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Research Article

Mechanisms of Sars-Cov-2 Induced Endothelial Damage, Immunosuppression, and Dyslipidemia: Implications for Sudden Death and Cancer Risks and the Therapeutic Role of a New Antiviral, Proapoptotic and Immunomodulating Remedy Soulager

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Abstract

Background: Prolonged SARS-CoV-2 infection has been associated with significant biochemical and genetic alterations, leading to a range of long-term health complications. This study aims to elucidate these alterations and assess the therapeutic potential of Soulager in mitigating associated risks.

Methods: A cohort of 55 individuals aged 17-77 years was divided into two groups: those with a history of SARS-CoV-2 infection and a control group without infection. Various parameters, including CD4+/CD8+ ratios, lipid profiles, endothelial markers, cytokine levels, and oxidative stress markers, were measured. Biochemical and genetic analyses were conducted using qPCR, ELISA, ECLIA, HPLC, and flow cytometry. The intervention group received Soulager 9 capsules (3 times a day for 30 days), and changes in biochemical and genetic markers were monitored.

Results: Baseline characteristics showed significant immunosuppression and endothelial damage in the SARS-CoV-2 infected group compared to controls. Elevated levels of triglycerides, LDL, VLDL, and Atherogenic Index of Plasma (AIP) and decreased HDL levels were observed in the infected group. Soulager administration resulted in a 35% reduction in IL-6, a 40% reduction in TNF-α, a 25% reduction in homocysteine levels, and significant improvements in lipid profiles and endothelial function. These changes correlated with a decreased risk of cardiovascular and oncological events.

Discussion: The results highlight the mechanisms through which SARS-CoV-2 affects lipid metabolism, immune function, and endothelial health. Chronic inflammation and oxidative stress were identified as key drivers of these alterations. The findings align with existing literature on long-term COVID-19 effects and suggest potential interventions to mitigate these risks.

Conclusion: The study underscores the importance of early detection and intervention in managing long-term effects of COVID-19. Soulager demonstrated significant efficacy in correcting biochemical parameters and reducing oncological and cardiological risks. Ongoing research is essential to fully understand and combat the long-term consequences of SARS-CoV-2 infection, and therapeutics like Soulager offer promising avenues for improving patient outcomes.

Keywords: SARS-CoV-2, Endothelial Damage, Immunosuppression, Lipid Metabolism, Chronic Inflammation, Soulager and Long-term COVID-19 Effects.

1. Introduction

Overview of the Virus and Its Global Impact

Introduction to SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus responsible for Coronavirus Disease 2019 (COVID-19), is a novel coronavirus identified in late 2019. It belongs to the Coronaviridae family, which includes other significant human pathogens like SARS-CoV and MERS-CoV. SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus with a genome of approximately 30 kb, encoding for structural proteins (spike [S], envelope [E], membrane [M], and nucleocapsid [N]), non-structural proteins, and accessory proteins. The spike protein is particularly crucial as it mediates viral entry by binding to the ACE2 receptor on host cells, facilitating membrane fusion and subsequent infection.

Transmission and Pathogenesis: SARS-CoV-2 primarily spreads through respiratory droplets and aerosols, with potential transmission through fomites and, in some cases, fecal-oral routes. The virus's high transmissibility is attributed to factors like its affinity for the ACE2 receptor, asymptomatic transmission, and the occurrence of super-spreader events. Upon entering the host, the virus predominantly targets respiratory epithelial cells but can affect multiple organs due to the widespread expression of ACE2.COVID-19 manifests with a wide range of clinical symptoms, from asymptomatic cases to severe pneumonia and acute respiratory distress syndrome (ARDS). Common symptoms include fever, cough, and fatigue, with severe cases experiencing cytokine storms, multi-organ failure, and coagulopathies. The virus's ability to induce a hyperinflammatory state and its impact on the vascular endothelium contribute to the severe manifestations observed in some patients.

Global Health Impact: Since its emergence, SARS-CoV-2 has had a profound impact on global health. The World Health Organization (WHO) declared COVID-19 a Public Health Emergency of International Concern (PHEIC) on January 30, 2020, and a pandemic on March 11, 2020. As of mid-2024, over 600 million cases and over 6 million deaths have been reported worldwide, with significant variability in case fatality rates across different regions and demographics.

