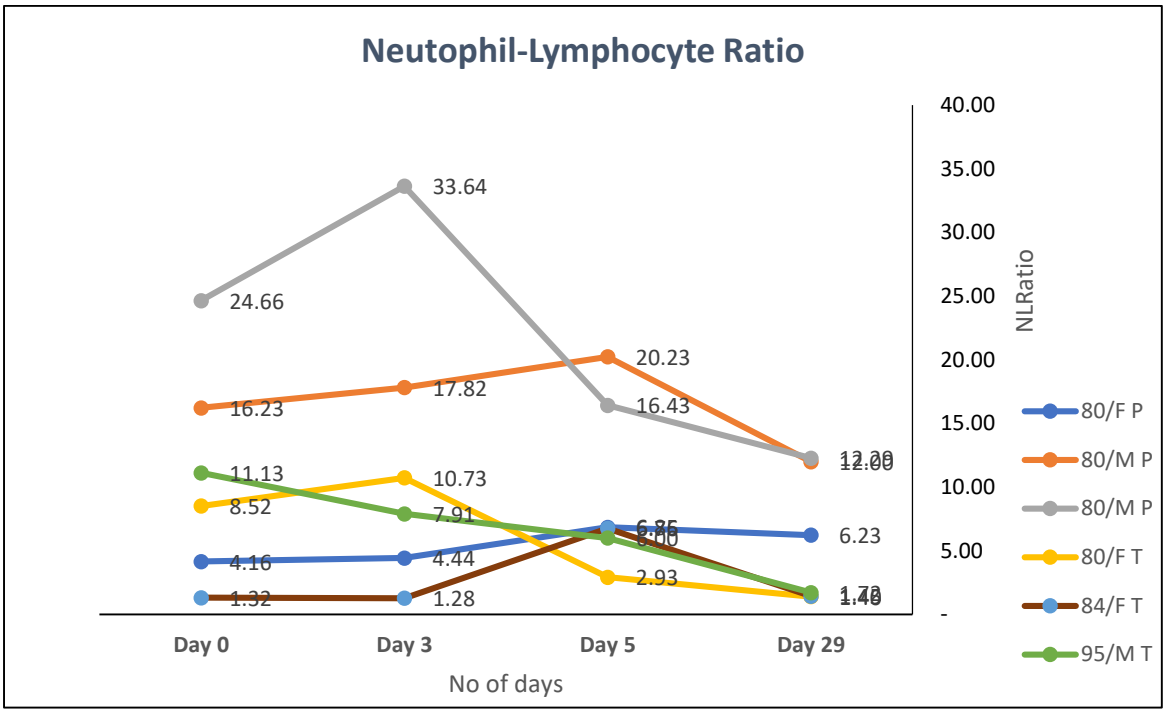


Figure 5: Neutrophil-lymphocyte ratio – in patients aged above 80 years of age
P- placebo, T- AEV01 treated group

In figure 5, it is seen that the neutrophil-lymphocyte ratio is markedly improved in the AEV01 group in patients above 80 years of age.

Table 4: Adverse events reported in both groups during study period

Sl. No	Adverse Events	Test Group (N)	Placebo group (N)
1	Gastrointestinal side effects	7	11
2	Hypoglycemia	3	0
3	Allergic/ Hypersensitivity reactions	0	0

There was no incidence of laboratory adverse events. However, there were hypoglycaemic episodes in 3 patients and gastrointestinal side effects in 7 patients treated with AEV01 as shown in table 4. 11 patients in placebo group reported gastrointestinal side-effects like nausea, dyspepsia and diarrhoea.

