Investigator's Brochure

CimetrA

Medical Oral Spray

Sponsor's Name: MGC Pharma Ltd

Product Name: CimetrA

Release Date: 5 Sep, 2021

Version: 04

authorization from MGC Pharma, except to the extent necessary to obtain informed consent from those persons to whom the device will be administered.

LIST OF ABBREVIATIONS

AE Adverse Event

ALT/SGPT Alanine Aminotransferase / Serum Glutamic Pyruvic Transaminase

API Active Pharmaceutical Ingredient

AST/SGOT Aspartate Aminotransferase / Serum Glutamic Oxaloacetic Transaminase

BMI Body mass Index

BSA Body Surface Area

BW Body Weight

CBC Complete Blood Count

GMP Good Manufacturing Practice

CI Confidence Interval

Cmax Maximum Plasma Concentration

CRP C-Reactive Protein

CTCAE Common Terminology Criteria for Adverse Events

EC Ethics Committee

ECG Electrocardiogram

EOT	End of Treatment
FU	Follow Up
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonization
IEC	International Expert Committee
IMP	Investigational Medicinal Product
kg	Kilogram
mg	Milligram
ml	Milliliter
MoA	Mechanism of Action
MoH	Israeli Ministry of Health
NA	Not Applicable
NGSP	National Glycohemoglobin Standardization Program
P-SLD	Placebo Sublingual Drops
PK	Pharmacokinetic(s)
PSQI	Sleep Disturbance Questionnaire
QoL	Quality of Life
RBC	Red Blood Cell
ROS	Reactive Oxygen Species
SAE	Serious Adverse Event

SD Standard Deviation

SOC Standard of Care

SOP Standard Operation Procedures

T1/2 Elimination half-life

TBD To be Determined

Tmax Time to maximum plasma concentration

WBC White Blood Cell (Count)

WHO World Health Organization

1. INTRODUCTION

According to the World Health Organization (WHO), viral diseases continue to emerge and represent a serious issue to public health. In the last twenty years, several viral epidemics such as the severe acute respiratory syndrome coronavirus (SARS-CoV) from 2002 to 2003, and H1N1 influenza in 2009, have been recorded. Most recently, the Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in Saudi Arabia in 2012. [1]

In a timeline that reaches the present day, an epidemic of cases with unexplained low respiratory infections detected in Wuhan, the largest metropolitan area in China's Hubei province, was first reported to the WHO Country Office in China, on December 31, 2019. Published literature can trace the beginning of symptomatic individuals back to the beginning of December 2019. As they were unable to identify the causative agent, these first cases (n=29) were classified as "pneumonia of unknown etiology." The Chinese Center for Disease Control and Prevention (CDC) and local CDCs organized an intensive outbreak investigation program. The etiology of this illness was attributed to a novel virus belonging to the coronavirus (CoV) family.

On February 11, 2020, the WHO Director-General, Dr. Tedros Adhanom Ghebreyesus, announced that the disease caused by this new CoV was a "COVID-19," which is the acronym of "coronavirus disease 2019". In the past twenty years, two additional CoVs epidemics have occurred. SARS-CoV provoked a large-scale epidemic beginning in China and involving two dozen countries with approximately 8000 cases and 800 deaths (fatality rate of 9,6%) [2], and the MERS-CoV that began in Saudi Arabia and has

approximately 2,500 cases and 800 deaths (fatality rate of 35%) and still causes as sporadic cases [3].

In a meeting on January 30, 2020, per the International Health Regulations (IHR, 2005), the outbreak was declared by the WHO a Public Health Emergency of International Concern (PHEIC) as it had spread to 18 countries with four countries reporting human-to-human transmission. An additional landmark occurred on February 26, 2020, as the first case of the disease, not imported from China, was recorded in the United States.

Initially, the new virus was called 2019-nCoV. Subsequently, the task of experts of the International Committee on Taxonomy of Viruses (ICTV) termed it the SARS-CoV-2 virus as it is very similar to the one that caused the SARS outbreak (SARS-CoVs).

The CoVs have become the major pathogens of emerging respiratory disease outbreaks. They are a large family of single-stranded RNA viruses (+ssRNA) that can be isolated in different animal species. [4]. For reasons yet to be explained, these viruses can cross species barriers and can cause, in humans, illness ranging from the common cold to more severe diseases such as MERS and SARS. Interestingly, these latter viruses have probably originated from bats and then moving into other mammalian hosts — the Himalayan palm civet for SARS-CoV, and the dromedary camel for MERS-CoV — before jumping to humans. The dynamics of SARS-Cov-2 are currently unknown, but there is speculation that it also has an animal origin.

The potential for these viruses to grow to become a pandemic worldwide represents a serious public health risk. Concerning COVID-19, the WHO raised the threat to the CoV epidemic to the "very high" level, on February 28, 2020. On March 11, as the number of COVID-19 cases outside China has increased 13 times and the number of countries involved has

tripled with more than 118,000 cases in 114 countries and over 4,000 deaths, WHO declared the COVID-19 a pandemic.

World governments are at work to establish countermeasures to stem the devastating effects and it has been estimated that strict shutdowns may have saved 3 million lives across 11 European countries [5]. Health organizations coordinate information flows and issues directives and guidelines to best mitigate the impact of the threat. At the same time, scientists around the world work tirelessly, and information about the transmission mechanisms, the clinical spectrum of disease, new diagnostics, and prevention and therapeutic strategies are rapidly developing. Many uncertainties remain with regard to both the virus-host interaction and the evolution of the pandemic, with specific reference to the times when it will reach its peak.

At the moment, the therapeutic strategies to deal with the infection are mostly supportive, and prevention aimed at reducing transmission in the community is our best weapon. Aggressive isolation measures in China have led to a progressive reduction of cases. From China, the disease spread to Europe. In Italy, in geographic regions of the north, initially, and subsequently throughout the peninsula, political and health authorities have made incredible efforts to contain a shock wave that has severely tested the health system. Afterward, the COVID-19 quickly crossed the ocean and as of June 20, 2020, about 2,282,000 cases (with 121,000 deaths) have been recorded in the US, whereas Brazil with more than 1,000,000 cases and about 50,000 deaths is the most affected state in South America and the second in the world after the US. Although over time the lethality rate (total number of deaths for a given disease in relation to the total number of patients) of COVID-19 has been significantly lower than that of the SARS and MERS epidemics, the transmission of the SARS-CoV-2 virus is much larger than that of the previous viruses, with a much higher total number of deaths. It has

been estimated that about one in five individuals worldwide could be at increased risk of severe COVID-19 disease if they become infected, due to underlying health conditions [6].

Preliminary data suggests the reported death rate ranges from 1% to 2% depending on the study and country. The majority of the fatalities have occurred in patients over 50 years of age. Young children appear to be mildly infected but may serve as a vector for additional transmission [1].

2. CYTOKINE STORM

The first reference to the term cytokine storm in the published medical literature appears to be by Ferrara et al. in 1993 in a discussion of graft vs. host disease; a condition in which the role of excessive and self-perpetuating cytokine release had already been under discussion for many years [7] [8]. The term next appeared in a discussion of pancreatitis in 2002, and in 2003 it was first used in reference to a reaction to an infection .[7]

It is believed that cytokine storms were responsible for the disproportionate number of healthy young adult deaths during the 1918 influenza pandemic, which killed 17 to 50 million people. In this case, a healthy immune system may have been a liability rather than an asset [9]. Preliminary research results from Taiwan also indicated this as the probable reason for many deaths during the SARS epidemic in 2003[10]. Human deaths from the bird flu H5N1 usually involve cytokine storms as well [11]. Cytokine storm has also been implicated in hantavirus pulmonary syndrome .[12]

In 2006, a study at Northwick Park Hospital in England resulted in all six of the volunteers given the drug theralizumab becoming critically ill, with multiple organ failure, high fever, and a systemic inflammatory response [13] [14]. Parexel, a company conducting trials for pharmaceutical companies, in

one of its documents, wrote about the trial and said theralizumab could cause a cytokine storm—the dangerous reaction the men experienced [15].

2.1. Covid-19–Associated Cytokine Storm

The severe acute respiratory syndrome coronavirus, associated coronavirus disease 2019 (COVID-19) illness is a syndrome of viral replication in concert with a host inflammatory response. The cytokine storm and viral evasion of cellular immune responses may play an equally important role in the pathogenesis, clinical manifestation, and outcomes of COVID-19. Systemic proinflammatory cytokines and biomarkers are elevated as the disease progresses towards its advanced stages, and correlate with worse chances of survival [16].

SARS-CoV-2 activates the innate immune system and results in a release of a large number of cytokines, including IL-6, which can increase vascular permeability and cause a migration of fluid and blood cells into the alveoli as well as the consequent symptoms such as dyspnea and respiratory failure [17]. The higher mortality is being linked to the result of ARDS (acute respiratory distress syndrome) aggravation and the tissue damage that can result in organ-failure and/or death [18].

According to studies published in October, 2020, ARDS is the cause of mortality in 70% of COVID-19 deaths [19]. In a cytokine plasma level analysis of those with severe Sars-CoV-2, the levels of many interleukins and cytokines are extremely elevated, showing evidence of a cytokine storm in those most harshly affected [18]. Additionally, postmortem examination of patients with COVID-19 has shown large accumulation of inflammatory cells in lung tissues, including macrophages and T-helper cells [20].

Covid-19, which is caused by SARS-CoV-2, is characterized by heterogeneous symptoms ranging from mild fatigue to life-threatening

pneumonia, cytokine storm, and multiorgan failure [21]. Cytokine storm was also reported in patients with SARS and was associated with poor outcomes [22]. Although the mechanisms of lung injury and multiorgan failure in Covid-19 are still under investigation, [23] reports of hemophagocytosis and elevated cytokine levels — as well as beneficial effects of immunosuppressant agents — in affected patients, particularly those who are the most severely ill, suggest that cytokine storm may contribute to the pathogenesis of Covid-19 [24] [25].

Serum cytokine levels that are elevated in patients with Covid-19–associated cytokine storm include interleukin-1β, interleukin-6, IP-10, TNF, interferon-γ, macrophage inflammatory protein (MIP) 1α and 1β, and VEGF [26] [27]. Higher interleukin-6 levels are strongly associated with shorter survival [28]. The relative frequencies of circulating activated CD4+ and CD8+ T cells and plasmablasts are increased in Covid-19 [29]. In addition to the elevated systemic cytokine levels and activated immune cells, several clinical and laboratory abnormalities, such as elevated CRP and d-dimer levels, hypoalbuminemia, renal dysfunction, and effusions, are also observed in Covid-19, as they are in cytokine storm disorders. Laboratory test results reflecting hyperinflammation and tissue damage were found to predict worsening outcomes in Covid-19 [30].

Although immunologic dysregulation has been observed in severe cases of Covid-19 [31], it is not known whether immune hyperactivity or a failure to resolve the inflammatory response because of ongoing viral replication or immune dysregulation underlies severe cases. The correlation between the nasopharyngeal viral load and cytokine levels (e.g., interferon-α, interferon-γ, and TNF), as well as a declining viral load in moderate but not severe cases, suggests that the immune response is positively associated with the viral burden [31]. Alternatively, the discoveries of inborn errors of type I interferon immunity and autoantibodies against type I interferons in the most severe

cases of Covid-19 suggest that an inadequate antiviral response may be contributory in some patients with Covid-19 [32] [33] [34]. Host immune responses and immune-related symptoms are extremely variable between asymptomatic patients (who have effective control of SARS-CoV-2) and patients with severe Covid-19 (who are unable to control the virus), which suggests that host immune dysregulation contributes to pathogenesis in some cases. Another hypothesized mechanism involves autoimmunity due to molecular mimicry between SARS-CoV-2 and a self-antigen. These mechanisms may be involved in subgroups of patients, such as children with postinfection multisystem inflammatory syndrome, a condition that seems to be ameliorated by immunomodulatory therapies such as intravenous immune globulin, glucocorticoids, and anti–interleukin-1 and anti–interleukin-6 therapies. Patients with multisystem inflammatory syndrome very clearly meet the definition of cytokine storm, since SARS-CoV-2 is no longer present; however, it is unclear whether the cytokine storm is a driver of Covid-19 or a secondary process. Furthermore, it is now clear that patients with SARS-CoV-2 infection can be asymptomatic or can have acute Covid-19 with heterogeneous severity, a chronic course of Covid-19, or multisystem inflammatory syndrome. A critical question concerns the factors that contribute to the severe cytokine storm–like phenotype observed in a small fraction of patients. Coexisting conditions such as hypertension, diabetes, and obesity are associated with more severe cases of Covid-19, possibly because of the preexisting chronic inflammatory state or a lower threshold for the development of organ dysfunction from the immune response.

Several important differences in therapeutic considerations should be noted between Covid-19–associated cytokine storm and many other cytokine storm disorders. First, cytokine storm triggered by infection with SARS-CoV-2 may require different therapies from those used for cytokine storm due to other

causes. Cytokines may be both a key component of the cytokine storm and an essential factor in the antimicrobial response. Thus, blocking cytokine signaling may actually impair clearance of SARS-CoV-2, increase the risk of secondary infections, and lead to worse outcomes, as seen with influenza virus [34]. Since interleukin-6 and other cytokines are potentially critical for both a healthy response to SARS-CoV-2 and a detrimental cytokine storm, it is particularly important that the right subgroups of patients with Covid-19 are selected for treatments at the right time. Despite positive anecdotal reports, two large, randomized, controlled trials of anti–interleukin-6 receptor antibody therapies did not show a survival benefit in hospitalized patients with Covid-19 [35] [36].

Second, the primary site of infection and disease most likely contributes to differences in immune responses and mechanisms underlying the cytokine storm, which have implications for treatment. For example, selective elimination of the primary viral reservoir is beneficial in patients with HHV-8–associated multicentric Castleman's disease but is not possible in patients with Covid-19.

Third, lymphopenia is not often observed in cytokine storm disorders, but it is a hallmark of severe Covid-19. It is currently unclear whether the lymphopenia observed in Covid-19 is due to tissue infiltration or destruction of lymphocytes.

Fourth, clotting issues can occur across cytokine storm disorders, but thromboembolic events appear to be more frequent in Covid-19–associated cytokine storm [37]. Finally, although cytokine panels have not been measured simultaneously on the same platform across Covid-19–associated cytokine storm and other cytokine storm disorders, preliminary results suggest that circulating levels of several cytokines, such as interleukin-6, as well as other inflammatory markers, such as ferritin, are less severely elevated in Covid-19

than in some of the other cytokine storm disorders [31]. Levels of inflammatory mediators in pulmonary tissue during infection with SARS-CoV-2 remain unknown.

Despite the many unknowns, a recent randomized, controlled trial showing that dexamethasone reduces mortality among the most severe cases of Covid-19, characterized by elevated CRP levels and supplemental oxygen requirements, and potentially worsens outcomes in milder cases suggests that excessive, late-stage inflammation contributes to mortality [25]. A meta-analysis of seven randomized trials showed that 28-day all-cause mortality in critically ill patients with Covid-19 was lower among those who were treated with glucocorticoids than among those who received usual care or placebo [38]. An observational study suggesting that patients with Covid-19 have a good response to glucocorticoids when the CRP level is high but a poor response when the level is low is consistent with these findings [39]. Further support comes from positive anecdotal reports of targeted antagonists against interleukin-1, granulocyte–macrophage colony-stimulating factor, and JAK1 and JAK2 in patients with Covid-19 [40] [41] [43]. Likewise, the observation that proinflammatory agents such as inhaled interferon-β have a positive effect if given early in the disease course is consistent with a model in which immunostimulation that enhances antiviral activity is helpful early (and probably harmful late), whereas immunosuppression is helpful late and harmful early. As with dexamethasone, the timing of treatment and selection of subgroups of patients included in studies will most likely have an effect on outcomes.

Despite unknowns regarding the role of immune dysregulation and cytokine storm in Covid-19, hundreds of immunomodulatory drugs are currently under investigation [40]. Many of these treatments have been used for other cytokine storm disorders. Canakinumab, an anti–interleukin-1β monoclonal antibody,

and anakinra are both being studied for Covid-19–induced ARDS. Acalabrutinib, a selective inhibitor of Bruton tyrosine kinase that regulates B-cell and macrophage signaling and activation, may have promise for dampening the hyperinflammatory response in Covid-19 [44]. JAK1 and JAK2 inhibitors, which are approved for the treatment of a number of autoimmune and neoplastic conditions, have the potential to inhibit signaling downstream of type I interferon, interleukin-6 (and other gp130 family receptors), interferon-γ, and interleukin-2, among other cytokines [45]. Much like anti–interleukin-6 antibody therapy, inhibition of Bruton tyrosine kinase and JAK could prove to be damaging or unhelpful if given too soon, when the immune response to SARS-CoV-2 is critical in controlling viral replication and clearance.

2.2. Treatment of Cytokine Storm

The general treatment strategy for cytokine storm involves supportive care to maintain critical organ function, control of the underlying disease and elimination of triggers for abnormal immune system activation, and targeted immunomodulation or nonspecific immunosuppression to limit the collateral damage of the activated immune system. As noted throughout this review, a number of drugs are effective across multiple disorders under the cytokine storm umbrella and still more may be effective in multiple conditions that have not yet been studied.

Given the growing number of new therapeutics targeting various aspects of the immune system and our ability to probe the biologic mechanisms of disease, further research should focus on the identification of drugs that can be used across cytokine storm disorders and precision diagnostics for selecting the right drugs for the right patients, regardless of the underlying condition [46] [47]. A study involving patients with systemic juvenile idiopathic

arthritis revealed subgroups of patients with cytokine profiles in which interleukin-6 and interleukin-18 predominated, pointing toward available therapeutic approaches [48]. Likewise, biomarkers were recently shown to effectively predict which patients with adult-onset Still's disease would have a response to anakinra or tocilizumab [49]. The progress made in precision oncology suggests that similar efforts across cytokine storm disorders are warranted to identify specific therapeutic targets and signatures of response to certain drugs that cross disease boundaries. JAK signaling is an interesting target in cytokine storm, because multiple cytokine–receptor pairs can be targeted simultaneously, an approach that may be effective for multiple diseases driven by different cytokines. In addition, plasma exchange and plasma filtration columns for the adsorption of cytokines are both under evaluation for cytokine storm disorders.