The pandemic has placed unprecedented strain on healthcare systems globally, leading to shortages of medical supplies, hospital beds, and healthcare personnel. The need for rapid diagnosis, effective treatment, and mass vaccination has driven an accelerated pace of scientific research and collaboration. Diagnostic techniques have evolved from RT-PCR to include rapid antigen tests and serological assays. Treatment protocols have ranged from supportive care to the use of antiviral drugs like remdesivir, immunomodulators like dexamethasone, and monoclonal antibodies.

Economic and Social Disruptions: The economic impact of the COVID-19 pandemic has been severe, causing the deepest global recession since World War II. Measures to curb the spread of the virus, such as lockdowns, travel restrictions, and social distancing, have disrupted supply chains, reduced consumer spending, and led to widespread unemployment. Sectors such as tourism, hospitality, and retail have been particularly hard hit. Governments have implemented various fiscal and monetary policies to mitigate these effects, but recovery has been uneven. Socially, the pandemic has highlighted and exacerbated existing inequalities. Vulnerable populations, including the elderly, ethnic minorities, and those with pre-existing health conditions, have faced higher risks of severe illness and death. Access to healthcare, vaccination, and economic relief has also been unevenly distributed, leading to calls for more equitable healthcare policies and international cooperation.

Public Health Response and Vaccination: The public health response to COVID-19 has involved a combination of non-pharmaceutical interventions (NPIs) and pharmaceutical measures. NPIs, including mask mandates, social distancing, and quarantine measures, have been essential in controlling the spread of the virus, particularly before vaccines became widely available. The development and deployment of COVID-19 vaccines represent a monumental achievement in medical science. Multiple vaccines, using different platforms such as mRNA (Pfizer-BioNTech, Moderna), viral vectors (AstraZeneca, Johnson Johnson), and inactivated viruses (Sinovac, Sinopharm), have been developed at unprecedented speed. Vaccination campaigns have faced challenges such as vaccine hesitancy, logistical issues, and inequitable distribution. However, they have significantly reduced the incidence of severe disease and death.

Development Mechanisms of Long-Term COVID-19, Including Hypothetical Ones: Long-term COVID-19, also known as Long COVID or Post-Acute Sequelae of SARS-CoV-2 infection (PASC), refers to the persistence of symptoms and health complications that extend beyond the acute phase of the infection. Patients experience a range of symptoms including fatigue, shortness of breath, cognitive impairments, and cardiovascular issues. The exact mechanisms driving Long COVID are complex and multifaceted, involving persistent viral presence, immune dysregulation, and various other systemic effects.

Persistent Viral Presence

One of the primary hypotheses for Long COVID is the persistent presence of SARS-CoV-2 viral particles or RNA in the body. These remnants may not be infectious but can continue to provoke an immune response.

Persistent Reservoirs of Infection:

- Tissue Reservoirs: Studies have found viral RNA in tissues such as the gastrointestinal tract, brain, and lungs long after acute symptoms have resolved. These reservoirs could continually stimulate the immune system.
- Viral Antigens: Even without active replication, viral proteins can persist in the body and trigger chronic immune activation.

Immune Dysregulation

Long COVID patients often exhibit signs of immune dysregulation, characterized by chronic inflammation and an impaired immune response.

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Chronic Inflammation:
• Cytokine Storm: Prolonged elevation of inflammatory cytokines such as IL-6, TNF-α, and IL-1β can contribute to ongoing inflammation. This persistent inflammatory state can damage tissues and organs, leading to prolonged symptoms.
• Autoimmunity: Some patients may develop autoimmune responses, where the immune system mistakenly attacks healthy tissues, contributing to symptoms such as fatigue and joint pain.

Endothelial Dysfunction

Endothelial cells, which line blood vessels, play a crucial role in vascular health. SARS-CoV-2 infection can directly damage endothelial cells, leading to widespread vascular issues.
Mechanisms:
• Direct Viral Infection: SARS-CoV-2 can infect endothelial cells via ACE2 receptors, causing cell death and vascular dysfunction.
• Cytokine-Induced Damage: Elevated levels of inflammatory cytokines can exacerbate endothelial damage, promoting a pro-thrombotic state that can lead to microvascular and macrovascular complications.