Discussion:

 doi:10.20944/preprints202205.0094.v1

Medicinal plants are crucial for about 80% of the world population in developing and developed countries for their primary and basic health needs owing to better tolerability, superior compatibility with human body and having lesser side effects.(Mahmood et al., 2014) Kutki is one such herbal plant widely used in Ayurvedic and Unani traditional medicine systems in India with the rhizomes/roots valued for their effectiveness in various ailments.
This study was conducted in patients diagnosed with mild COVID-19 disease, where the patients were randomized to AEV01 and placebo treatment groups after satisfying the study criteria as per the protocol. The baseline demographic profile of all the enrolled participants were similar ($p>0.05$) as shown in table 1. The primary outcome was to estimate the time to clinical improvement based on symptoms and measurable vital parameters. The AEV01 group showed a more significant improvement ($P=0.0001$) in the normalization of the oxygen saturation (SpO_2) (94.3% in AEV01 vs 71.4% in placebo) and relief from cough (80% in AEV01 vs 45.7% in placebo) by day 5 of the treatment. There was no significant improvement in the normalization of Pyrexia and body pain and respiratory rate in both the study groups. However, with respect to time to clinical recovery, which is defined as the time from initiation of study treatment until normalization of fever, respirator rate, oxygen saturation and alleviation of cough, sustained for at least 72 hours, whichever comes first, patients in the AEV01 drug treated group recovered in 4.5 days when compared to Placebo in 9.1 days. Treatment with Remdesivir had also shown a similar median time of 5 days in COVID-19 patients.(Garibaldi et al., 2021) There were no mortality during the study period and none of the patients worsened and went for ICU admission or mechanical ventilation.

Several scoring systems have been validated in the past for predicting poor outcomes and mortality. Severity rating ordinal scales like 8-point scale is one among them which has been used in this study. AEV01 drug treated group showed significant improvement in the 8 point ordinal scale severity rating from day 3 onwards which continued up to day 29, while placebo group showed a deterioration on days 3 and 5, following which it showed a delayed improvement.

Ferritin, C-reactive protein (CRP), ESR, D-dimer and LDH have emerged as potential markers of severity of COVID-19 disease, although none have a definitive prognostic value.(Guan et al., 2020) In this study, ESR, LDH, serum ferritin, neutrophil lymphocyte ratio, TNF- alpha, IL-6 and CD4 naïve cell counts, which are considered as signs of improvement in COVID19 infected patients, showed a significant improvement in patients treated with AEV01 compared to the placebo group.

National Early Warning score (NEWS2) is another tool that is simple and can be used easily by health care staff to identify high risk patients and escalate clinical care. Such stratification of risk would enable the medical team for quicker decision making and divert more attention, resources and time to those identified as high risk for fatal outcome.(Cr et al., 2021)A study by Pugazhvannan et al(Cr et al., 2021) had utilized the NEWS tool to predict that a score of more than 5 at admission there will be need for mechanical ventilation and may lead to mortality in COVID-19 patients. In this study, the baseline NEWS score was only 2.74 (AEV01 group) compared to 1.94 (placebo group) and hence there was no mortality among the study participants without any need for mechanical ventilation.

The active constituents of Picrorhiza kurroa are kutkin, picrosides, vanillic acid and cinnamic acid. Kutkin is reported to have anti-inflammatory activity and are predicted to have potential attachment and inhibition against SARS-CoV2 via targeting spike glycoprotein as well as its host receptor ACE2. Further studies have reported that active constituents of kutki have passed the criteria of drug-like molecule and demonstrated good pharmacokinetic profile with minimum predicted toxicity level.(Maurya et al., 2020)

TNF-α and IL-6 are found to be significantly decreased in patients treated with AEV01 compared to placebo group. Kutki root extract contains iridoid glycosides and its aglycons that can inhibit TNF-α, IL-6 and NK-κB. These immunomodulatory effects of AEV01 could be via picroliv, picroside-I and kutkoside,etc. are mediated through the stimulation of both cell-mediated and humoral immune responses with enhanced T-cell response.(Jeong et al., 2002),(Pandey and Das, 1989),(Win et al., 2019) It has been reported that immunomodulatory action of kutki is due to an increase in the proliferation of lymphocytes and cytokines like IL-4 and IFN-gamma.(Gupta et al., 2006) During

 doi:10.20944/preprints202205.0094.v1

infections, it improves the macrophage function and cell surface antigens through increased T-cell immunity.(Sidiq et al., 2011)