2.3. Prevention of Cytokine Storm

However, the main question is why some patients are more predisposed to cytokine storm respect others. Different genetic mutations may also represent a risk factor for the severe disease course and the occurrence of cytokine storm in COVID-19. Notably, data obtained from a global population indicate that allelic alterations in cytokine genes showed a sharp latitudinal impact [50] [51]. Geographical latitude is the main environmental factor that is affected by our evolutionary history with respect to environmental selection. The latitude is therefore associated with a variety of factors comprising genetic background, biometeorological factors, and socio−economic influences. Regarding the role of biometeorological factors, the sunlight has a pivotal role for the synthesis of Vitamin D, which in turn plays a key role in preserving the immune homeostasis. Genetic factors are known to account for up to 28% of

inter−individual variability in serum 25(OH)D concentrations [52]. Genetic as well as individual differences of vitamin D status have been reported across various populations [53]. In the light of this, it is possible to postulate that there is a possibility that vitamin D status may have some influence on geographical variance of COVID−19. [54]

Furthermore, deficiency in vitamin D may lead to increased autoimmunity and elevated susceptibility to infections. Indeed, Vitamin D inhibit the production of proinflammatory cytokines (i.e., TNF−α and IFN−γ) and stimulate the release of anti−inflammatory cytokines. Vitamin D decreases the risk of microbial infection and death through different mechanism. A recent review categorized those mechanisms into three groups, including a physical barrier as well as innate and adaptative immunity [55]. COVID-19 viruses disrupt junction integrity, increasing the susceptibility to the infection by the virus and other microorganisms [56], while vitamin D supports the maintenance of cell junctions integrity [57]. Vitamin D may be valuable in controlling the cytokine storm and the outcome of COVID−2019 patients. Its deficiency leads to greater risk, and supplements of Vitamin D could thus be potentially used [58].

Cytokine regulation, however, depends on different upstream regulators, such as Toll-like Receptors (TLRs), and these interrelate with other components of innate immune system, such as complement elements. TLRs are a family of innate immune sensor proteins exerting a key function in infection, inflammation and immunity processes [59]; TLR pathway may be significantly implicated in cytokine storm occurring during COVID-19 infection. To date, there are no studies regarding the role of TLR signaling in SARS−CoV−2 infection. Previous studies indicate, however, that genetic variation within TLRs or TLR signaling affected SARS−CoV infection [60] [61] [62] [63].

Moreover, the complement system interacts with TLRs, and it is thus involved in higher susceptibility to the infection and cytokine storm activation [64]. In fact, a recent study reported that the complement system represents a crucial host mediator of SARS−CoV infection. SARS−CoV−infected C3–/– mice exhibited less respiratory impairment and lowered levels of chemokines and cytokines in the organs [65]. In addition, hyperactivation of the complement system was reported in COVID−19 patients, and the highly pathogenic coronavirus N protein exacerbated MASP−2−mediated complement activation [66]. Overall, the complement system is crucially involved in the stimulation of the cytokine storm and inflammation in SARS−CoV−2 infection.

2.4. COVID-19 Experimental and Clinical Investigations

Data concerning the correlation between COVID-19 and cytokine/chemokine dysregulation are still limited, but the current available in vitro and clinical studies suggest a likeness with what was reported after SARS and MERS infections.

So far, few studies into SARS-CoV-2 infection have been reported. One interesting study compared SARS-CoV-2 and SARS-CoV behavior in the pulmonary tissue. The research group inoculated the viruses in ex vivo human pulmonary tissue samples and reported that SARS-CoV-2 was more efficient than SARS-CoV in both replicating and infecting human lung tissues. Additionally, SARS-CoV-2 infection was less competent in inducing the expression of any IFNs, suggesting that SARS-CoV and SARS-CoV-2 may differ in their capability to control proinflammatory cytokines and chemokines release. Indeed, SARS-CoV infection increased 11 out of the 13 proinflammatory factors tested in this study, while SARS-CoV-2 upregulated only five of them (i.e., CXCL10, IL6, CCL2, CXCL1, and CXCL5) despite replicating more efficiently. The expression of 12 out of 19 among IFNs and

cytokines/chemokines genes tested was substantially lower in SARS-CoV-2-infected human samples than SARS-CoV-infected samples. Notably, CXCL8 transcription was increased only by SARS-CoV, but not SARS-CoV-2 infection, while the opposite for CXCL10 was detected [67].

Another research group isolated SARS-CoV-2 from a patient with established COVID-19 and compared virus tropism and replication competence with SARS, MERS, and 2009 pandemic influenza H1N1 (H1N1pdm) in ex vivo samples of human lung and bronchus. To assess extrapulmonary infection, the authors used ex vivo cultures of human conjunctiva epithelium (potential portals of infection for SARS-CoV-2) and human colorectal adenocarcinoma cell lines [68]. SARS-CoV-2 was able to infect mucus-secreting, ciliated, and club cells of bronchial epithelium type 1 pneumocytes in the lung and the conjunctival mucosa. In the bronchus, SARS-CoV-2 replication was higher than SARS and similar to MERS and lower than H1N1pdm. In the lungs, SARS-CoV-2 replication was comparable to SARS and H1N1pdm but lower than MERS. In conjunctiva, SARS-CoV-2 replication was superior to SARS-CoV. SARS-CoV-2 was less effective in inducing proinflammatory cytokines than H1N1 and MERS. Both SARS-CoV and SARS-CoV-2 are thus comparably replicated in the alveolar epithelium; SARS-CoV-2 is replicated more extensively in the bronchus than SARS-CoV. These findings support valuable insights into the transmissibility of SARS-CoV-2 infection and dissimilarities with other respiratory pathogens [69].

In a retrospective study, the clinical and immunological features of 21 patients (17 male and four female) affected by COVID-19 were evaluated. These patients were classified in different degrees of severity, according to the guidelines of the National Health Commission of China. In particular, the 11 patients with severe form exhibited considerably elevated serum levels of IL-6, IL-10, and TNF-α in parallel to the reduced absolute number of T

lymphocytes, CD4 + T cells, and CD8 + T cells with respect with moderate cases. This retrospective observational study suggests that SARS-CoV-2 infection may involve principally T lymphocytes, particularly CD4 + and CD8 + T cells, leading to decreased T lymphocytes number as well as IFN-γ production by CD4 + T cells. These potential immunological markers can be relevant due to their association with COVID-19 disease severity [70].

To characterize the transcriptional signatures of host inflammatory response to SARS-CoV-2, Xiong and collaborators performed a transcriptome sequencing of different proinflammatory genes from RNAs isolated from the broncho-alveolar lavage fluid and peripheral blood mononuclear cells of COVID-19 patients. This analysis showed distinct host inflammatory cytokine profiles to SARS-CoV-2 infection and supports the association between COVID-19 pathogenesis and aberrant cytokine release; CXCL10 in particular was upregulated in peripheral blood mononuclear cells, but no up-regulation of CXCL10 gene in broncho-alveolar lavage fluid was detected. Additionally, SARS-CoV-2 induced the activation in lymphocytes of numerous genes involved in apoptosis and P53 pathways, leading to the assumption that this activity may be the primary cause of lymphopenia frequently detected in COVID-19 cases. The transcriptome sequencing analysis of COVID-19 patients represents a significant source for clinical guidance on anti-inflammatory treatment and to understand the molecular mechanisms of host response [71].

Another study, involving 65 SARS-CoV-2-positive patients, revealed that the absolute numbers of CD4 + and CD8 + T cells and B cells progressively diminished in relation to increased severity of disease [72]. Furthermore, Yang and collaborators analyzed 48 circulating cytokines from 53 COVID-19 patients (34 severe cases), and 14 resulted higher in patients with severe COVID-19 clinical history. Among them, CXCL10, CCL7, and IL-1 receptor

antagonist were the ones strongly related to severity illness and, even more significantly, CXCL10 levels were the only one to be positively and significantly correlated with the viral load [73].

In 70 patients who survived severe COVID-19 pneumonia, 66 showed significant damage as revealed by CT scans taken before hospital release. The injury varied from dense clumps of tissue obstructing blood vessels of the alveoli to tissue lesions. The tissue lesions may represent signs of chronic lung disease and may be irreversible, rendering the patient frail [74]. Furthermore, people who survived ARDS due to COVID-19 may have lasting pulmonary scarring [75] .If pulmonary tissues are replaced with scar tissues, they are no longer functional as normal lung tissues, which may lead to poor gas exchange. Similar damage has been documented also in survivors of MERS and SARS even if those illnesses attacked only one lung.

It is important to consider several factors in managing cytokine storm. Neutralization of a particular cytokine whose level is elevated in the circulation with an existing agent (anti–interleukin-6, anti-TNF, anti–interferon-γ, or anti–interleukin-1β antibody) will not always be effective, and blocking a cytokine with a low or normal circulating level can be effective if it is a key component of the hyperinflammatory circuit or if its level is potentially elevated in tissue.

In addition, the various therapies mentioned in this review have distinctive side-effect and risk profiles. All targeted agents have target-specific risks, and combination therapy has more potential risks than single-agent therapy. Furthermore, pathologic hyperinflammation itself is an immunodeficiency that can put patients at risk for infections, and immunosuppressive agents most likely increase the risk further. In this age of cytokine profiling and individualized medicine, patients must be monitored and given appropriate

prophylaxis when treated empirically, and randomized, controlled trials should always be performed to assess efficacy and safety.

Advancing the research and treatment of cytokine storm will require pooling of samples for “omics” studies and collaboration among experts across conditions. The introduction of an International Classification of Diseases, 10th Revision, code for cytokine release syndrome in 2021 should facilitate electronic health record–based research into its natural history, pathogenesis, and treatments. Once sufficient scientific progress has been achieved toward biomarker-guided, individualized treatment of cytokine storm, reliable, quick, and accessible assays will be needed to measure soluble mediators of inflammation in plasma and tissues.

3. CIMETRA

CimetrA is comprised of natural active ingredients and formulated in microscopic structures known as micelles. The elements forming the structure of these micelles are in themselves also of exclusively natural origin. The unique formulation is endowed with highly desirable pharmacological features, providing otherwise unattainable high bioavailability to the active ingredients it is designed to deliver.

The investigational medicinal product, CimetrA (MGCAU00CS03), is an oromucosal spray, suspension, containing two active drug substances: curcumin (CUR) and boswelic acid (BA). Medicinal product contains also L-ascorbic acid, Sodium benzoate, Potassium sorbate, Kolliphor® RH 40 (PEG 40), Kollisolv® PEG 400, Kollidon® 12 PF (PVP) and Aqua purificata as excipients. There is one strength, 7mg/g CUR and 15 mg/g BA.
CimetrA investigational medicinal product is intended to be used for imunomodulatory indication. CimetrA-1 recommended daily dose is 2 ml, delivered twice daily. One spray equals to 200 µL, for one dose are required 5 sprays.

CimetrA-2 recommended daily dose is 1.4 ml, delivered twice daily. One spray equals to 140 µL, for one dose are required 5 sprays.

Product is packed in a multidose container with a spray pump. It is administered by spraying into the oral cavity. Spray pump on product bottle enables dosing 200 µl of product per spray. The product should be stored in the room temperature.

There is also investigational medicinal product MGCAU00CS02-ArtemiC containing Artemisinin as the third active drug substance along CUR and BA. MGCAU00CS02 have been tried in pre-clinical and clinical studies, but the new formulation CimetrA was more effective so it continued in phase IIB .

APIs:

Drug substance is containing of at least 95 % of Curcuminoids with at least 60 % of Curcumin as a main curcuminoid, accompanied with demethoxycurcumin and bisdemethoxycurcumin. Since the main curcuminoid is curcumin, the expression curcumin is used for both the drug substance and its main ingredient. Details on Curcumin as main drug substance ingredient and other curcuminoids are presented in the IPMD.

Boswellic acid is the second active drug substance in CimetrA drug product. Drug substance is containing of at least 85 % of total boswellic acids with at least 30 % of 3-Acetyl Keto Beta Boswellic Acid (AKBA) as the main boswellic ingredient. Details on AKBA are presented in the IMPD.

Batch formula for CimetrA, MGCAU00CS03

Name of Ingredient	**Quantity per 800 g batch (g)**
CUR	5.6
BA	12
Ascorbic acid	4
Sodium benzoate	0.6
Potassium sorbate	0.6

Name of Ingredient	Quantity per 800 g batch (g)
Kolliphor® RH 40	192
Kollisolv® PEG 400	48
Kollidon® 12 PF	4
Aqua purificata	533.2

The pharmacological features of CimetrA may be estimated on the basis of available literature, since the extensive technically challenging studies have yet to be completed. An outstanding feature of the two active ingredients is that they all are poorly and inconsistently soluble in aqueous media on their own, as described for curcumin (Modasiya and Patel 2012, Mohanty, Das et al. 2012, Prasad, Tyagi et al. 2014), Boswellia Serrata extracts (Abdel-Tawab, Werz et al. 2011). This issue has been addressed by formulating them in the SNEDDS with the purpose of rendering them soluble with satisfactory bioavailability (Buya, Beloqui et al. 2020). Not less important is the improved consistency of the ingredients' pharmacokinetics with increased exposure due to prolonged presence in the circulation which has regularly been observed when changing from poorly soluble formulations to their nano-encapsulated modifications (Bilia, Piazzini et al. 2017, Bilia, Bergonzi et al. 2020). These qualities can be crudely inferred from descriptions of similar formulations of curcumin (Rai, Pandit et al. 2015, Chen, Liang et al. 2021) and boswellia (Bairwa and Jachak 2015, Meins, Behnam et al. 2018, including combined encapsulation of curcumin (Lapenna, Bilia et al. 2009, Bilia, Bergonzi et al. 2020) that have been tested already with the findings published in the accessible literature.

The available data also support the safety of the active ingredients – curcumin and Boswellia when administered as nano-encapsulated drugs. This has been demonstrated in preclinical studies performed with CimetrA at relevant concentration both in-vitro and in-vivo. Furthermore, a study describing delivery of much larger doses of 80 mg nano-encapsulated curcumin to human subjects twice daily over 3 weeks has been published without report of any significant adverse effects (Tahmasebi, El-Esawi et al. 2020). Boswellia has also been

studied in doses far in excess of those that can be attained by CimetrA in its recommended regimen with no evidence of toxicity (Efferth and Oesch 2020), indicating that the expected increased bioavailability (Bairwa and Jachak 2016) of this ingredient secondary to the nano-formulation is safe to the degree that it places no restriction on the treatment. With the advent of nano-technology it has become possible to circumvent the barriers to the administration with significantly higher bioavailability and pharmacokinetic consistency (Isacchi, Arrigucci et al. 2011, Isacchi, Bergonzi et al. 2012). Notably, it is accepted that micellar structures have improved access to the sites of inflammation associated with increased permeability (Bilia, Piazzini et al. 2017), as expected in conditions of acute lung injury of the nature encountered in COVID-19 pneumonia (Luo, Yu et al. 2020). The study drug is formulated in micelles.

The micellar structure of the drug ensures the high mucosal absorption in the oral spray formulation. This way of administration was checked in preclinical and clinical studies and showed a safety and efficacy.

Study product doses are defined according to the following –

Arm 1: CimetrA-1, with a total dose containing a combination of Curcuma longa rhizome dry extract 28 mg, Boswellia serrata resin dry extract 60 mg in spray administration – divided in 4 separate doses given as an add on therapy, 4 doses over 48 hours (day 1 and day 2), twice a day (morning and evening)

Arm 2: CimetrA-2, with a total dose containing a combination of Curcuma longa rhizome dry extract 19.6 mg, Boswellia serrata resin dry extract 42 mg in spray administration – divided in 4 separate doses given as an add on therapy, 4 doses over 48 hours (day 1 and day 2), twice a day (morning and evening).

4. PRE-CLINICAL DATA

4.1. Acute Tox Study

The study was performed on December 2020, in the pre clinical facility - SIA - Science in Action, Ness Ziona, Israel. Science in Action is accredited for OECD principles of Good Laboratory Practice ENV/MC/CHEM (98)17 for toxicity studies; however, this study does not follow the complete GLP regulations, and is thus considered a non-GLP study. The study follows this protocol and the Science in Action SOPs.

The objective of this study was to evaluation the safety and toxicity of ArtemiC spray in lower doses by splash route of administration into the oral cavity.

Animal handling was performed according to guidelines of the National Institute of Health (NIH) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Animals were housed in polyethylene cages (3 per cage) measuring 35 × 30 × 15 cm, with stainless steel top grill facilitating pelleted food and drinking water in plastic bottle; bedding: steam sterilized clean paddy husk are used and bedding material is changed along with the cage at least twice a week. The study performed under the approval by "The Israel Board for Animal Experiments", in compliance with "The Israel Animal Welfare Act" and Ethics Committee.

Each group 3+3 rats were treated in splash route of administration into the oral cavity on day 1 with the experimental substances.

Each animal was weighed prior to treatment.

Group 1 (n=3M + n=3F): 50ul saline per rat

Group 2 (n=3M + n=3F): 48ug ArtemiC / per Kg rat

Group 3 (n=3M + n=3F): 96ug ArtemiC / per Kg rat

Group 4 (n=3M + n=3F): 192ug ArtemiC / per Kg rat

During all 7 days of the experiment the animals were monitored for observation and weight to detect the appearance of abnormal clinical signs.