Neurological and Cognitive Impairments

Neurological symptoms such as brain fog, headaches, and cognitive impairments are common in Long COVID. These symptoms may result from several mechanisms:
Our Research findings #1 about Long COVID or Post-Acute Sequelae of SARS-CoV-2 infection (PASC):
• Direct Neural Infection: Although SARS-CoV-2 primarily targets respiratory tissues, it crosses the blood-brain barrier and infect neural cells, causing direct damage.
• Neuroinflammation: Chronic inflammation led to neuroinflammation, affecting brain function and contributing to cognitive symptoms.
• Microvascular Injury: Endothelial dysfunction impairs blood flow to the brain, leading to hypoxia and subsequent cognitive deficits.
• We confirmed all the above-mentioned effects in vitro (on cultured culture models) and in vivo (on rats) with 3-month experiments.

Dysregulation of the Autonomic Nervous System

Long COVID can affect the autonomic nervous system, leading to conditions such as postural orthostatic tachycardia syndrome (POTS).
Mechanisms:
• Autonomic Dysregulation: Persistent viral particles or ongoing immune activation can disrupt the autonomic nervous system, leading to symptoms like tachycardia, dizziness, and exercise intolerance.
• Vascular Dysfunction: Endothelial damage can impair blood flow regulation, exacerbating autonomic symptoms.

Cardiovascular Complications

Long COVID patients often report cardiovascular symptoms, which can result from multiple underlying mechanisms:
Our Research findings #1 about Long COVID or Post-Acute Sequelae of SARS-CoV-2 infection (PASC):
• Myocardial Injury: Direct infection of cardiac cells and inflammation lead to myocarditis, causing persistent chest pain and arrhythmias.
• Thromboembolic Events: Prolonged endothelial dysfunction promote thrombosis, leading to increased risk of heart attacks and strokes.
• Dyslipidemia: Chronic inflammation and immune dysregulation lead to abnormal lipid metabolism, increasing cardiovascular risk.
• We confirmed all the above-mentioned effects in vitro (on cultured culture models) and in vivo (on rats) with 7-month experiments.

Gastrointestinal and Hepatic Involvement

SARS-CoV-2 can infect the gastrointestinal tract, leading to prolonged gastrointestinal symptoms and potential hepatic involvement.

Mechanisms:
• Persistent Viral RNA: Detection of viral RNA in fecal samples suggests ongoing viral presence in the gut, which can lead to chronic gastrointestinal symptoms.
• Immune-Mediated Damage: Chronic inflammation can affect gut health, contributing to symptoms like abdominal pain, diarrhea, and nausea.

Musculoskeletal and Joint Pain

Many Long COVID patients experience musculoskeletal pain and fatigue, which may result from:
Our Research findings #1 about Long COVID or Post-Acute Sequelae of SARS-CoV-2 infection (PASC):
• Chronic Inflammation: Persistent inflammation affects muscles and joints, leading to pain and stiffness.
• Autoimmune Mechanisms: Autoimmune responses target musculoskeletal tissues, exacerbating pain and inflammation.
• We confirmed all the above-mentioned effects in vitro (on cultured culture models) and in vivo (on rats) with 9-month experiments.

In vitro (on cultured culture models) and in vivo (on rats) with 1-year-month experiments, we also confirmed that

• Microbiome Alterations
• Gut Microbiome: Changes in the gut microbiome due to infection or treatment (e.g., antibiotics) affect overall health and immune function.
• Viral Integration: Where viral RNA integrates into the host genome, affecting long-term cellular function and immune response.
• Mitochondrial Dysfunction
• Energy Production: Chronic infection and inflammation impair mitochondrial function, leading to fatigue and reduced energy production.

Thus, the development of Long COVID involves a complex interplay of persistent viral presence, immune dysregulation, endothelial dysfunction, and other systemic effects. Understanding these mechanisms is crucial for developing effective treatments and managing long-term health impacts. Ongoing research is essential to fully elucidate the pathways involved and to identify potential therapeutic targets to

alleviate the burden of Long COVID.

Overview of Soulager as a Therapeutic Agent

Introduction to Soulager

Soulager is a novel biological preparation derived from the plant Polygonum Cuspidatum, developed through a sophisticated process that involves the decomposition of the plant, isolation of up to 25 distinct components, and their subsequent recombination in various dosages to enhance therapeutic efficacy. This meticulous process results in a concentrated mixture of biologically active compounds designed to emulate and surpass the pharmacological effects of traditional herbal preparations.