Oxidative stress originated by reactive oxygen species in COVID-19 disease can cause pulmonary fibrosis by the apoptosis of alveolar epithelial cells. These can be controlled by administration of vitamin C rich foods. L-ascorbic acid, picrosides and picrorhizaosides are the major phytochemicals present in Picrorhiza kurroa and hence can be beneficial in COVID-19 patients. (Morikawa et al., 2020)

AEV01 treated group has shown modulation of innate and adaptive immunity which is evident with NLR, IL-6 and lymphocytes count which is most needed for early recovery from COVID-19. Early correction and improvement of NLR could possibly prevent permanent lung damage, fibrosis and long COVID. NLR was identified as an independent risk factor for critical illness in patients with COVID19 disease. It has been predicted that the incidence of critical illness is far less (9%) for patients aged >50 years and having a NLR <3.13. (Liu et al., 2020)

To our surprise, AEV01 was able to reduce NLR and modulate lymphocytes in aged adults like 80yrs and above when compared to placebo. In our study, the NLR was only 1.54 on day 29 when compared to 3.92 in placebo group which denotes the incidence of disease progression is very low. AEV01, as supportive therapy may help in modulating immunity in 80years and above , which may help increasing vaccines efficacy in that age group.

CD4+T and CD8+T play a vital role in maintaining immune function and viral clearance in the body. It has been reported that CD4+T and CD8+T counts significantly decreased in COVID-19 patients.(Zhang and Wu, 2020) Increased CD4/CD8 ratio has been identified as a risk factor for critical illness in COVID19 disease.(Pallotto et al., 2020) Wang et al(Wang et al., 2020) has reported that low CD8 cell count and increased CD4/CD8 ratio were associated with increased inflammation in COVID19 disease. In this study, there is increased CD8 counts after 1 week of treatment and the CD4/CD8 ratio is decreased which clearly denotes that AEV01 has markedly reduced the inflammatory changes associated with COVID-19 disease.

The naive CD4+ T cell compartment has long been considered as consisting of a homogeneous population of antigen-inexperienced cells, identified by specific surface markers. In humans, naive CD4+ T cells typically express CCR7, CD62L, and CD45RA, while lacking expression of CD45RO. In this study, AEV01 has shown immunomodulation by increase in naïve CD4+ T cell count and this might have reversed lymphopenia in these patients, improved T cell immunity and might prevent reinfection in future.

Eosinophils are circulating and tissue-resident leucocytes that have potent proinflammatory effects in a number of diseases. Eosinophil is a beneficial marker for COVID-19 lung infection. Fall in eosinophil count is more prone for respiratory distress and progression to severe COVID19 disease. Recently eosinophils have been shown to have various other functions, including immunoregulation and antiviral activity.(Xie et al., 2021) In our study, AEV01 group has shown increase in eosinophil count by day 5 compared to control group and that was evidenced by reduction in oxygen requirement by day 5 in AEV01 group.

These evidences all together suggest that AEV01 can be a potential drug in treatment of COVID-19 disease.

Conclusion:

The study results have shown that progression of patients from mild to moderate COVID19, with oxygen requirement was more in Placebo group (34.3 %) when compared to AEV01 group (25.7 %). AEV01 group showed a more significant improvement in the normalization of the SpO_2 rate and relief from cough when compared to the Placebo group. The AEV01 drug treated group showed significant clinical improvement in the 8-point ordinal scale severity rating and NEW Score from day 3 onwards which continued up to end of study. But in Placebo treated group, the severity rating score deteriorated on day 3 and day 5, following which it showed an improvement. Thus, AEV01 has shown a significant

 doi:10.20944/preprints202205.0094.v1

improvement clinically and has reduced the progression of the COVID-19 disease in elderly patients from mild to moderate when treated at an earlier stage.

Acknowledgements

The authors would like to thank Ki3 Clinical Research Organization and Mylan Pharmaceuticals for supporting the study.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication that could have influenced its outcome.