After 7 days blood samples from all rats were taken for a full panel of hematology and chemistry. Blood 0.2 for an unbound EDTA test for hematology panel, and 0.5 ml for a separable gel test tube, to 0.25 ml serum for a panel of blood chemistry composition

After blood collection the animals were sacrificed and the organs: brain, lungs, heart, liver, spleen and kidneys were removed, weighted and kept in formalin 4%, and sent for pathological examination

4.1.1. *Results*

Table 1. Animal Weight (gram)

		Day	**0**	**2**	**5**	**7**
	Group	**Rat No**	**body weight**	**body weight**	**body weight**	**body weight**
Vehicle Saline	**1M**	11	248	269	280	289
		12	245	265	277	283
		13	254	276	285	295
		AVERAGE	**249**	**270**	**280.67**	**289**
		SEM	**2.16**	**2.62**	**1.91**	**2.83**

		Day	**0**	**2**	**5**	**7**
	Group	**Rat No**	**body weight**	**body weight**	**body weight**	**body weight**
	1F	14	193	199	202	203
		15	202	209	209	210
		16	196	203	206	208
		AVERAGE	**197**	**203.67**	**205.67**	**207**
		SEM	**2.16**	**2.37**	**1.66**	**1.70**
Low dose 8ul	**2M**	21	264	286	293	302
		22	260	276	286	298
		23	254	273	281	292
		AVERAGE	**259.33**	**278.33**	**286.67**	**297.33**
		SEM	**2.37**	**3.21**	**2.84**	**2.37**
	2F	24	198	203	205	207
		25	201	212	215	219
		26	206	212	216	218
		AVERAGE	**201.67**	**209**	**212**	**214.67**
		SEM	**1.91**	**2.45**	**2.87**	**3.14**
	3M	31	269	284	299	311

		Day	0	2	5	7
	Group	Rat No	body weight	body weight	body weight	body weight
Mid dose 16ul		32	242	263	275	284
		33	251	264	282	293
		AVERAGE	**254**	**270.33**	**285.33**	**296**
		SEM	**6.48**	**5.58**	**5.82**	**6.48**
	3F	34	193	200	208	214
		35	195	203	205	206
		36	197	204	206	207
		AVERAGE	**195**	**202.33**	**206.33**	**209**
		SEM	**0.94**	**0.98**	**0.72**	**2.05**
High dose 32ul	**4M**	41	244	262	274	285
		42	253	270	284	293
		43	251	271	280	293
		AVERAGE	**249.33**	**267.67**	**279.33**	**290.33**
		SEM	**2.23**	**2.33**	**2.37**	**2.18**
	4F	44	192	202	203	206
		45	200	205	205	204

		Day	0	2	5	7
	Group	Rat No	body weight	body weight	body weight	body weight
		46	201	212	210	210
		AVERAGE	**197.67**	**206.33**	**206**	**206.67**
		SEM	**2.33**	**2.42**	**1.70**	**1.44**

Figure 1. Male Weight

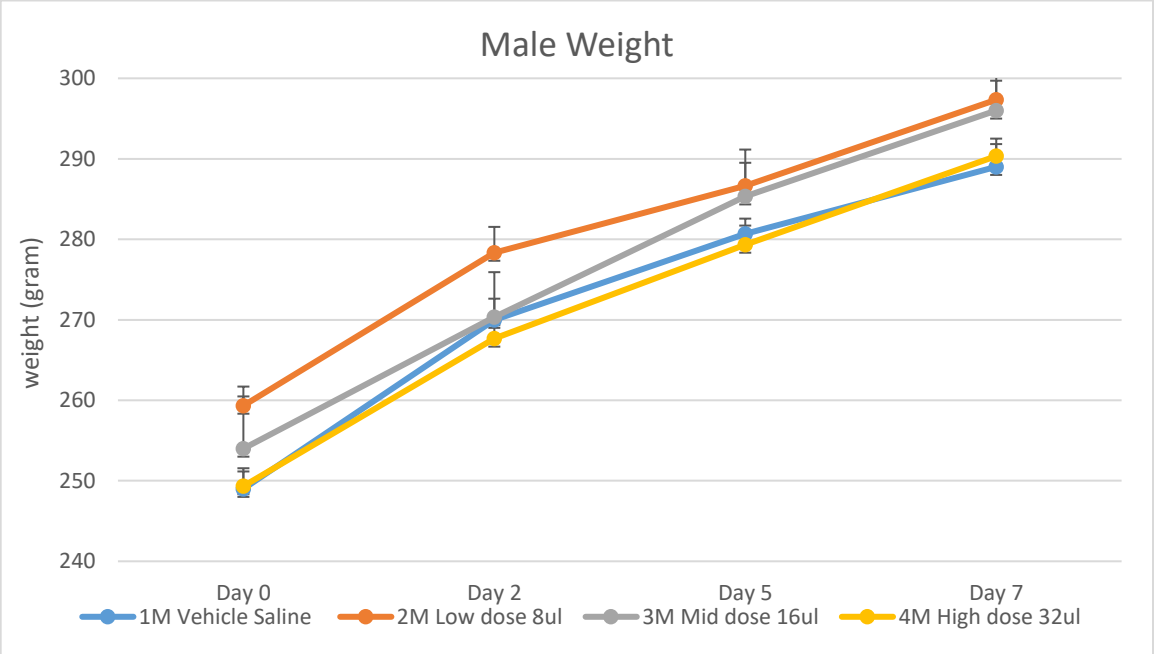


Figure 2. Female Weight

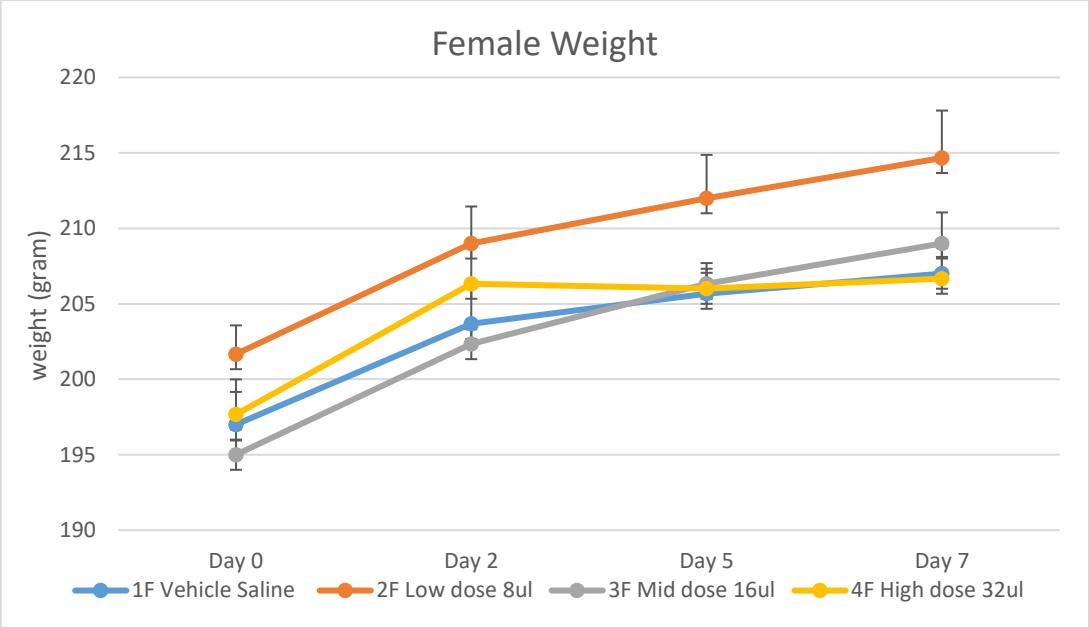


Table 2. Organ Weight (gr.)

		Day	22/09/20					
	Group	rat No	Brain	Heart	Lungs	Spleen	Kidneys	Liver
Vehicle Saline	**1M**	11	1.695	1.199	2.86	0.854	2.418	14.311
		12	1.811	1.213	2.434	0.775	2.189	10.619
		13	1.757	1.231	2.683	0.855	2.099	12.515
		AVERAGE	**1.754**	**1.214**	**2.659**	**0.828**	**2.235**	**12.482**
		SEM	**0.027**	**0.008**	**0.101**	**0.022**	**0.078**	**0.870**
	1F	14	1.743	0.811	2.435	0.831	1.431	7.5
		15	1.765	0.81	2.643	0.65	1.483	8.139

		16	1.514	0.878	2.96	0.683	1.602	7.927
		AVERAGE	**1.674**	**0.833**	**2.679**	**0.721**	**1.505**	**7.855**
		SEM	**0.066**	**0.018**	**0.125**	**0.045**	**0.041**	**0.153**
Low dose 8ul	**2M**	21	1.743	1.134	2.592	0.854	2.478	12.714
		22	1.732	1.279	2.67	0.882	2.229	12.623
		23	1.828	1.042	2.73	0.94	2.252	13.721
		AVERAGE	**1.768**	**1.152**	**2.664**	**0.892**	**2.320**	**13.019**
		SEM	**0.025**	**0.056**	**0.033**	**0.021**	**0.065**	**0.287**
	2F	24	1.7	0.834	2.173	0.676	1.25	8.014
		25	1.75	0.913	2.35	0.787	1.378	8.329
		26	1.755	0.876	2.223	0.722	1.392	7.544
		AVERAGE	**1.735**	**0.874**	**2.249**	**0.728**	**1.340**	**7.962**
		SEM	**0.014**	**0.019**	**0.043**	**0.026**	**0.037**	**0.186**
Mid dose 16ul	**3M**	31	1.752	1.225	2.54	1.077	2.362	13.411
		32	1.73	1.214	2.233	0.839	1.989	12.266
		33	1.75	1.11	2.789	0.842	2.048	12.034
		AVERAGE	**1.744**	**1.183**	**2.521**	**0.919**	**2.133**	**12.570**
		SEM	**0.006**	**0.030**	**0.131**	**0.064**	**0.095**	**0.348**

	3F	34	1.575	0.865	2.175	0.726	1.45	7.781
		35	1.782	0.828	2.3	0.643	1.401	7.392
		36	1.59	0.838	2.389	0.581	1.337	7.562
		AVERAGE	**1.649**	**0.844**	**2.288**	**0.650**	**1.396**	**7.578**
		SEM	**0.054**	**0.009**	**0.051**	**0.034**	**0.027**	**0.092**
High dose 32ul	**4M**	41	1.695	1.044	2.398	0.834	2.197	11.018
		42	1.875	1.171	2.371	0.884	2.137	11.011
		43	1.903	1.032	2.424	0.817	2.165	11.264
		AVERAGE	**1.824**	**1.082**	**2.398**	**0.845**	**2.166**	**11.098**
		SEM	**0.053**	**0.036**	**0.012**	**0.016**	**0.014**	**0.068**
	4F	44	1.669	1.071	2.33	0.714	1.708	8.376
		45	1.708	0.913	2.317	0.554	1.333	7.759
		46	1.715	0.859	2.362	0.63	1.392	7.646
		AVERAGE	**1.697**	**0.948**	**2.336**	**0.633**	**1.478**	**7.927**
		SEM	**0.012**	**0.052**	**0.011**	**0.038**	**0.095**	**0.185**

Figure 3. Organs Weight- Male (gr.)

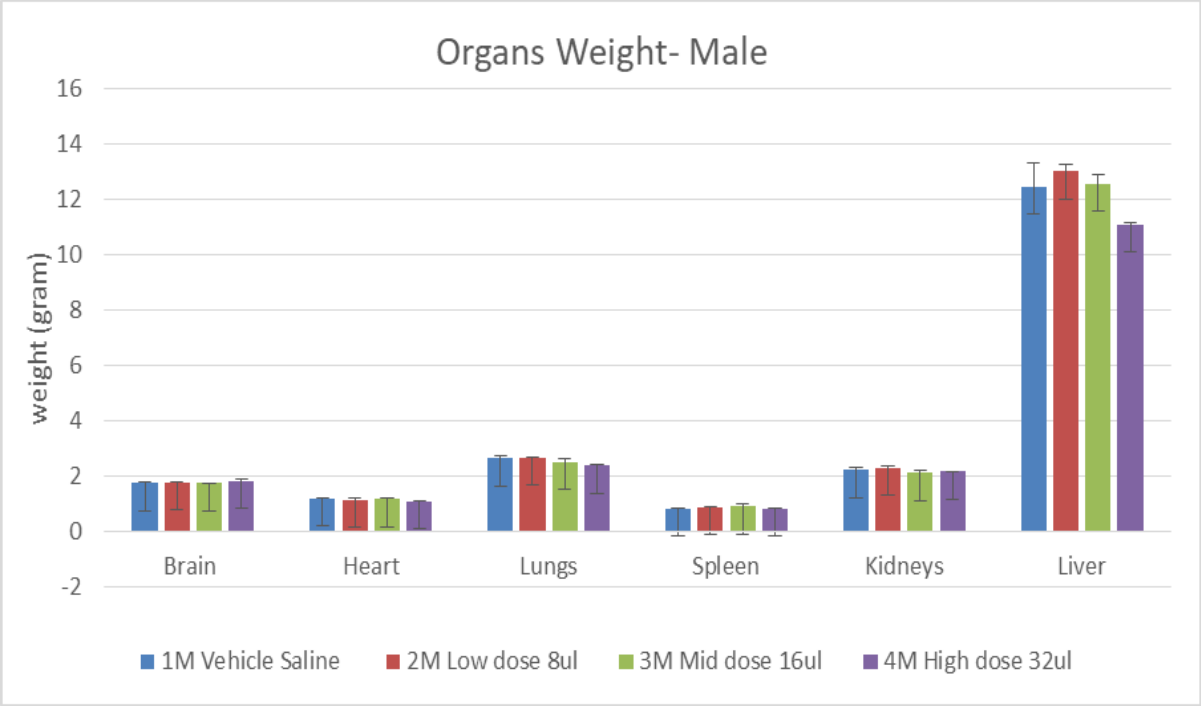

Organs Weight- Male
16
14
12
10
8
6
4
2
0
-2
weight (gram)
Brain
Heart
Lungs
Spleen
Kidneys
Liver
1M Vehicle Saline
2M Low dose 8ul
3M Mid dose 16ul
4M High dose 32ul

Figure 4. Organs Weight- Female (gr.)

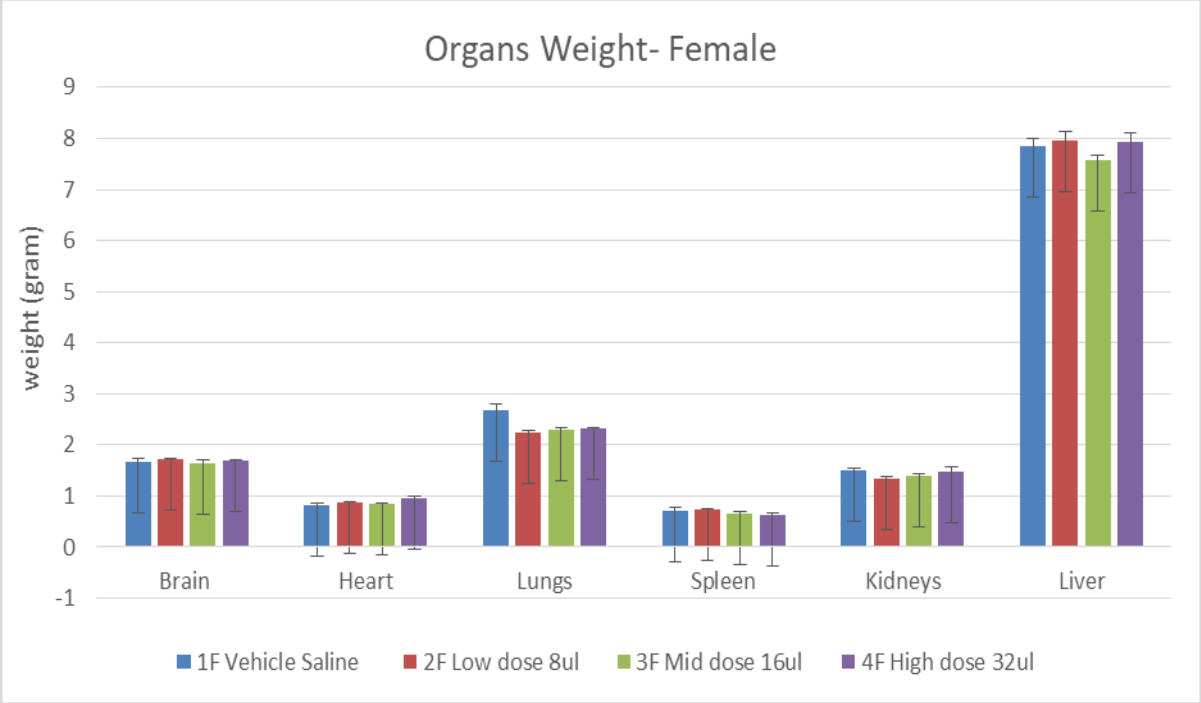


Table 3. Hematology Results

		Animal	WBC	RBC	HGB	HCT	MCV	MCH	MCHC
		ID	**10*3/µl**	**10*6/µl**	**g/dl**	**%**	**FL**	**pg**	**g/dl**
Vehicle Saline	**1M**	11	10.04	7.93	15.6	51.4	64.8	19.7	30.4
		12	9.44	8.33	15.5	49.6	59.5	18.6	31.3
		13	3.99	5.31	10.7	33.8	63.7	20.2	31.7
		AVERAGE	**7.82**	**7.19**	**13.93**	**44.93**	**62.67**	**19.50**	**31.13**
		SEM	**1.57**	**0.77**	**1.32**	**4.56**	**1.32**	**0.39**	**0.31**

		Animal	WBC	RBC	HGB	HCT	MCV	MCH	MCHC
		ID	**10*3/µl**	**10*6/µl**	**g/dl**	**%**	**FL**	**pg**	**g/dl**
	1F	14	9.5	8.03	15.6	51.3	63.9	19.4	30.4
		15	5.66	8.52	15.8	51.3	60.2	18.5	30.8
		16	6.96	8.63	16.4	52.1	60.4	19	31.5
		AVERAGE	**7.37**	**8.39**	**15.93**	**51.57**	**61.50**	**18.97**	**30.90**
		SEM	**0.92**	**0.15**	**0.20**	**0.22**	**0.98**	**0.21**	**0.26**
Low dose 8ul	**2M**	21	9.07	7.9	15.9	52.1	65.9	20.1	30.5
		22	14.39	8.75	15.7	50	57.1	17.9	31.4
		AVERAGE	**11.11**	**8.92**	**16.87**	**54.07**	**60.80**	**18.93**	**31.17**
		SEM	**1.35**	**0.53**	**0.87**	**2.51**	**2.15**	**0.52**	**0.28**
	2F	24	8.2	8.63	15.1	47.8	55.4	17.5	31.6
		25	8.1	9.12	15.8	48.3	57.4	18.4	31.5
		26	7.26	8.91	16.6	54.3	60.9	18.6	30.6
		AVERAGE	**7.85**	**8.89**	**15.83**	**50.13**	**57.90**	**18.17**	**31.23**
		SEM	**0.24**	**0.12**	**0.35**	**1.71**	**1.31**	**0.28**	**0.26**
	3M	31	9.15	5.9	12.3	43.2	73.2	20.8	28.5
		32	10.58	9.13	17.4	55.1	60.4	19.1	31.6