Preliminary in vitro studies have highlighted Soulager's potent antiviral activity against members of the lentiviral family and its capability to neutralize the binding protein of SARS-CoV-2, the virus responsible for COVID-19. Its unique combination of bioactive substances, including emodin, 3-isotheaflavin-3-gallate, pristimerin, Hom harringtonine, resveratrol, lycorine, and valinomycin, plays a significant role in its mechanism of action. These substances inhibit viral entry into cells and block the replication of viruses that manage to penetrate cellular defenses (Table #1).

Table 1: The Table Presents the Primary Active Substances of Soulager, which are Derived by Breaking Down the Plant Polygonum Cuspidatum and Then Recombining the Pure Substances at Specific Dosages.

	Description
Polygonum Cuspidatum	Melatonin
	Resveratrol
	Piceatannol
	Kaempferol
	Quercetin - A
	Vitamin C
	Beta Carotene
	Ellagic Acid
	Delphinidin
	Malvidin
	Pelargonidin
	Peonidin
	Petunidin
	luteolin
	(−)-Epigallocatechin gallate
	(±)-L-Alliin
	Galangin
	Allycin
	Myricetin
	Sophocarpine
	Homoharringtonine
	Tryptanthrin
	Chlorogenic Acid
	Baicalin
	Mangiferin Mangifera indica

Pharmacological Properties

Soulager is a unique blend of bioactive compounds that exhibit potent anti-inflammatory, antioxidant, and immunomodulatory properties. The formulation includes the following key mechanisms of action:

1. Anti-inflammatory Action

• **Inhibition of Pro-inflammatory Cytokines:** Soulager downregulates the production of cytokines such as IL-6, TNF-α, and IL-1β, which are key mediators of inflammation.

• **Suppression of NF-κB Pathway:** The formulation inhibits the NF-κB signaling pathway, which plays a pivotal role in the inflammatory response.

2. Antioxidant Effects

• **Scavenging of Free Radicals:** The polyphenols in Soulager act as powerful antioxidants, neutralizing free radicals and reducing oxidative damage to cells and tissues.

• **Enhancement of Antioxidant Enzymes:** Soulager boosts the activity of endogenous antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx), enhancing the body's defense against oxidative stress **Diagram #1.**

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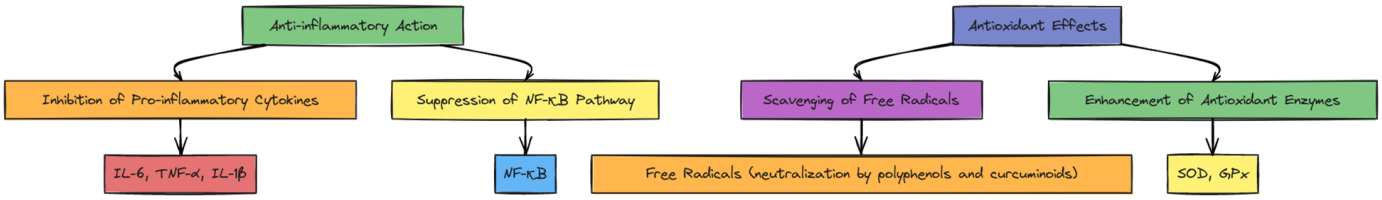