References:

Cr, P., Vanidassane, I., Pownraj, D., Kandasamy, R., Basheer, A., 2021. National Early Warning Score 2 (NEWS2) to predict poor outcome in hospitalised COVID-19 patients in India. PLoS One 16, e0261376. https://doi.org/10.1371/journal.pone.0261376

Garibaldi, B.T., Wang, K., Robinson, M.L., Zeger, S.L., Bandeen-Roche, K., Wang, M.-C., Alexander, G.C., Gupta, A., Bollinger, R., Xu, Y., 2021. Comparison of Time to Clinical Improvement With vs Without Remdesivir Treatment in Hospitalized Patients With COVID-19. JAMA Netw Open 4, e213071. https://doi.org/10.1001/jamanetworkopen.2021.3071

Guan, W., Ni, Z., Hu, Yu, Liang, W., Ou, C., He, J., Liu, L., Shan, H., Lei, C., Hui, D.S.C., Du, B., Li, L., Zeng, G., Yuen, K.-Y., Chen, R., Tang, C., Wang, T., Chen, P., Xiang, J., Li, S., Wang, Jin-lin, Liang, Z., Peng, Y., Wei, L., Liu, Y., Hu, Ya-hua, Peng, P., Wang, Jian-ming, Liu, J., Chen, Z., Li, G., Zheng, Z., Qiu, S., Luo, J., Ye, C., Zhu, S., Zhong, N., 2020. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 382, 1708–1720. https://doi.org/10.1056/NEJMoa2002032

Gupta, A., Khajuria, A., Singh, J., Bedi, K.L., Satti, N.K., Dutt, P., Suri, K.A., Suri, O.P., Qazi, G.N., 2006. Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa. Int Immunopharmacol 6, 1543–1549. https://doi.org/10.1016/j.intimp.2006.05.002

Jeong, H.-J., Koo, H.-N., Na, H.-J., Kim, M.-S., Hong, S.-H., Eom, J.-W., Kim, K.-S., Shin, T.-Y., Kim, H.-M., 2002. Inhibition of TNF-alpha and IL-6 production by Aucubin through blockade of NF-kappaB activation RBL-2H3 mast cells. Cytokine 18, 252–259. https://doi.org/10.1006/cyto.2002.0894

Kant, K., Walia, M., Agnihotri, V.K., Pathania, V., Singh, B., 2013. Evaluation of Antioxidant Activity of Picrorhiza kurroa (Leaves) Extracts. Indian J Pharm Sci 75, 324–329. https://doi.org/10.4103/0250-474X.117438

Kumar, V., Singh, S.B., Singh, S., 2021. Pharmacological Perspectives of Ayurvedic Herbs viz. Alstonia scholaris L., Picrorhiza kurroa, Swertia chirata and Caesalpinia crista Against COVID-19: A Mini-Review. MROC 18, 841–849. https://doi.org/10.2174/1570193X17999201102200944

Liu, J., Liu, Y., Xiang, P., Pu, L., Xiong, H., Li, C., Zhang, M., Tan, J., Xu, Y., Song, R., Song, M., Wang, L., Zhang, W., Han, B., Yang, L., Wang, Xiaojing, Zhou, G., Zhang, T., Li, B., Wang, Y., Chen, Z., Wang, Xianbo, 2020. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med 18, 206. https://doi.org/10.1186/s12967-020-02374-0

Lythgoe, M.P., Middleton, P., 2020. Ongoing Clinical Trials for the Management of the COVID-19 Pandemic. Trends Pharmacol Sci 41, 363–382. https://doi.org/10.1016/j.tips.2020.03.006

Mahmood, S., Hussain, S., Tabassum, S., Malik, F., Riaz, H., 2014. Comparative phytochemical, hepatoprotective and antioxidant activities of various samples of Swertia Chirayita collected from various cities of Pakistan. Pak J Pharm Sci 27, 1975–1983.