		Animal	WBC	RBC	HGB	HCT	MCV	MCH	MCHC
		ID	**10*3/µl**	**10*6/µl**	**g/dl**	**%**	**FL**	**pg**	**g/dl**
Mid dose 16ul		33	10.23	7.9	14.4	46.4	58.7	18.2	31
		AVERAGE	**9.99**	**7.64**	**14.70**	**48.23**	**64.10**	**19.37**	**30.37**
		SEM	**0.35**	**0.77**	**1.21**	**2.90**	**3.74**	**0.62**	**0.78**
	3F	34	5.1	8.83	16.4	52	58.9	18.6	31.5
		35	5.27	9.03	16.6	51.8	57.4	18.4	32
		36	6.69	7.54	14.4	45.3	60.1	19.1	31.8
		AVERAGE	**5.69**	**8.47**	**15.80**	**49.70**	**58.80**	**18.70**	**31.77**
		SEM	**0.41**	**0.38**	**0.57**	**1.80**	**0.64**	**0.17**	**0.12**
High dose 32ul	**4M**	41	11.48	8.02	15.8	50.5	63	19.7	31.3
		42	8.55	9.36	17.4	53.6	57.3	18.6	32.5
		43	7.67	7.87	14.6	47.1	59.8	18.6	31
		AVERAGE	**9.23**	**8.42**	**15.93**	**50.40**	**60.03**	**18.97**	**31.60**
		SEM	**0.94**	**0.39**	**0.66**	**1.53**	**1.35**	**0.30**	**0.37**
	4F	44	4.12	9.86	18.5	58.5	59.3	18.8	31.6
		45	6.63	8.5	16.1	51.7	60.8	18.9	31.1
		46	5.24	8.77	15.5	50.1	57.1	17.7	30.9

		Animal	WBC	RBC	HGB	HCT	MCV	MCH	MCHC
		ID	10*3/µl	10*6/µl	g/dl	%	FL	pg	g/dl
		AVERAGE	5.33	9.04	16.70	53.43	59.07	18.47	31.20
		SEM	0.59	0.34	0.75	2.10	0.88	0.31	0.17

Table 3. (continue) Hematology Results

		Animal	Neut	Bands	Lymph	Mono	Eos	Baso	Plate
		ID	%	%	%	%	%	%	10*3/µl
Vehicle Saline	1M	11	10	0	88	1	1	0	774
		12	8	0	90	0	2	0	1020
		13	19	0	79	1	1	0	889
		AVERAGE	**12.33**	**0**	**85.67**	**0.67**	**1.33**	**0**	**894.33**
		SEM	**2.76**	**0**	**2.76**	**0.27**	**0.27**	**0**	**58.02**
	1F	14	34	0	65	1	0	0	599
		15	12	0	88	0	0	0	817
		16	14	0	86	0	0	0	517
		AVERAGE	**20.00**	**0**	**79.67**	**0.33**	**0**	**0**	**644.33**
		SEM	**5.73**	**0**	**6.01**	**0.27**	**0**	**0**	**73.09**

		Animal	Neut	Bands	Lymph	Mono	Eos	Baso	Plate
		ID	**%**	**%**	**%**	**%**	**%**	**%**	**10*3/µl**
Low dose 8ul	**2M**	21	18	0	82	0	0	0	1042
		22	12	0	88	0	0	0	720
		23	16	0	84	0	0	0	893
		AVERAGE	**15.33**	**0**	**84.67**	**0**	**0**	**0**	**885.00**
		SEM	**1.44**	**0**	**1.44**	**0**	**0**	**0**	**75.97**
	2F	24	8	0	92	0	0	0	442
		25	8	0	86	0	0	0	564
		26	7	0	93	0	0	0	432
		AVERAGE	**7.67**	**0**	**90.33**	**0**	**0**	**0**	**479.33**
		SEM	**0.27**	**0**	**1.78**	**0**	**0**	**0**	**34.65**
Mid dose 16ul	**3M**	31	16	0	84	0	0	0	1109
		32	8	0	92	0	0	0	791
		33	18	0	82	0	0	0	739
		AVERAGE	**14.00**	**0**	**86.00**	**0**	**0**	**0**	**879.67**
		SEM	**2.49**	**0**	**2.49**	**0**	**0**	**0**	**94.42**
	3F	34	16	0	84	0	0	0	760

		Animal	Neut	Bands	Lymph	Mono	Eos	Baso	Plate
		ID	**%**	**%**	**%**	**%**	**%**	**%**	**10*3/µl**
		35	12	0	88	0	0	0	511
		36	12	0	88	0	0	0	835
		AVERAGE	**13.33**	**0**	**86.67**	**0**	**0**	**0**	**702.00**
		SEM	**1.09**	**0**	**1.09**	**0**	**0**	**0**	**79.95**
High dose 32ul	**4M**	41	20	0	80	0	0	0	573
		42	26	0	74	0	0	0	579
		43	16	0	84	0	0	0	808
		AVERAGE	**20.67**	**0**	**79.33**	**0**	**0**	**0**	**653.33**
		SEM	**2.37**	**0**	**2.37**	**0**	**0**	**0**	**63.16**
	4F	44	9	0	91	0	0	0	590
		45	18	0	82	0	0	0	828
		46	24	0	76	0	0	0	492
		AVERAGE	**17.00**	**0**	**83.00**	**0**	**0**	**0**	**636.67**
		SEM	**3.56**	**0**	**3.56**	**0**	**0**	**0**	**81.46**

Table 3. (continue) Hematology Results

		Animal	Neutr Abs	Bands Abs	Lymph Abs	Mono Abs	Eos Abs	Basos Abs
		ID	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**
Vehicle Saline	**1M**	11	1	0	8.84	0.1	0.1	0
		12	0.76	0	8.5	0	0.19	0
		13	0.76	0	3.15	0.04	0.04	0
		AVERAGE	0.84	0	6.83	0.05	0.11	0
		SEM	0.07	0	1.50	0.02	0.04	0
	1F	14	3.23	0	6.18	0.1	0	0
		15	0.68	0	4.98	0	0	0
		16	0.97	0	5.99	0	0	0
		AVERAGE	1.63	0	5.72	0.03	0	0
		SEM	0.66	0	0.30	0.03	0	0
Low dose 8ul	**2M**	21	1.63	0	7.44	0	0	0
		22	1.73	0	12.66	0	0	0
		23	1.58	0	8.28	0	0	0
		AVERAGE	1.65	0	9.46	0	0	0
		SEM	0.04	0	1.32	0	0	0
	2F	24	0.66	0	7.54	0	0	0

		Animal	Neutr Abs	Bands Abs	Lymph Abs	Mono Abs	Eos Abs	Basos Abs
		ID	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**
		25	1.23	0	7.32	0	0	0
		26	0.51	0	6.75	0	0	0
		AVERAGE	0.80	0	7.20	0	0	0
		SEM	0.18	0	0.19	0	0	0
Mid dose 16ul	**3M**	31	1.46	0	7.69	0	0	0
		32	0.85	0	9.73	0	0	0
		33	1.84	0	8.39	0	0	0
		AVERAGE	**1.38**	**0**	**8.60**	**0**	**0**	**0**
		SEM	**0.24**	**0**	**0.49**	**0**	**0**	**0**
	3F	34	0.82	0	4.28	0	0	0
		35	1	0	4.64	0	0	0
		36	0.8	0	5.89	0	0	0
		AVERAGE	**0.75**	**0**	**4.94**	**0**	**0**	**0**
		SEM	**0.05**	**0**	**0.40**	**0**	**0**	**0**
	4M	41	2.3	0	9.18	0	0	0
		42	2.22	0	6.33	0	0	0

		Animal	Neutr Abs	Bands Abs	Lymph Abs	Mono Abs	Eos Abs	Basos Abs
		ID	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**	**10*3/µl**
High dose 32ul		43	1.23	0	6.44	0	0	0
		AVERAGE	**1.92**	**0**	**7.32**	**0**	**0**	**0**
		SEM	**0.28**	**0**	**0.76**	**0**	**0**	**0**
	4F	44	0.37	0	3.75	0	0	0
		45	1.19	0	5.44	0	0	0
		46	1.26	0	3.98	0	0	0
		AVERAGE	**0.94**	**0**	**4.39**	**0**	**0**	**0**
		SEM	**0.23**	**0**	**0.43**	**0**	**0**	**0**

Table 4. Chemistry Results

		Animal	Creat	Calc	Phos	Gluc	Urea	Chol	TP
		ID	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**g/dl**
Vehicle Saline	**1M**	11	0.29	10.97	9.1	134	31.9	134	6.05
		12	0.27	10.92	9.1	123	35.7	97	6.12
		13	0.29	10.97	9.2	125	46.7	141	6.18

		Animal	Creat	Calc	Phos	Gluc	Urea	Chol	TP
		ID	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**g/dl**
		AVERAGE	**0.28**	**10.95**	**9.13**	**127.33**	**38.10**	**124.00**	**6.12**
		SEM	**0.01**	**0.01**	**0.03**	**2.76**	**3.62**	**11.15**	**0.03**
	1F	14	0.31	10.5	8.4	124	36	112	6.39
		15	0.31	10.37	7.9	111	36.8	116	6.84
		16	0.28	10.39	7.2	132	34	118	6.48
		AVERAGE	**0.30**	**10.42**	**7.83**	**122.33**	**35.60**	**115.33**	**6.57**
		SEM	**0.01**	**0.03**	**0.28**	**5.00**	**0.68**	**1.44**	**0.11**
Low dose 8ul	**2M**	21	0.3	10.95	10	118	41.5	126	6.22
		22	0.36	10.8	10.1	155	36.1	128	6.41
		23	0.23	10.88	9	126	33.7	106	6.1
		AVERAGE	**0.30**	**10.88**	**9.70**	**133.00**	**37.10**	**120.00**	**6.24**
		SEM	**0.03**	**0.04**	**0.29**	**9.18**	**1.88**	**5.73**	**0.07**
	2F	24	0.31	11.26	7.6	118	39.3	158	6.58
		25	0.39	11.07	8.5	129	35.6	114	6.38
		26	0.33	11.52	7.4	124	37.9	128	6.64
		AVERAGE	**0.34**	**11.28**	**7.83**	**123.67**	**37.60**	**133.33**	**6.53**

		Animal	Creat	Calc	Phos	Gluc	Urea	Chol	TP
		ID	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**g/dl**
		SEM	**0.02**	**0.11**	**0.28**	**2.60**	**0.88**	**10.60**	**0.06**
Mid dose 16ul	**3M**	31	0.28	11.21	10.2	119	35.9	113	6.19
		32	0.26	11.1	9.2	103	34.6	117	6.22
		33	0.28	11.47	9.1	123	33.5	109	6.19
		AVERAGE	**0.27**	**11.26**	**9.50**	**115.00**	**34.67**	**113.00**	**6.20**
		SEM	**0.01**	**0.09**	**0.29**	**4.99**	**0.57**	**1.89**	**0.01**
	3F	34	0.27	10.52	7.4	132	29.3	99	5.99
		35	0.29	11.22	7.4	126	32.6	93	6.73
		36	0.34	10.94	7.9	135	41	147	6.89
		AVERAGE	**0.30**	**10.89**	**7.57**	**131.00**	**34.30**	**113.00**	**6.54**
		SEM	**0.02**	**0.17**	**0.14**	**2.16**	**2.84**	**13.95**	**0.23**
High dose 32ul	**4M**	41	0.33	10.58	10	131	30.5	110	5.97
		42	0.33	10.99	9.2	121	29.4	114	6.46
		43	0.39	11.28	10.8	153	41.5	132	6.67
		AVERAGE	**0.35**	**10.95**	**10.00**	**135.00**	**33.80**	**118.67**	**6.37**
		SEM	**0.02**	**0.17**	**0.38**	**7.72**	**3.15**	**5.52**	**0.17**

		Animal	Creat	Calc	Phos	Gluc	Urea	Chol	TP
		ID	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**	**g/dl**
	4F	44	0.3	11.11	7.8	135	39.3	109	6.4
		45	0.34	11.04	8.3	138	37.5	130	6.61
		46	0.38	11.26	7.4	134	38.7	153	6.66
		AVERAGE	**0.34**	**11.14**	**7.83**	**135.67**	**38.50**	**130.67**	**6.56**
		SEM	**0.02**	**0.05**	**0.21**	**0.98**	**0.43**	**10.37**	**0.07**

Table 4. (continue) Chemistry Results

		Animal	Alb	Glob	T. Bil	Alk Phos	LDH	SGOT	SGPT
		ID	**g/dl**	**g/dl**	**mg/dl**	**IU/L**	**IU/L**	**IU/L**	**IU/L**
Vehicle Saline	**1M**	11	4.1	1.95	0.04	178	1657	121	58
		12	4.4	1.72	0.04	228	1500	120	61
		13	4.5	1.68	0.01	286	1620	110	80
		AVERAGE	**4.33**	**1.78**	**0.03**	**230.67**	**1592.33**	**117.00**	**66.33**
		SEM	**0.10**	**0.07**	**0.01**	**25.48**	**38.69**	**2.87**	**5.62**
	1F	14	4.7	1.69	0.06	140	1564	125	58

		Animal	Alb	Glob	T. Bil	Alk Phos	LDH	SGOT	SGPT
		ID	**g/dl**	**g/dl**	**mg/dl**	**IU/L**	**IU/L**	**IU/L**	**IU/L**
		15	5	1.84	0.06	146	1424	109	63
		16	4.8	1.68	0.05	130	782	84	52
		AVERAGE	**4.83**	**1.74**	**0.06**	**138.67**	**1103.00**	**106.00**	**57.67**
		SEM	**0.07**	**0.04**	**0.00**	**3.81**	**226.98**	**9.74**	**2.60**
Low dose 8ul	**2M**	21	4.3	1.92	0.05	229	1307	121	56
		22	4.5	1.91	0.04	266	2705	299	101
		23	4.5	1.6	0.03	225	1134	102	60
		AVERAGE	**4.43**	**1.81**	**0.04**	**240.00**	**1715.33**	**174.00**	**72.33**
		SEM	**0.05**	**0.09**	**0.00**	**10.66**	**406.08**	**51.23**	**11.74**
	2F	24	4.8	1.78	0.04	172	484	96	68
		25	4.6	1.78	0.05	155	1010	211	99
		26	4.9	1.74	0.04	160	661	89	56
		AVERAGE	**4.77**	**1.77**	**0.04**	**162.33**	**718.33**	**132.00**	**74.33**
		SEM	**0.07**	**0.01**	**0.00**	**4.12**	**126.17**	**32.29**	**10.46**
	3M	31	4.3	1.89	0.02	212	1329	110	70
		32	4.5	1.72	0.01	226	1591	110	60

		Animal	Alb	Glob	T. Bil	Alk Phos	LDH	SGOT	SGPT
		ID	**g/dl**	**g/dl**	**mg/dl**	**IU/L**	**IU/L**	**IU/L**	**IU/L**
Mid dose 16ul		33	4.3	1.89	0	268	578	75	61
		AVERAGE	**4.37**	**1.83**	**0.01**	**235.33**	**1166.00**	**98.33**	**63.67**
		SEM	**0.05**	**0.05**	**0.00**	**13.74**	**247.87**	**9.53**	**2.60**
	3F	34	4.5	1.49	0	156	838	103	64
		35	4.8	1.93	0.02	197	612	91	56
		36	4.8	2.09	0.03	180	1281	114	65
		AVERAGE	**4.70**	**1.84**	**0.02**	**177.67**	**910.33**	**102.67**	**61.67**
		SEM	**0.08**	**0.15**	**0.01**	**9.71**	**160.43**	**5.42**	**2.33**
High dose 32ul	**4M**	41	4.2	1.77	0.01	211	1337	143	65
		42	4.5	1.96	0.06	209	1084	201	75
		43	4.6	2.07	0.05	322	2029	270	107
		AVERAGE	**4.43**	**1.93**	**0.04**	**247.33**	**1483.33**	**204.67**	**82.33**
		SEM	**0.10**	**0.07**	**0.01**	**30.49**	**230.61**	**29.97**	**10.34**
	4F	44	4.7	1.7	0	146	346	83	70
		45	4.9	1.71	0.09	109	333	82	50
		46	4.9	1.76	0.05	162	614	175	77

		Animal	Alb	Glob	T. Bil	Alk Phos	LDH	SGOT	SGPT
		ID	**g/dl**	**g/dl**	**mg/dl**	**IU/L**	**IU/L**	**IU/L**	**IU/L**
		AVERAGE	**4.83**	**1.72**	**0.05**	**139.00**	**431.00**	**113.33**	**65.67**
		SEM	**0.05**	**0.02**	**0.02**	**12.81**	**74.77**	**25.18**	**6.61**

Table 4. (continue) Chemistry Results

		Animal	Trig	CPK	Na	K	Cl	GGTP
		ID	**mg/dl**	**IU/L**	**mmol/L**	**mmol/L**	**mmol/L**	**IU/L**
Vehicle Saline	**1M**	11	111	940	140	5.7	97	0
		12	78	891	141	5.9	95	0
		13	111	598	141	5.5	96	0
		AVERAGE	**100.00**	**809.67**	**140.67**	**5.70**	**96.00**	**0.00**
		SEM	**8.98**	**87.18**	**0.27**	**0.09**	**0.47**	**0.00**
	1F	14	53	860	141	5.7	98	0
		15	63	533	140	5.3	95	0
		16	62	285	141	4.8	99	0
		AVERAGE	**59.33**	**559.33**	**140.67**	**5.27**	**97.33**	**0.00**
		SEM	**2.60**	**135.95**	**0.27**	**0.21**	**0.98**	**0.00**