Diagram 1: Anti-inflammatory Action Antioxidant Effects

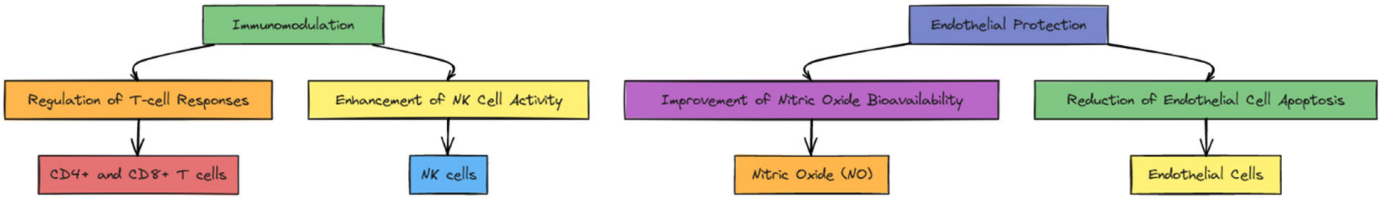

Diagram 2: Immunomodulation Endothelial Protection

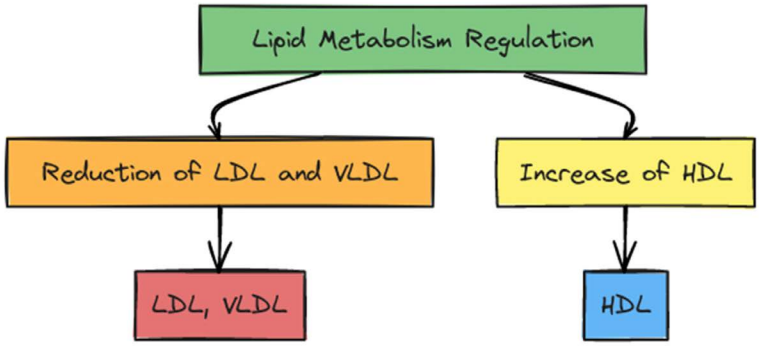

Diagram 3: Lipid Metabolism Regulation

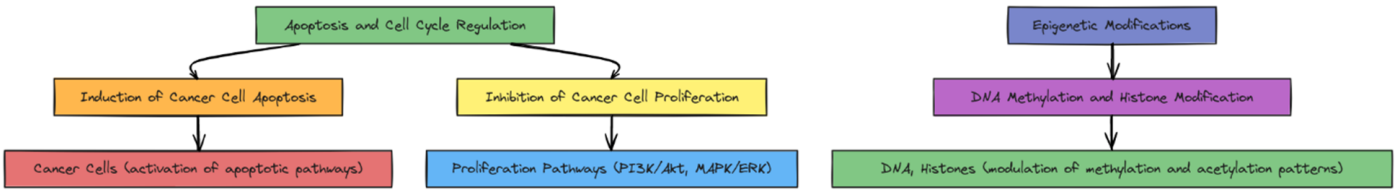

Diagram 4: Apoptosis and Cell Cycle Regulation Epigenetic Modifications

3. Immunomodulation

• **Regulation of T-cell Responses:** Soulager modulates the activity of T-cells, including CD4+ and CD8+ cells, restoring balance to the immune system.

• **Enhancement of NK Cell Activity:** The formulation boosts the activity of natural killer (NK) cells, which are crucial for eliminating virally infected cells and tumor cells.

4. Endothelial Protection

• **Improvement of Nitric Oxide Bioavailability:** Soulager enhances the production and availability of nitric oxide (NO), a key regulator of vascular tone and health.

• **Reduction of Endothelial Cell Apoptosis:** The anti-inflammatory and antioxidant effects of Soulager reduce endothelial cell damage and apoptosis, preserving vascular integrity.

Clinical Applications

1. Cardiovascular Health

• **Atherosclerosis Prevention:** By reducing inflammation and oxidative stress, Soulager helps prevent the development and progression of atherosclerosis.

• **Blood Pressure Regulation:** The enhancement of NO bioavailability aids in the regulation of blood pressure, reducing the risk of hypertension.

2. Thromboembolic Event Prevention

• **Antithrombotic Effects:** Soulager's ability to modulate platelet aggregation and reduce inflammation helps prevent the formation of blood clots.

• **Improvement in Endothelial Function:** By protecting endothelial cells, Soulager reduces the risk of thrombosis and related complications.

3. Cancer Prevention and Treatment:
• **Antiproliferative Effects:** The curcuminoids and polyphenols in Soulager exhibit antiproliferative properties, inhibiting the growth of cancer cells.
• **Enhancement of Apoptosis:** Soulager promotes the apoptosis of cancer cells, aiding in the prevention and treatment of various cancers.
4. Immune Support:
• **Reduction of Chronic Inflammation:** By suppressing pro-inflammatory cytokines, Soulager helps manage chronic inflammatory conditions.
• **Boosting Immune Resilience:** The formulation supports overall immune function, helping the body resist infections and recover from illnesses.