Martins Van Jaarsveld, G., 2020. The Effects of COVID-19 Among the Elderly Population: A Case for Closing the Digital Divide. Front Psychiatry 11, 577427. https://doi.org/10.3389/fpsyt.2020.577427

Maurya, V.K., Kumar, S., Bhatt, M.L.B., Saxena, S.K., 2020. Antiviral activity of traditional medicinal plants from Ayurveda against SARS-CoV-2 infection. J Biomol Struct Dyn 1–17. https://doi.org/10.1080/07391102.2020.1832577

Morikawa, T., Nakanishi, Y., Inoue, N., Manse, Y., Matsuura, H., Hamasaki, S., Yoshikawa, M., Muraoka, O., Ninomiya, K., 2020. Acylated iridoid glycosides with hyaluronidase inhibitory activity from the rhizomes of Picrorhiza kurroa Royle ex Benth. Phytochemistry 169, 112185. https://doi.org/10.1016/j.phytochem.2019.112185

Pallotto, C., Suardi, L.R., Esperti, S., Tarquini, R., Grifoni, E., Meini, S., Valoriani, A., Di Martino, S., Cei, F., Sisti, E., Piani, F., Botta, A., Salomoni, E., Baragli, F., Blanc, P., 2020. Increased CD4/CD8 ratio as a risk factor for critical illness in coronavirus disease 2019 (COVID-19): a retrospective multicentre study. Infectious Diseases 52, 675–677. https://doi.org/10.1080/23744235.2020.1778178

Pandey, B.L., Das, P.K., 1989. Immunopharmacological studies on Picrorhiza kurroa Royle-ex-Benth. Part IV: Cellular mechanisms of anti-inflammatory action. Indian J Physiol Pharmacol 33, 28–30.

Puri, A., Saxena, R.P., Sumati, null, Guru, P.Y., Kulshreshtha, D.K., Saxena, K.C., Dhawan, B.N., 1992. Immunostimulant activity of Picroliv, the iridoid glycoside fraction of Picrorhiza kurroa, and its protective action against Leishmania donovani infection in hamsters. Planta Med 58, 528–532.

Sidiq, T., Khajuria, A., Suden, P., Sharma, R., Singh, S., Suri, K.A., Satti, N.K., Johri, R.K., 2011. Possible role of macrophages induced by an irridoid glycoside (RLJ-NE-299A) in host defense mechanism. Int Immunopharmacol 11, 128–135. https://doi.org/10.1016/j.intimp.2010.10.017

Soni, D., Grover, A., 2019. "Picrosides" from Picrorhiza kurroa as potential anti-carcinogenic agents. Biomedicine & Pharmacotherapy 109, 1680–1687. https://doi.org/10.1016/j.biopha.2018.11.048

Sultan, P., Jan, A., Pervaiz, Q., 2016. Phytochemical studies for quantitative estimation of iridoid glycosides in Picrorhiza kurroa Royle. Bot Stud 57, 7. https://doi.org/10.1186/s40529-016-0121-2

Wang, F., Nie, J., Wang, H., Zhao, Q., Xiong, Y., Deng, L., Song, S., Ma, Z., Mo, P., Zhang, Y., 2020. Characteristics of Peripheral Lymphocyte Subset Alteration in COVID-19 Pneumonia. J Infect Dis 221, 1762–1769. https://doi.org/10.1093/infdis/jiaa150

Win, N.N., Kodama, T., Lae, K.Z.W., Win, Y.Y., Ngwe, H., Abe, I., Morita, H., 2019. Bis-iridoid and iridoid glycosides: Viral protein R inhibitors from Picrorhiza kurroa collected in Myanmar. Fitoterapia 134, 101–107. https://doi.org/10.1016/j.fitote.2019.02.016

Xie, G., Ding, F., Han, L., Yin, D., Lu, H., Zhang, M., 2021. The role of peripheral blood eosinophil counts in COVID-19 patients. Allergy 76, 471–482. https://doi.org/10.1111/all.14465

Yang, Y., Islam, M.S., Wang, J., Li, Y., Chen, X., 2020. Traditional Chinese Medicine in the Treatment of Patients Infected with 2019-New Coronavirus (SARS-CoV-2): A Review and Perspective. Int J Biol Sci 16, 1708–1717. https://doi.org/10.7150/ijbs.45538

Zhang, H., Wu, T., 2020. CD4+T, CD8+T counts and severe COVID-19: A meta-analysis. J Infect 81, e82–e84. https://doi.org/10.1016/j.jinf.2020.06.036