		Animal	Trig	CPK	Na	K	Cl	GGTP
		ID	**mg/dl**	**IU/L**	**mmol/L**	**mmol/L**	**mmol/L**	**IU/L**
Low dose 8ul	**2M**	21	130	1169	141	6.3	97	0
		22	135	6495	141	6.2	95	0
		23	88	644	139	5.5	96	0
		AVERAGE	**117.67**	**2769.33**	**140.33**	**6.00**	**96.00**	**0.00**
		SEM	**12.17**	**1526.02**	**0.54**	**0.21**	**0.47**	**0.00**
	2F	24	86	284	142	4.8	96	0
		25	89	3958	142	5.1	98	0
		26	113	304	141	5.3	97	0
		AVERAGE	**96.00**	**1515.33**	**141.67**	**5.07**	**97.00**	**0.00**
		SEM	**6.98**	**997.23**	**0.27**	**0.12**	**0.47**	**0.00**
Mid dose 16ul	**3M**	31	175	708	140	5.5	96	0
		32	134	678	141	5.9	96	0
		33	151	233	141	5.5	95	0
		AVERAGE	**153.33**	**539.67**	**140.67**	**5.63**	**95.67**	**0.00**
		SEM	**9.71**	**125.40**	**0.27**	**0.11**	**0.27**	**0.00**
	3F	34	91	701	140	5	98	0

		Animal	Trig	CPK	Na	K	Cl	GGTP
		ID	**mg/dl**	**IU/L**	**mmol/L**	**mmol/L**	**mmol/L**	**IU/L**
		35	99	256	139	5.2	97	0
		36	104	656	140	5.7	98	0
		AVERAGE	**98.00**	**537.67**	**139.67**	**5.30**	**97.67**	**0.00**
		SEM	**3.09**	**115.48**	**0.27**	**0.17**	**0.27**	**0.00**
High dose 32ul	**4M**	41	66	1264	141	5.9	97	0
		42	91	2819	141	6.3	96	0
		43	103	8618	142	6.9	96	0
		AVERAGE	**86.67**	**4233.67**	**141.33**	**6.37**	**96.33**	**0.00**
		SEM	**8.90**	**1827.04**	**0.27**	**0.24**	**0.27**	**0.00**
	4F	44	88	471	141	5.2	97	0
		45	50	364	139	5.2	97	0
		46	63	3088	140	5.5	98	0
		AVERAGE	**67.00**	**1307.67**	**140.00**	**5.30**	**97.33**	**0.00**
		SEM	**9.10**	**727.26**	**0.47**	**0.08**	**0.27**	**0.00**

Table 5. Urine examination

	Group	Rat No	Leukocytes (cell/µl)	Urobilinogen (mg/dL)	Bilirubin (mg/dL)	Blood (cell/µl)	Nitrite
Vehicle Saline	**1M**	11	±15	+2	negative	negative	negative
		12	±15	+2	negative	negative	negative
		13	±15	+2	negative	negative	negative
	1F	14	±15	+2	negative	negative	negative
		15	±15	+2	negative	negative	negative
		16	±15	+2	negative	negative	negative
Low dose 8ul	**2M**	21	±15	+2	negative	negative	negative
		22	±15	+2	negative	negative	negative
		23	±15	+2	negative	negative	negative
	2F	24	±15	+2	negative	negative	negative
		25	±15	+2	negative	negative	negative
		26	±15	+2	negative	negative	negative
Mid dose 16ul	**3M**	31	±15	+2	negative	negative	negative
		32	±15	+2	negative	negative	negative
		33	±15	+2	negative	negative	negative
	3F	34	±15	+2	negative	negative	negative

		35	±15	+2	negative	negative	negative
		36	±15	+2	negative	negative	negative
High dose 32ul	**4M**	41	±15	+2	negative	negative	negative
		42	±15	+2	negative	negative	negative
		43	±15	+2	negative	negative	negative
	4F	44	±15	+2	negative	negative	negative
		45	±15	+2	negative	negative	negative
		46	±15	+2	negative	negative	negative

Table 5. (continue) Urine examination

	Group	**Rat No**	**pH**	**Specific Gravity**	**Protein (mg/dL)**	**Glucose (mg/dL)**	**Keytone (mg/dL)**
Vehicle Saline	**1M**	11	7	1.03	+30	negative	negative
		12	6.5	1.03	+30	negative	negative
		13	6.5	1.03	+30	negative	negative
	1F	14	6.5	1.03	+30	negative	negative
		15	7	1.03	+30	negative	negative
		16	6.5	1.03	+30	negative	negative

Low dose 8ul	**2M**	21	6.5	1.03	+30	negative	negative
		22	6.5	1.03	+30	negative	negative
		23	6.5	1.03	+30	negative	negative
	2F	24	6.5	1.03	+30	negative	negative
		25	7	1.03	+30	negative	negative
		26	6.5	1.03	+30	negative	negative
Mid dose 16ul	**3M**	31	6.5	1.03	+30	negative	negative
		32	7	1.03	+30	negative	negative
		33	7	1.03	+30	negative	negative
	3F	34	6.5	1.03	+30	negative	negative
		35	6.5	1.03	+30	negative	negative
		36	6.5	1.03	+30	negative	negative
High dose 32ul	**4M**	41	6.5	1.03	+30	negative	negative
		42	6.5	1.03	+30	negative	negative
		43	7	1.03	+30	negative	negative
	4F	44	7	1.03	+30	negative	negative
		45	6.5	1.03	+30	negative	negative
		46	6.5	1.03	+30	negative	negative

Table 6. Ophthalmological examination

	Group	Rat No	Ophthalmological Examination
Vehicle Saline	**1M**	11	normal
		12	normal
		13	normal
	1F	14	normal
		15	normal
		16	normal
Low dose 8ul	**2M**	21	normal
		22	normal
		23	normal
	2F	24	normal
		25	normal
		26	normal
Mid dose 16ul	**3M**	31	normal
		32	normal
		33	normal
	3F	34	normal

		35	normal
		36	normal
High dose 32ul	**4M**	41	normal
		42	normal
		43	normal
	4F	44	normal
		45	normal
		46	normal

Histopathology results

Samples (n=168) of liver, heart, brain, spleen, spinal cord (cervical, thoracal and lumbar), sciatic nerve, kidney (L+R), lungs and tongue from 24 rats were harvested, fixed in 4% formaldehyde, arrived to Patho-Logica in the fixative and kept in the fixative for 48 hours, for further fixation. Then, the tissues were trimmed, put in embedding cassettes and processed routinely for paraffin embedding. Seven cassettes were prepared per animal. Paraffin blocks were cut at approximately 4 microns thickness. The sections were put on glass slides and stained with Hematoxylin & Eosin (H&E). Pictures were taken using Olympus microscope (BX60, serial NO. 7D04032) at objective magnification of X4 and X10 and microscope's Camera (Olympus DP73, serial NO. OH05504).

Histological evaluation

The H&E stained slides were examined, described and scored by the study Pathologist, using a semi-quantitative grading of five grades (0-4), for the severity of the pathological changes (Schafer et al.):

Grade 0 – The tissue appears normal, without any changes at all.

Grade 1 – Minimal pathological findings .

Grade 2 – Mild pathological findings.

Grade 3 – Moderate pathological findings .

Grade 4 – Severe pathological findings .

The histopathological evaluation included a comparison between treated and control or naïve animals. Pathological findings were described, scored and demonstrated in representative histological pictures.

Upon the client requirement and only in specific TOX studies NOAEL and LOAEL values were determined following examination of all samples from control and treated groups, and comparison between all treated groups (control, low, intermediate and high doses) .

Results

Histopathology

- In general, the H&E stained sections didn't show any pathological changes in all tested animal samples .
- The spleen of all tested animals were reactive, showing a marked proliferation of the white pulp lymphocytes. However, this is not considered

as a pathological finding and is most probably not related to the tested treatment .

- In the tongue's muscles of all samples, a population of mast cells was observed. These cells are resident of this area, therefore this finding is not considered as a pathological one.
- In all the spinal cords, the white matter appeared diffusely vacuolated. This change is regarded as an artifact due to the decalcification process. These changes were not observed in the brans sections .
- The pathological evaluation, including grading, in individual animals in the different groups is given below in Table 7.

Table 7. A semi-quantitative analysis of the histological findings, using a scoring scale (see details in M&M)

Group / treatment	Animal NO.	Brain	Heart	Lungs	Liver	Kidneys	Spleen	Spinal cord	Sciatic nerve	Tongue
G1	11	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0
	13	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0

Table 7. A semi-quantitative analysis of the histological findings, using a scoring scale (see details in M&M)

Group / treatment	**Animal NO.**	**Brain**	**Heart**	**Lungs**	**Liver**	**Kidneys**	**Spleen**	**Spinal cord**	**Sciatic nerve**	**Tongue**
	15	0	0	0	0	0	0	0	0	0
	16	0	0	0	0	0	0	0	0	0
G1	**Mean**	0	0	0	0	0	0	0	0	0
N=6	**SD**	0	0	0	0	0	0	0	0	0
G2	21	0	0	0	0	0	0	0	0	0
	22	0	0	0	0	0	0	0	0	0
	23	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0	0	0
	26	0	0	0	0	0	0	0	0	0
G2	**Mean**	0	0	0	0	0	0	0	0	0

Table 7. A semi-quantitative analysis of the histological findings, using a scoring scale (see details in M&M)

Group / treatment	**Animal NO.**	**Brain**	**Heart**	**Lungs**	**Liver**	**Kidneys**	**Spleen**	**Spinal cord**	**Sciatic nerve**	**Tongue**
N=6	**SD**	0	0	0	0	0	0	0	0	0
G3	31	0	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	0
	33	0	0	0	0	0	0	0	0	0
	34	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0
	36	0	0	0	0	0	0	0	0	0
G3	**Mean**	0	0	0	0	0	0	0	0	0
N=6	**SD**	0	0	0	0	0	0	0	0	0
Group 4	41	0	0	0	0	0	0	0	0	0
	42	0	0	0	0	0	0	0	0	0
	43	0	0	0	0	0	0	0	0	0

Table 7. A semi-quantitative analysis of the histological findings, using a scoring scale (see details in M&M)

Group / treatment	Animal NO.	Brain	Heart	Lungs	Liver	Kidneys	Spleen	Spinal cord	Sciatic nerve	Tongue
	44	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0
	46	0	0	0	0	0	0	0	0	0
G4	**Mean**	0	0	0	0	0	0	0	0	0
N=6	**SD**	0	0	0	0	0	0	0	0	0

Representative Histological photographs

H&E staining; Objective magnification X4 .

Figure 5. Group 4M. Animal #42, Brain - hippocampus. No pathological nor cytotoxic changes.

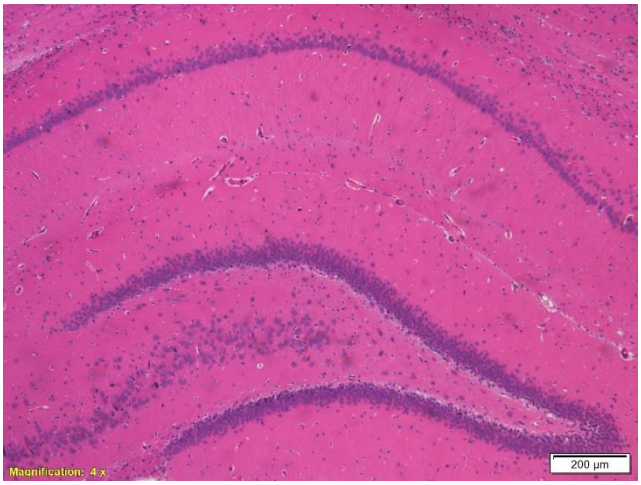


Figure 6. Group 4M. Animal #42, Heart. No pathological nor cytotoxic changes.

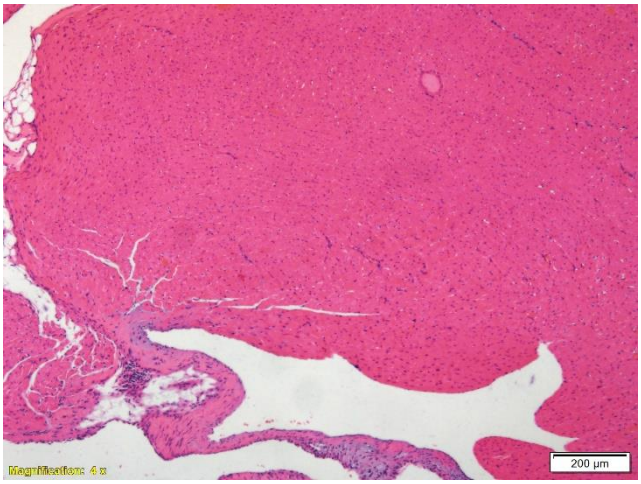


Figure 7. Group 4M. Animal #42, Lung. No pathological nor cytotoxic changes.

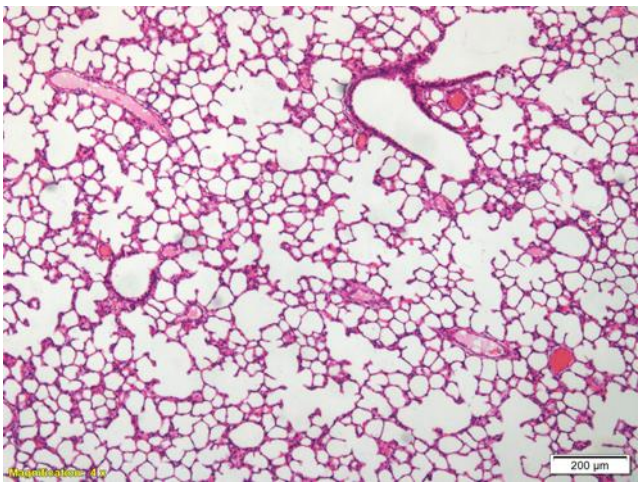


Figure 9. Group 4M. Animal #42, Liver. No pathological nor cytotoxic changes.

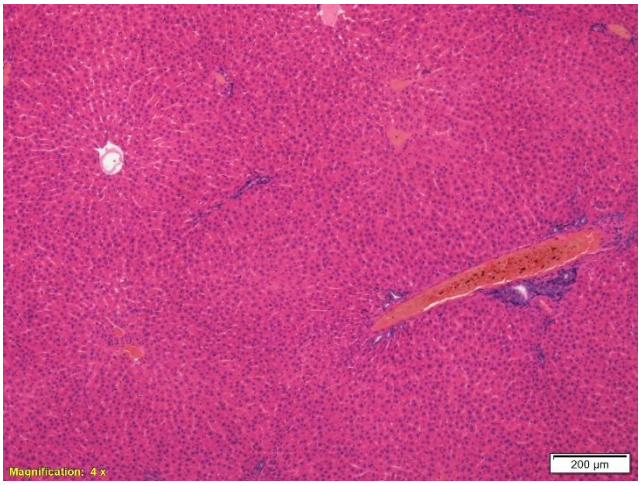


Figure 9. Group 4M. Animal #42, Kidney. No pathological nor cytotoxic changes.

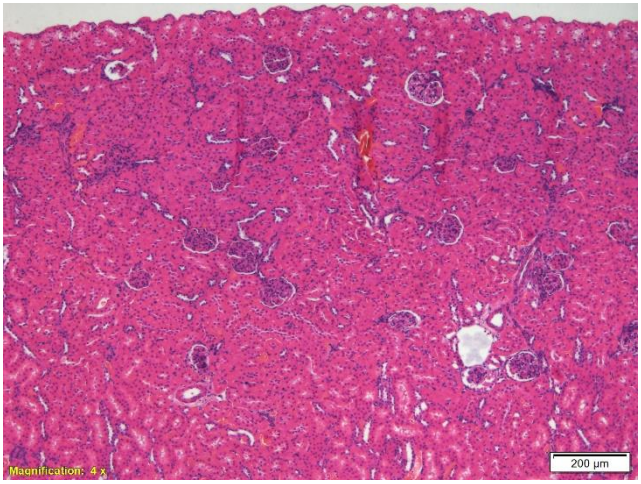


Figure 10. Group 4M. Animal #42, Spleen. No pathological nor cytotoxic changes. The white pulp looks active.

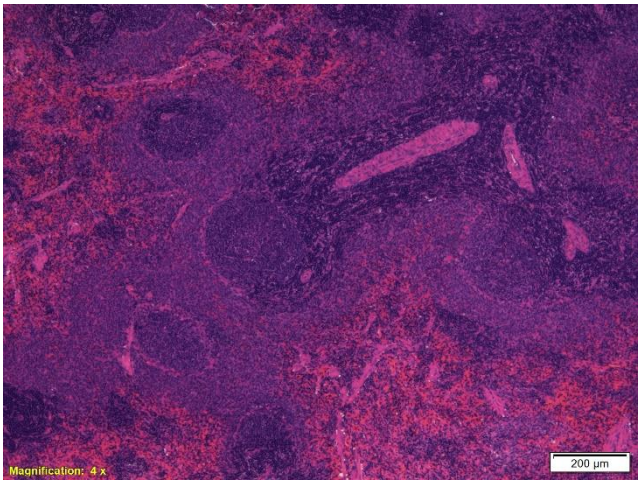


Figure 11. Group 4M. Animal #42, Spinal cord, cervical segment. No pathological nor cytotoxic changes. The white matter showes vacuolization, due to a technical artifact.

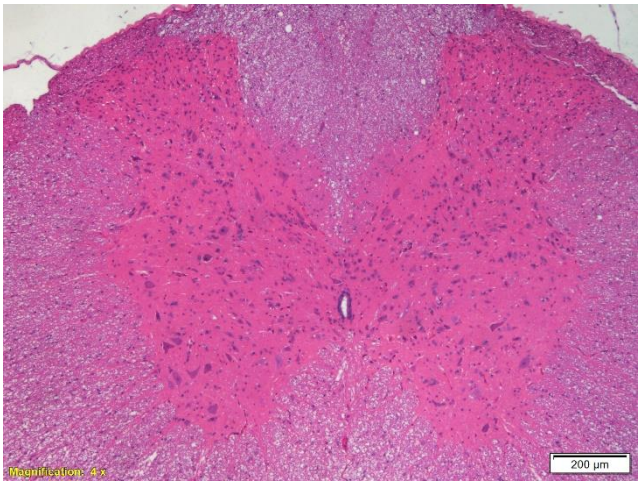


Figure 12. Group 4M. Animal #42, Sciatic nerve. No pathological nor cytotoxic changes.

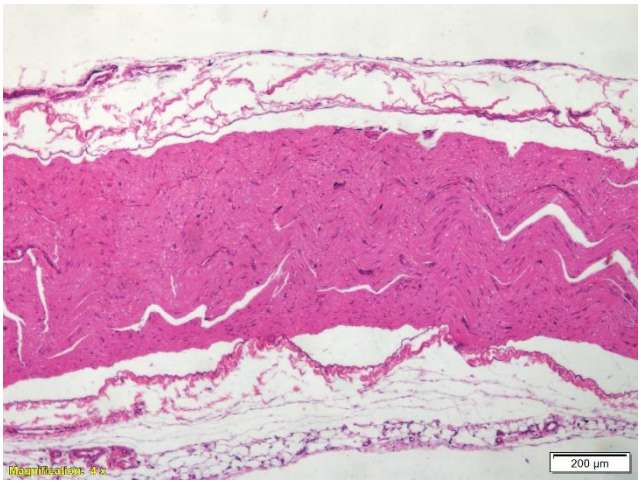
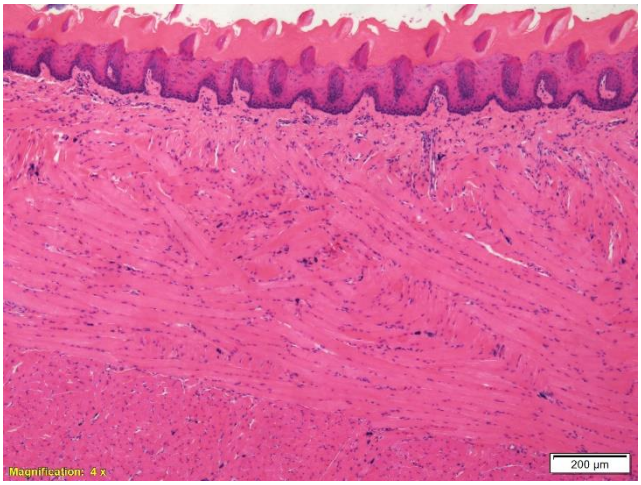


Figure 9. Group 4M. Animal #42, Tongue. No pathological nor cytotoxic changes. Arrows indicating some mast cells (resident cells) in the striated muscle.



Summary and Conclusions

All samples looked normal and didn't show any pathological changes .

4.2. In vitro study

The goal of the study was to examine the effect of ArtemiC and their components on human peripheral blood mononuclear cells (PBMCs) viability and their capacity to attenuate inflammatory response upon stimulation with E coli-derived Lipopolysaccharide (LPS).

Preparation of ArtemiC and their components dilutions in culture medium as indicated in table 8.

Table 8: ArtemiC and its components dilutions in culture medium

Name	**Stock concentration (%)**	**Stock conc. (mg/ml)**	**Target conc. in culture media (ug/ml)**	Dilution factor

Full formulation	ArtemiC	Artemisinin	0.6	6	3.704	1620
		M vitamin C	1.5	15	9.259	1620
		Dissolved vitamin C	4.5	45	27.778	1620
		Olibanum (Boswellia)	1.5	15	9.259	1620
		Curcumin	2	20	12.346	1620
Mono components		Artemisinin	2	20	3.704	5400
	mix Vitamin C 6%	M vitamin C + Dissolved vitamin C	1.5	15	9.259	1620
			4.5	45	27.778	1620
		Olibanum (Boswellia)	2.5	25	9.259	2700
		Curcumin	6	60	12.346	4860

Procedure:

PBMCs were thawed and seeded at a density of 5x105 cells/well in 100µl of culture medium.

PBMCs were incubated at 370C in 5% CO2 for 18 hours.

For viability assay, after incubation period, the cells were centrifuged at 300g for 8 minutes and culture media was exchanged with culture media containing test items alone or in combination as indicated in table 9.

Table 9: Treatment groups

Group	Treatment
1	ArtemiC
2	Artemisinin
3	Curcumin
4	Boswellia
5	Vitamin C mix
6	Artemisinin + Curcumin
7	Artemisinin + Boswellia
8	Artemisinin + Vitamin C mix
9	Artemisinin + Curcumin + Vitamin C mix
10	Artemisinin + Curcumin + Boswellia
11	Boswellia + Curcumin + Vitamin C mix

12	Boswellia +Artemisinin + Vitamin C mix
13	Curcumin + Boswellia- **CimetrA**
14	Curcumin + Vitamin C mix
15	Boswellia + Vitamin C mix
16	Vehicle

The treatments were performed in triplicates.

Wells with cells in culture media containing cell culture grade water (vehicle) will serve as control. PBMCs were incubated with test items/control for 24 hours at 370C in 5% CO2. At the end of incubation period, PBMCs were subjected to viability evaluation using RealTime-Glo(TM) MT Cell Viability Assay, according to manufacturer's instructions. For examination of anti-inflammatory effect, PBMCs were pre-treated with test items individually or in combinations, as indicated in table 2 above and incubated for 3h at 370C in 5% CO2. After incubation period, PBMCs were stimulated with 100ng/ml LPS with or w/o test items and incubated for 24h at 370C in 5% CO2. Additional set of PBMCs pretreated with ArtemiC formulation was concomitantly stimulated with 10ng/ml LPS with ArtemiC formulation and incubated for 24h at 370C in 5% CO2. PBMCs treated with LPS 10ng/ml alone served as control. At the end of incubation period, conditioned media

was collected and subjected to cytokine analysis using Human Magnetic Luminex Assay kit for TNF-α, IL-1Ra, IL-1β, IL-6 and IL-2, according to manufacturer instructions.

Results

Table 9: PBMCs viability

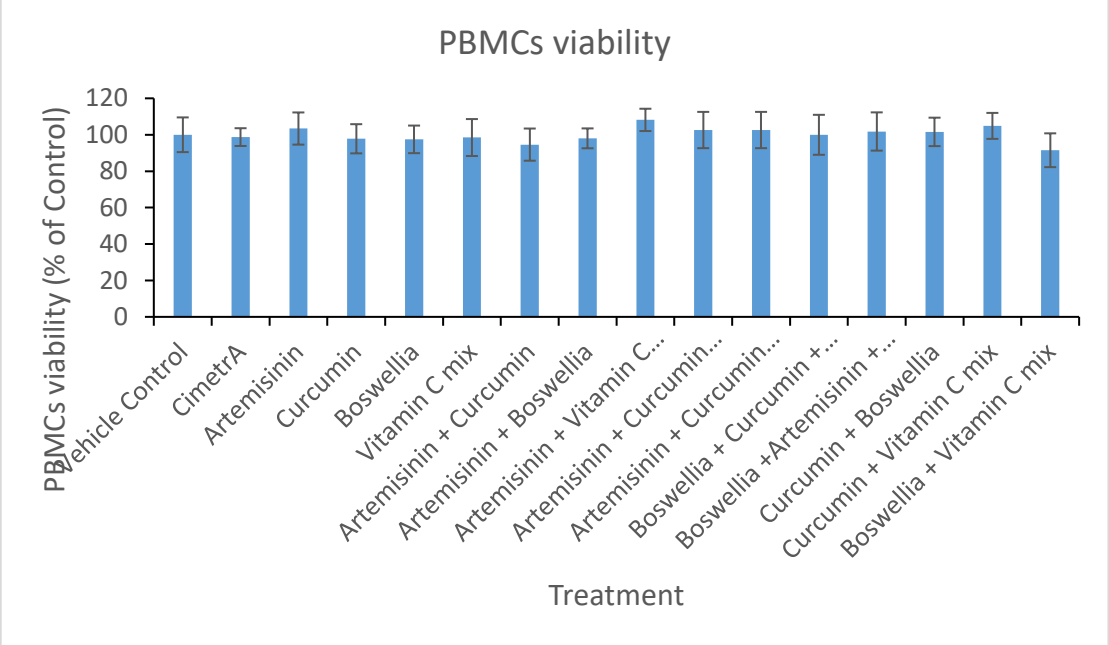
Sample name	**Luminescence reads**	**Luminescence reads w/o background**	**cell viability (%)**	**Mean cell viability (%)**	**Std. cell viability**
Vehicle Control	6690	6250.33	99.68	**100.00**	9.53
	5988	5548.33	88.49		
	7452	7012.33	111.83		
ArtemiC	6277	5837.33	93.09	**98.72**	4.87
	6816	6376.33	101.69		
	6796	6356.33	101.37		
Artemisinin	6847	6407.33	102.18	103.42	8.83
	7513	7073.33	112.81		
	6414	5974.33	95.28		
Curcumin	7152	6712.33	107.05	97.80	8.01
	6267	5827.33	92.93		
	6297	5857.33	93.41		

Boswellia	6141	5701.33	90.93	97.47	7.58
	7072	6632.33	105.77		
	6442	6002.33	95.73		
Vitamin C mix	7331	6891.33	109.90	98.47	10.14
	6395	5955.33	94.98		
	6117	5677.33	90.54		
Artemisinin + Curcumin	5811	5371.33	85.66	94.59	8.83
	6918	6478.33	103.32		
	6383	5943.33	94.78		
Artemisinin + Boswellia	6721	6281.33	100.18	98.03	5.46
	6841	6401.33	102.09		
	6197	5757.33	91.82		
Artemisinin + Vitamin C mix	7503	7063.33	112.65	108.17	6.12
	6785	6345.33	101.20		
	7379	6939.33	110.67		
	6715	6275.33	100.08	102.35	2.65
	6817	6377.33	101.71		

Artemisinin + Curcumin + Vitamin C mix	7040	6600.33	105.26		
Artemisinin + Curcumin + Boswellia	6861	6421.33	102.41	102.60	9.96
	7504	7064.33	112.66		
	6255	5815.33	92.74		
Boswellia + Curcumin + Vitamin C mix	5927	5487.33	87.51	99.98	10.98
	7224	6784.33	108.20		
	6975	6535.33	104.23		
Boswellia +Artemisinin + Vitamin C mix	7240	6800.33	108.45	101.80	10.51
	6063	5623.33	89.68		
	7165	6725.33	107.26		
Curcumin + Boswellia- **CimetrA**	6569	6129.33	97.75	101.58	7.80
	6486	6046.33	96.43		
	7372	6932.33	110.56		
Curcumin + Vitamin C mix	6521	6081.33	96.99	104.87	7.14
	7134	6694.33	106.76		
	7392	6952.33	110.88		

Boswellia + Vitamin C mix	6814	6374.33	101.66	91.51	9.28
	5673	5233.33	83.46		
	6046	5606.33	89.41		
background	483	439.67	NA	NA	NA
	447				
	389				

Fig. 10. PBMC viability



Cytokine secretion

TNF-a, IL-1RA, IL-1b, IL-6 and IL-2 concentration in PBMCs conditioned medium upon the pre-treatment with test items for 3 hours and concomitant

stimulation with LPS for 24 hours was evaluated using Human Magnetic Luminex Assay.

Fig. 11. TNF-α concentration

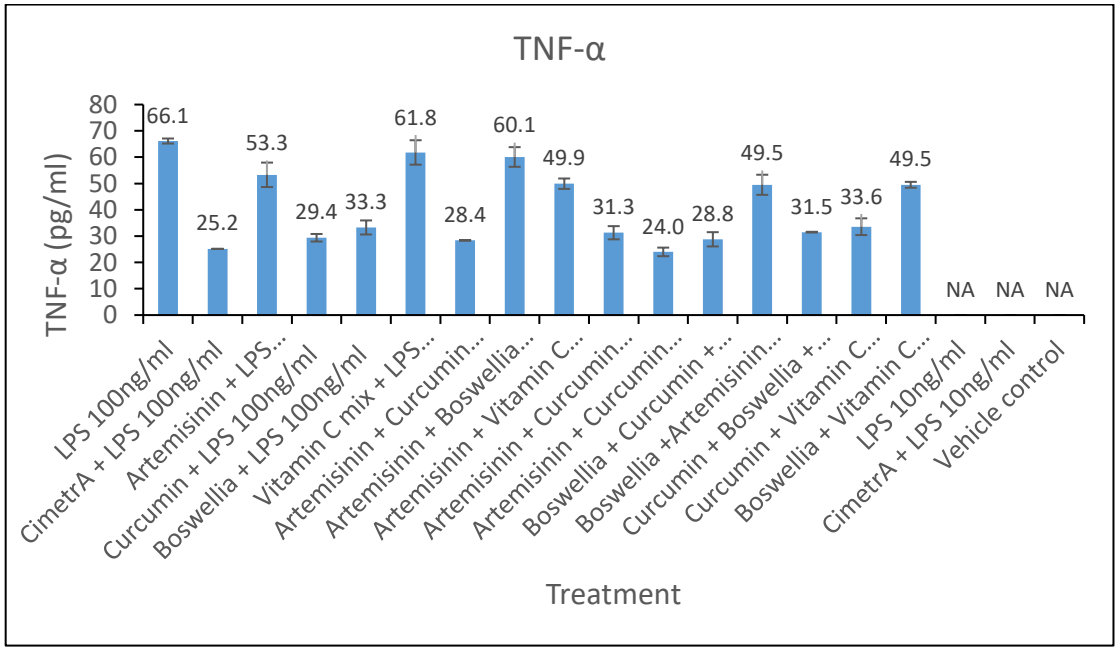


Fig. 12. IL-6 concentration

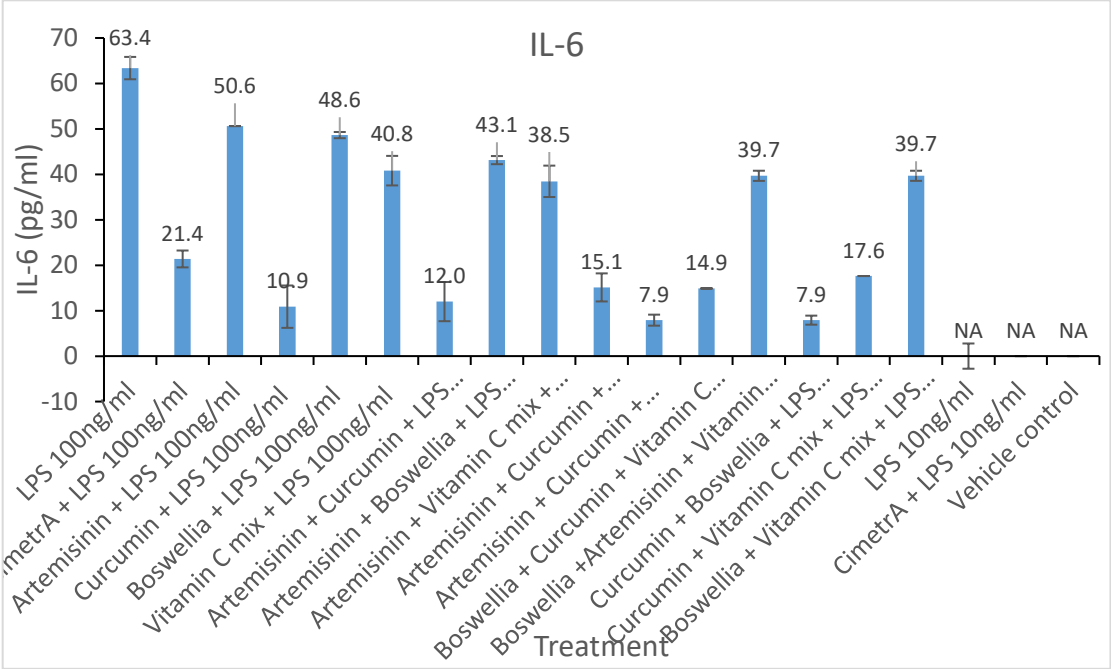


Fig. 13. IL-1β concentration

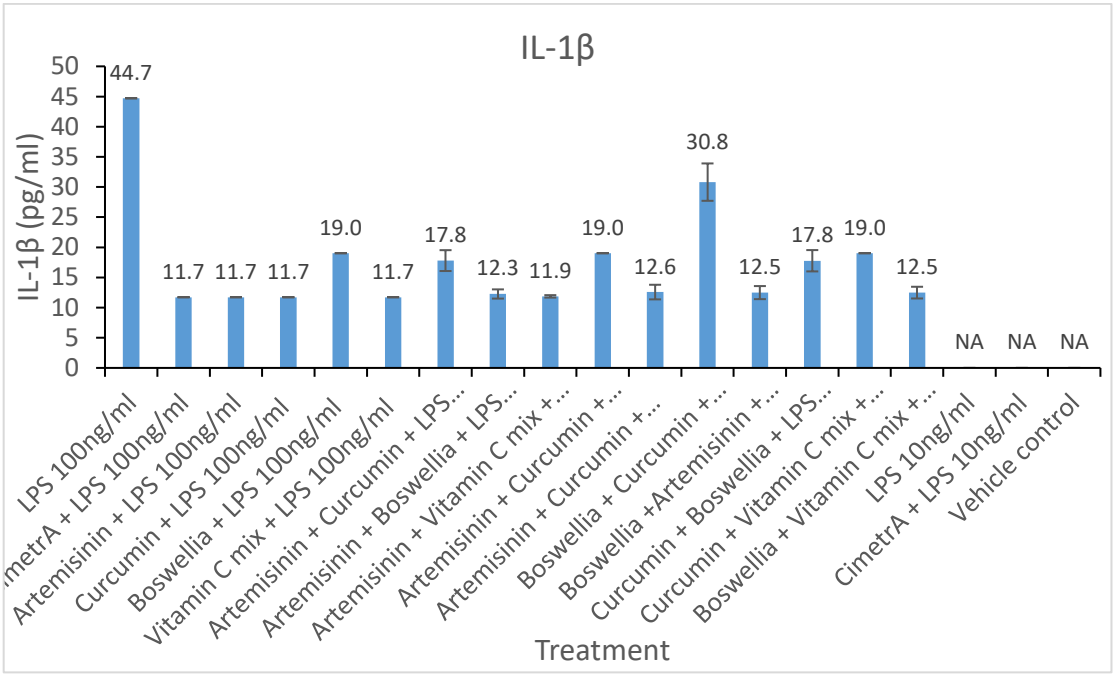


Fig. 14. IL -1Ra concentration

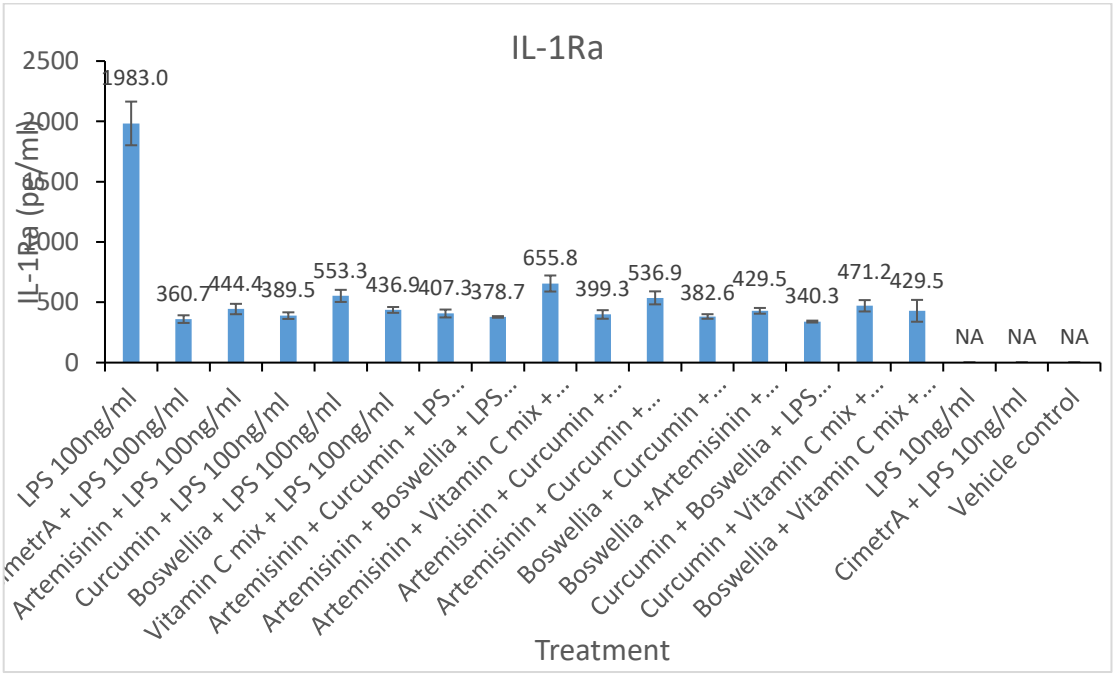
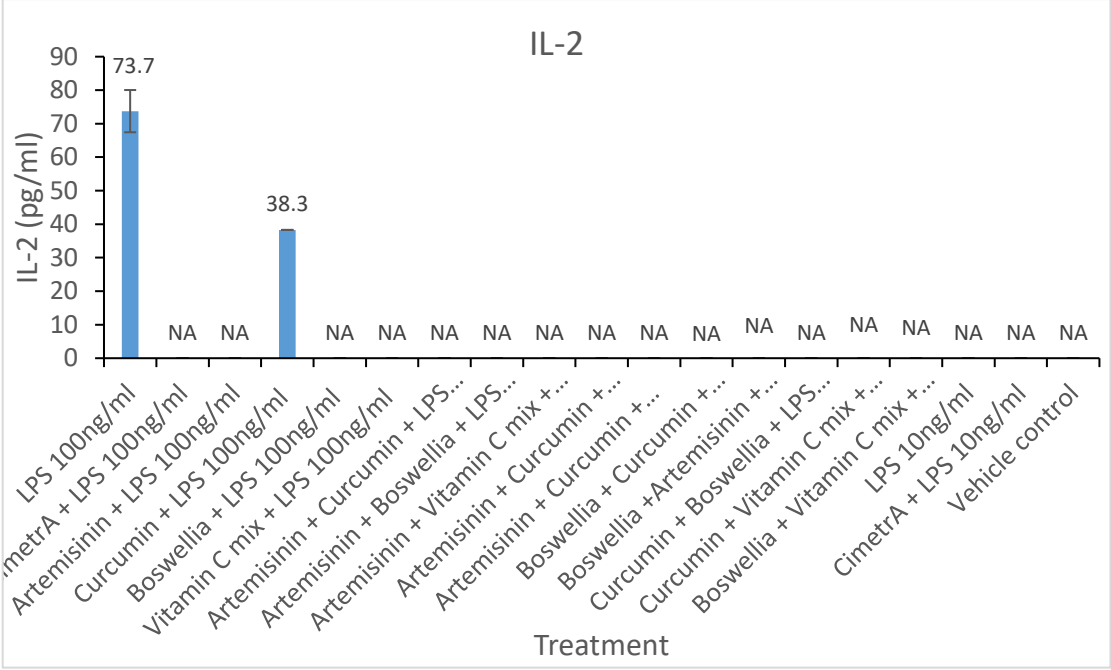


Fig. 15. IL-2 concentration



Conclusions: in the in vitro study on the induced cell culture, ArtemiC demonstrated the ability to prevent a cytokine storm.

5. CLINICAL DATA

Randomized Controlled clinical trial on moderate hospitalized COVID-19 patients was managed in Israel and India. 50 patients enrolled to the study, 33 in the treatment group and 17 in the placebo group. 40 patients were recruited in 3 hospitals in Israel and 10 – in one hospital in India.

ArtemiC was administrated in the dosage of Artemisinin 12 mg, Curcumin 40 mg, Boswellia 30 mg and Vitamin C 120 mg, in spray administration – divided in 4 separate doses given as an add on therapy, 4 doses over 48 hours (day 1 and day 2), twice a day (morning and evening). The study drug dose was defined based on the Acute Tox preclinical study.

Study purpose – This study was designed to evaluate the safety and efficacy of ArtemiC on patients diagnosed with COVID-19.

Study endpoints –

The primary outcomes:

- Time to clinical improvement, defined as a national Early Warning Score 2 (NEWS2) of </= 2 Maintained for 24 Hours in comparison to routine treatment
- Percentage of participants with definite or probable drug related adverse events

The secondary outcomes:

- Time until negative PCR
- Proportion of participants with normalization of fever and oxygen saturation through day 14 since onset of symptoms
- COVID-19 related survival
- Incidence and duration of mechanical ventilation
- Incidence of Intensive Care Init (ICU) stay
- Duration of ICU stay
- Duration of time on supplemental oxygen
- Additional Data will be recorded to complete the Core Set of Outcomes

Study population:

Inclusion Criteria:

1. Confirmed SARS-CoV-2 infection.

2. Hospitalized patient with COVID-19 of moderate stable or worsening severity not requiring ICU admission, and on the other hand not experiencing clinical improvement under ongoing standard care.

3. Age: 18 years old and above.

4. Subjects must be under observation or admitted to a controlled facility or hospital (home quarantine is not sufficient).

5. Ability to receive treatment by spray into the oral cavity

Exclusion Criteria:

1. Tube feeding or parenteral nutrition.

2. Patient who need oxygen supply beyond use of nozzles or simple mask as per score 4.

3. Respiratory decompensation requiring mechanical ventilation.

4. Uncontrolled diabetes type 2.

5. Autoimmune disease.

6. Pregnant or lactating women.

7. Need for admission to ICU in the course of the present hospitalization at any time prior to completion of the recruitment to the study.

8. Any condition which, in the opinion of the Principal Investigator, would prevent full participation in this trial or would interfere with the evaluation of the trial endpoints.

Methodology:

Multi-center-controlled study. 50 adult patients who suffer from COVID-19 infection. Safety was assessed through collection and analysis of adverse events, blood and urine laboratory assessments and vital signs. After Screening visit, the study drug was administrated twice a day morning and evening (every 12 hours) during (day 1 and day 2) The patients was randomized 2:1 for study drug and Standard of Care and or placebo and Standard of care respectively. The study last 2 weeks until conclusion on day 15 or until discharge from hospital, whichever occurs later. In case of hospital discharge within the study period, follow up continued per protocol until day 15 wherever the subject will be located. In event of a prolonged hospitalization beyond 15 days, subjects continued to be monitored for safety and endpoints until discharge.

Baseline characteristic of patients are presented in table 10.

Table 10 Demographics and Baseline Characteristics (ITT Population)

Population	**Treatment Group**		**Total, n (%) (N=50)**
	Active (N=33)	**Placebo (N=17)**	
Age (years)			
Mean (SD)	52 (14)	53 (14)	53 (14)
Median	49.5	55	51
Minimum, Maximum	30,84	22,74	22, 84
Sex, n (%)			
Male	17 (52)	8 (47)	25 (50)
Female	15 (46)	9 (53)	24 (48)
Not documented	1 (3)	-	1 (2)
Race, n (%)			
Asian	9 (27)	1 (6)	10 (20)
White	23 (70)	16 (94)	39 (78)
African	1 (3)	-	1 (2)
Smoker, n (%)			
Current	5 (15)	3 (18)	8 (16)
Past	1 (3)	3 (18)	4 (8)
Never	26 (79)	11 (65)	37 (75.5)
Alcohol consumer, n (%)			

Table 10 Demographics and Baseline Characteristics (ITT Population)

Occasional	2 (6)	0	2 (4)
Weekly	0 (0)	1 (6)	1 (2)
Never	30 (91)	16 (94)	46(92)
NEWS2			
Mean (SD)	1.5 (2.0)	1.9 (2.1)	1.6 (2.0)
Median	1	1	1
Minimum, Maximum	0, 7	0, 7	0 , 7
NEWS2, n (%)			
0	15 (45.4)	4 (23.5)	19 (38.0)
1	7 (21.2)	6 (35.3)	13 (26.0)
2	3 (9.0)	3 (17.6)	6 (12.0)
3	2 (6.0)	1 (5.9)	3 (6.0)
4	2 (6.0)	1 (5.9)	3 (6.0)
5	2 (6.0)	-	2 (4.0)
6	1 (3.0)	1 (5.9)	2 (4.0)
7	1 (3.0)	1 (5.9)	2 (4.0)
PCR COVID-19, n (%)			
Positive	32 (97.0)	17 (100)	49 (98.0)
Unknown	-	1 (3.0)	1 (2.0)
Supplemental O_2, n (%)	4 (12.1)	3 (17.6)	7 (14.0)
Systolic blood pressure, mmHg			
Mean (SD)	125.3 (18.0)	128.9 (24.7)	126.6 (20.4)
Median	125	123	124
Min, Max	98, 163	97, 190	97, 190
Pulse, mmHg			
Mean (SD)	78.4 (13.1)	73.5 (14.6)	76.7 (13.6)
Median	80	73	75
Minimum, Maximum	59, 116	38, 105	38, 116
Temperature, °C			
Mean (SD)	36.9 (0.5)	36.8 (0.5)	36.8 (0.5)
Median	36.8	36.8	36.8
Minimum, Maximum	36.0, 39.4	36.0, 37.9	36.0, 39.4
Saturation, %			
Mean (SD)	96.6 (2.1)	94.9 (4.5)	96.2 (3.4)
Median	97	96	97
Minimum, Maximum	91, 100	80, 100	80, 100
Comorbidities, n (%)			
Myocardial infarction	3 (9.1)	3 (17.6)	6 (12.0)
Percutaneous coronary intervention	2 (6.1)	3 (17.6)	5 (10.0)
Cerebrovascular accident	-	2 (11.8)	2 (4.0)
Atrial fibrillation	2 (6.1)	0	2 (4.0)

Table 10 Demographics and Baseline Characteristics (ITT Population)

Ischemic heart disease	1 (3.0)	2 (11.8)	3 (6.0)
Heart failure	3 (9.1)	3 (17.6)	6 (12.0)
Type 2 diabetes mellitus	11 (33.3)	11 (64.7)	22 (44.0)
Hypertension	13 (39.4)	10 (58.8)	23 (46.0)
Hyperlipidemia	11 (33.3)	10 (58.8)	21 (42.0)
Chronic obstructive pulmonary disease	2 (6.1)	1 (5.9)	3 (6.0)
Pulmonary arterial hypertension	2 (6.1)	1 (5.9)	3 (6.0)
Surgery	2 (6.1)	4 (23.5)	6 (12.0)
Gasterology	7 (21.2)	3 (17.6)	10 (20.0)
Neurological	3 (9.1)	4 (23.5)	7 (14.0)
Anemia	9 (27.3)	8 (47.0)	17 (34.0)
Obesity	11 (33.3)	5 (29.4)	16 (32.0)
Fatty liver	1 (3.0)	3 (17.6)	4 (8.0)
Asthma	3 (9.1)	1 (5.9)	4 (8.0)
Renal failure	2 (6.0)	1 (5.9)	3 (6.0)
Cataract	-	3 (17.6)	3 (6.0)
Lower back pain	2 (6.0)	1 (5.9)	3 (6.0)

Table 10. Baseline characteristic of treatment and placebo groups.

Efficacy data

NEWS score analysis

All 33 patients in the treatment group had NEWS scores less or equal than 2 in the last measurement, compare to only 12 out of 17 in the placebo group; P=0.015.

There was no different in the mean NEWS score in visit one, while there was significant lower score in the last measurement. See table 11 below.

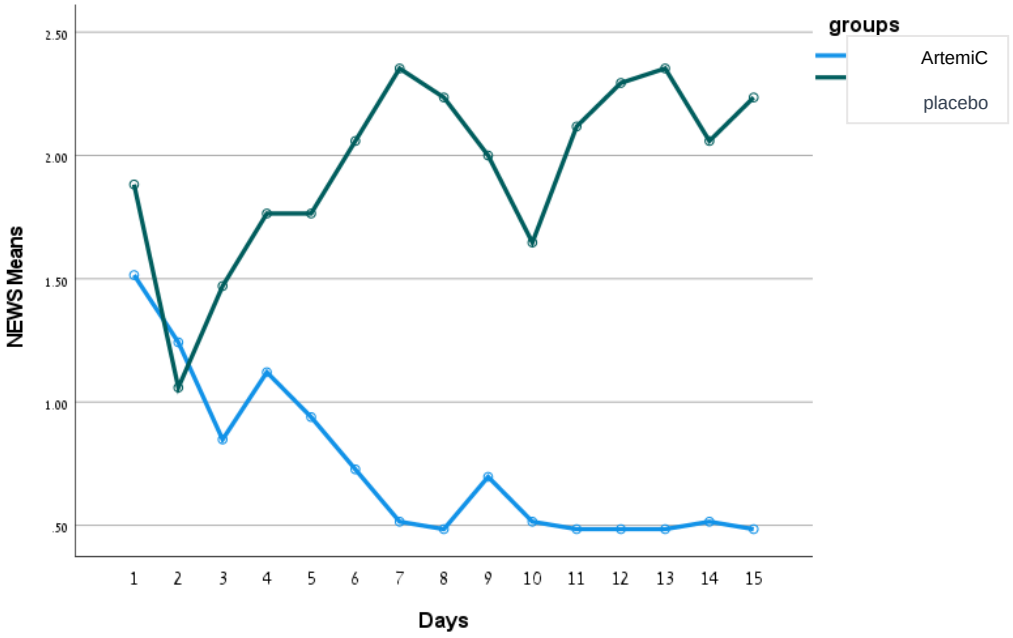
Table 11. NEWS Score change in both study groups

Group Statistics

	groups	N	Mean	Std. Deviation	Std. Error Mean	P
sum_news.1	ArtemiC	33	1.5152	2.00189	.34848	
	placebo	17	1.8824	2.05798	.49913	0.546
last_news	ArtemiC	33	.5152	.66714	.11613	
	placebo	17	2.2353	3.19236	.77426	0.042

In an ITT analysis using imputation by last observation carried forward, there was significant different between the groups.

Fig. 16. Daily change of NEWS Score in both study groups



Room air or supplemental O2 by patients is presented in Table 12.

Table 12. Room air or supplemental O2 by patients.

	Visit 1	*During study*	*Last observed*
ArtemiC *n=33*	*4 patients*	*4 patients*	*0 patients*
Placebo n=17	*3 patients*	*5 patients*	*4 patients*

Safety data

No drug related SAEs or AEs were reported during the study.

9 patients out of 50 reported the following side effects - abdominal pain, chest pain, anemia, cough, bradycardia, increase in BUN, decrease in blood clot values, sepsis infection, increase in blood leukocyte level. Those effects were defined as not related to the study drug adverse events that related to the medical condition of the patients before the hospitalization or due to COVID-19 diagnosis.

Based on the published literature, we can suppose the following potential safety information - Curcumin seems to be generally well tolerated. The most common side effects observed in clinical studies are gastrointestinal and include constipation, dyspepsia, diarrhea, distension, gastroesophageal reflux, nausea, vomiting, yellow stool and stomach ache. These adverse events are defined as rare, and may affect up to 1 in 10,000 people.

Boswellia is also well tolerated. The most common published side effects are related to the potential ability of Boswellia to stimulate blood flow in the uterus and pelvis. It can accelerate menstrual flow and may induce miscarriage in pregnant women. Other possible side effects of boswellia include nausea, acid reflux, diarrhea and skin rashes. These adverse events are defined as rare and may affect up to 1 in 10,000 people.

There is no scientific data about the specific requirements in case of intoxication by curcumin or Boswellia, usual recommendation propose to contact physician in these cases.

Summary of SAEs during the study is presented in Table 13.

Table 13. Safety data summary.

	ArtemiC	***Placebo***
Number of patients	*9 patients: (max severity)* *Mild 4* *Moderate 4* *Severe 1*	*7 patients: (max severity)* *Mild 5* *Moderate - 0* *Severe 2*
Number of events	*13 events:* *Mild 6* *Moderate 5* *severe 2*	*16 events:* *Mild 13* *Moderate 1* *Severe 2*
C01-001		*Worsening of abcess (mild)*
C01-002		*Exacerbation of desaturation+ (moderate)* *Acute respiratory..(severe)+* *Melena(mild)+*

		Tachycardia(mild)+ *AF (mild)*
C02-005	*Worsening of leuckocytosis(mild)+* *Exacerbation of musculoskeletal pain(moderate)+* *Abdominal pain(mild)+* *Worsening of hypokalemia (moderate)*	
C02-013	*Bradycaradia(mild)*	
C02-014	*Elevated BUN(mild)*	
C02-019		*Hypokalemia(mild)+* *Hypophosphatemia(mild)+* *Lymphopenia(mild)+* *Thrombocytopenia(mild)+* *Hypokalemia(mild)+* *Hypophosphatemia(mild)*
C02-023		*Constipation (mild)*
C04-002	*MSSA infection(severe)+*	

Both files	*Septic shok (severe)*	
C04-012		*Constipation (mild)*
C02-002	*Worsening of pannus(mild)*	
C02-003		*(ARDS) (severe)*
C02-004	*Worsening of anemia(moderate)*	
C02-006		*Fall (mild)*
C02-008	*Worsening of cough(moderate)*	
Co2-009	*Pneumonia(moderate)*	
C02-010	*Chest pain (mild)*	

Conclusions: Phase II clinical study demonstrated full safety and efficacy profile of ArtemiC, supported by the preclinical study results.

As defined by the GCP, in the case of overdosing or adverse reactions, the patients should be examined and followed up till the end of the adverse event. All the relevant information should be reported in the CRF of the study.

5.1. Summary of Data and Guidelines for the Investigator

During Phase II study on 50 COVID-19 patients no drug related adverse events were reported. The full safety profile is presented above.

It is important to say that both active ingredients of the study drug is defined as GRAS and found to be safe with no adverse events in clinical studies. Based on the literature, the following data regarding the potential risks of the Curcumin and Boswellia is available –

1. Madhu K at al., Rahmani et al. and Selvi et al. reported gastrointestinal adverse events related to diarrhea, nausea and abdominal pain
2. Chuengsamarn et al. reported hot flashes and constipation that were observed in the clinical trial in type 2 diabetes patients.

In the case of overdose, treatment with antiviral therapy will have to be discontinued and the participant should be carefully monitored for potential adverse events and receive appropriate medical treatment according to their clinical condition.

6. PACKAGING AND LABELING OF THE DRUG PRODUCT

6.1. Formulation change

Artemisinin is excluded from the finished product due to insufficient compatibility with other active ingredients. Artemisinin has shown poor stability in low pH water environment, what is caused by the API boswellic acids and antioxidant vitamin C. This decision is supported by the preclinical data of the in vitro tests of **CimetrA** and its compounds in different combinations, presented in the section of Preclinical experiments of the IB. The analysis of the formulation ultimately clarified that the artemisinin component with which it was formulated was unstable to the extent that neither it, nor its metabolites were present in the preparations used in the phase 2 trial.

As a part of the development process for the multi APIs medical products, the Sponsor continue the preclinical program and based on the in vivo preclinical study, presented in Section 4.2 of the Investigator Brochure, performed

additional preclinical trials in the new formulation, named CimetrA, which does not include Artemisinin.

The results of the study are presented below.

The in vitro trial aimed to check the anti-inflammatory activity of ArtemiC versus CimetrA in the related to COVID-19 cytokines and chemokines was performed in Science in Action Lab, Ness Ziona, Israel.

The objective of this study was to examine the effect of ArtemiC and CimetrA on human peripheral blood mononuclear cells (PBMCs) viability and their capacity to attenuate inflammatory response upon stimulation with E coli-derived lipopolysaccharide (LPS).

The study demonstrated the superiority of the formulation named CimetrA (based on Curcumin and Boswellia) on ArtemiC (based on Artemisinin, Curcumin ,Boswellia and vitamin C) in the prevention of uncontrolled COVID-19 related cytokines and chemokines production.

The results are presented below.

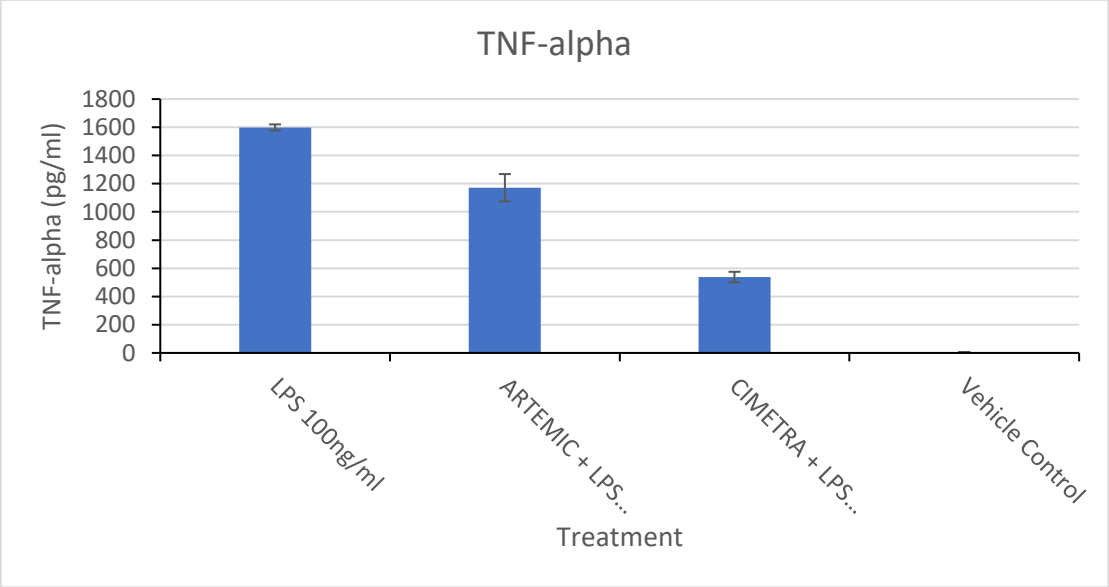


Fig 19. Concentration of TNF-alpha in PBMCs supernatant

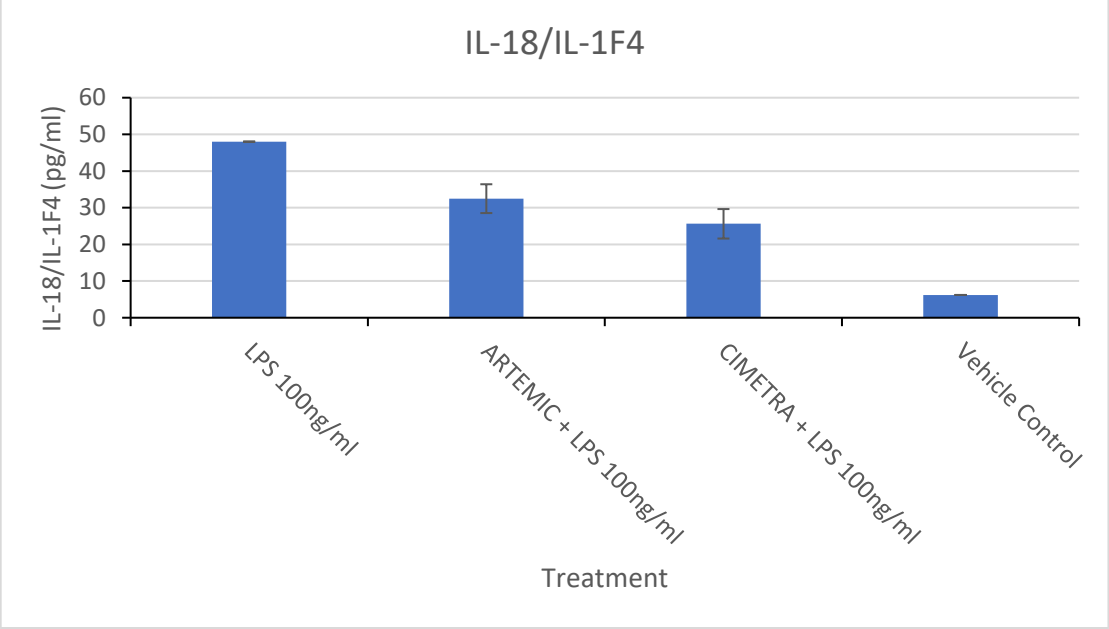


Fig 20. Concentration of IL-18/IL-1F4 in PBMCs supernatant

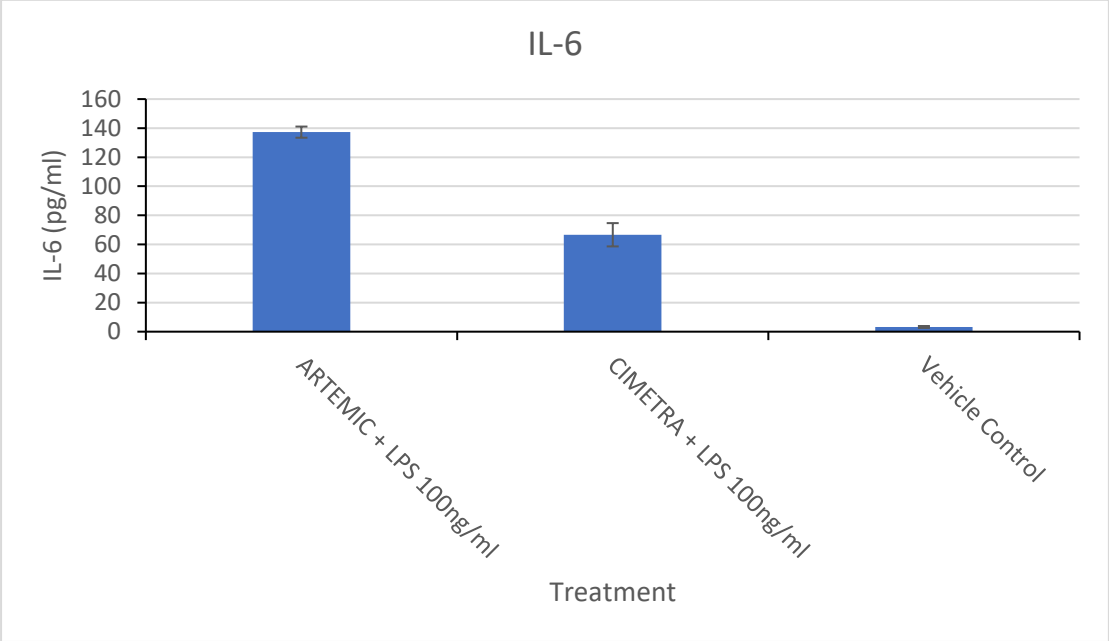


Fig 21. Concentration of IL-6 in PBMCs supernatant

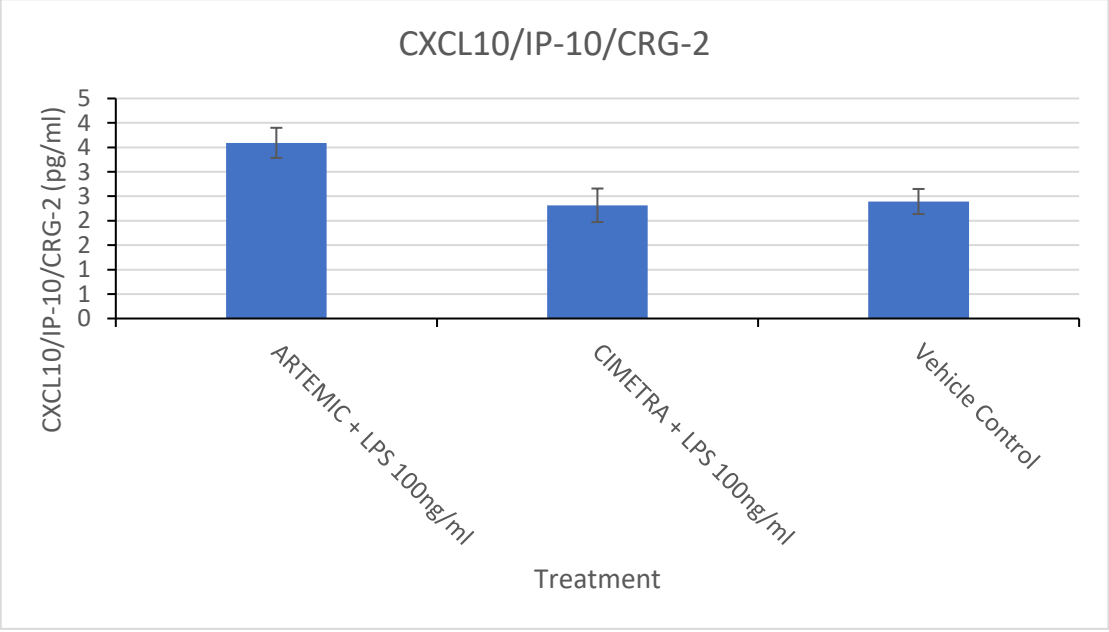


Fig 22. Concentration of CXCL10/IP-10/CRG-2 in PBMCs supernatant

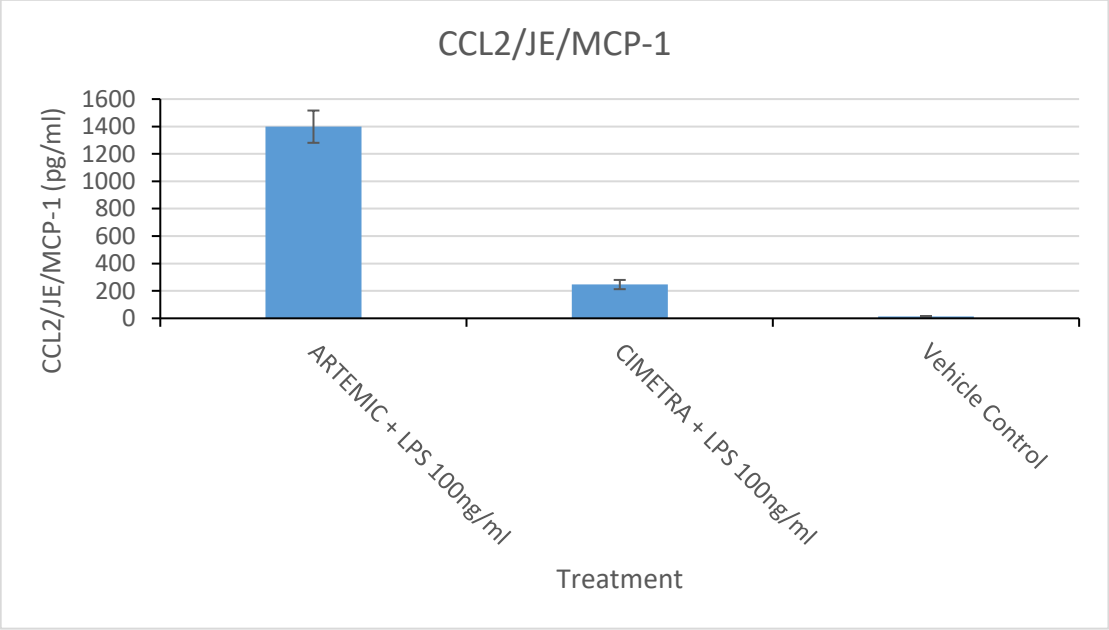


Fig 23. Concentration of CCL2/JE/MCP-1 in PBMCs supernatant

Based on the data presented above, MGC Pharma decided to continue the clinical program (phase IIb) with more active formulation, CimetrA, which answers all the goals of the cytokine storm prevention in COVID-19 patients needs. This formulation is developed and manufactured under EU-GMP conditions and certificates in the EU-certified manufacturing facility of MGC Pharma.

6.2. Labelling and Storage

CimetrA formulations will be kept in a non-transparent spray bottles at room temperature and labeled according to the GCP and local MoH requirements.

CimetrA formulations will be packaged and identified by two-part labels. The first part will be firmly attached to the bottle while the second part will be

separable and will be attached to the subject's drug accountability record in the CRF when CimetrA is administered. The label will contain the following information:

- Protocol number
- Dose
- Lot number
- Date of manufacture
- Retest date
- Sponsor’s name
- Instructions for use and storage
- “Investigational Use Only” statement

6.3. Supply, Distribution and Shipment

MGC and/or its designee will supply the investigational products to the clinical site. The investigational product will be manufactured by MGC under EU-GMP manufacturing conditions and will be in sufficient supply for the completion of the trial.

Each shipment sent for the study will contain a shipment form describing the content of shipment. This form will assist in maintaining current and accurate inventory records. When a shipment is received, the investigator, coordinator or pharmacist will verify its content and acknowledge receipt of the investigational product supplies by signing the shipment form and faxing it back to the attention of a MGC representative. If, upon arrival at the investigational site, the investigational product supplies appear to be damaged, or missing, MGC should be contacted immediately.

6.4. Storage, Dispensing and Return of the Investigational Products

All investigational products sent to the study center must be stored under the specified conditions in a secure area accessible only to the investigator and designated site personnel. All investigational products should be stored and inventoried according to applicable government regulations and study procedures. Temperature should be maintained between 20 - 25°C.

The empty bottles must be returned to the Sponsor for destruction or discarded as chemically hazardous waste according to local regulations.

6.5. Accountability and Compliance of Investigational Products

Subject compliance with the investigational product dosing regimen will be assessed by study staff administrating the investigational products.

A drug accountability log will be used at the study center to keep accurate records of investigational product inventories at the center (date and quantity received by the Investigator, dates of administration to the subjects, dates when unused products returned to the Sponsor or alternatively disposed of).

The investigator or designated staff member will be responsible for maintaining accurate records of the quantity and dates of all investigational product supplies received, administered, and returned. The quantity of investigational product lost, missing, destroyed, etc. must also be accounted for and documented. At the end of the study reconciling the delivery records with those of usage and returned stocks must be possible. Accounts must be given on any discrepancies.

Government regulations require that all investigational product materials not used in clinical trials be returned to the Sponsor before or at the completion

of the study. The investigator will return the designated copies of the completed dispensing and inventory record as indicated on the form.

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