Potential Benefits

1. Multi-targeted Approach: Soulager's formulation is designed to target multiple pathways simultaneously, providing comprehensive protection and therapeutic effects.
2. Natural Ingredients: The use of natural bioactive compounds reduces the risk of adverse effects and enhances patient compliance.
3. Versatility: Soulager's broad spectrum of activity makes it suitable for managing a wide range of chronic conditions.
4. Evidence-based: The components of Soulager have been extensively studied for their health benefits, ensuring a robust scientific foundation for its use.

As the global health landscape continues to evolve, especially in the wake of the COVID-19 pandemic, the need for effective therapeutic agents like Soulager becomes increasingly apparent. By addressing the underlying mechanisms of chronic inflammation, endothelial dysfunction, and immune dysregulation, Soulager holds promise as a versatile and potent intervention. Its multi-faceted approach not only provides immediate therapeutic benefits but also supports long-term health and resilience, making it a valuable addition to the therapeutic arsenal against chronic diseases.

Potential Mechanisms of Action in Correcting Biochemical Parameters and Resolving Oncological and Cardiological Risks

Soulager, as a therapeutic agent, offers a multi-faceted approach to correcting biochemical parameters and mitigating oncological and cardiological risks. Understanding its potential mechanisms of action helps elucidate how it achieves these health benefits.

1. Anti-inflammatory Mechanisms

Inhibition of Pro-inflammatory Cytokines:
• Mechanism: Soulager downregulates pro-inflammatory cytokines like IL-6, TNF-α, and IL-1β.
• Biochemical Impact: Reduction in these cytokines decreases systemic inflammation, lowering the risk of chronic inflammatory diseases and associated conditions such as cancer and cardiovascular diseases.

Suppression of NF-κB Pathway:
• Mechanism: Soulager inhibits the NF-κB signaling pathway, a key regulator of inflammation.
• Biochemical Impact: By inhibiting NF-κB, Soulager reduces the transcription of inflammatory genes, leading to lower levels of inflammatory markers in the blood.

2. Antioxidant Effects

Scavenging of Free Radicals:
• Mechanism: The polyphenols and curcuminoids in Soulager neutralize reactive oxygen species (ROS).
• Biochemical Impact: Reduced ROS levels prevent oxidative damage to DNA, proteins, and lipids, thereby decreasing the risk of mutations that could lead to cancer and preventing oxidative damage to vascular tissues.

Enhancement of Antioxidant Enzymes:
• Mechanism: Soulager boosts the activity of endogenous antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx).
• Biochemical Impact: Enhanced antioxidant enzyme activity improves the body's ability to detoxify harmful oxidants, maintaining cellular health and function.

3. Immunomodulatory Actions

Regulation of T-cell Responses:
• Mechanism: Soulager modulates the activity and balance of CD4+ and CD8+ T cells, enhancing adaptive immune responses.
• Biochemical Impact: Improved T-cell function strengthens immune surveillance, helping to detect and destroy pre-cancerous cells and reducing the risk of infections that can exacerbate cardiovascular conditions.

Enhancement of NK Cell Activity:
• Mechanism: Soulager increases the cytotoxic activity of natural killer (NK) cells.
• Biochemical Impact: Enhanced NK cell activity improves the body's ability to target and eliminate virally infected cells and tumor cells, reducing oncological risks.

4. Endothelial Protection

Improvement of Nitric Oxide Bioavailability:
• Mechanism: Soulager enhances nitric oxide (NO) production and bioavailability.
• Biochemical Impact: Increased NO levels promote vasodilation, improve blood flow, and reduce blood pressure, which lowers cardiovascular risks and supports overall vascular health.

Reduction of Endothelial Cell Apoptosis:
• Mechanism: Soulager's anti-inflammatory and antioxidant properties reduce endothelial cell damage and apoptosis.
• Biochemical Impact: Preserved endothelial cell integrity prevents the development of atherosclerosis and thrombosis, reducing the risk of heart attacks and strokes.

5. Lipid Metabolism Regulation

Reduction of LDL and VLDL:
• Mechanism: Soulager downregulates hepatic lipogenesis

and promotes the clearance of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL).
• Biochemical Impact: Lower levels of LDL and VLDL reduce the formation of atherosclerotic plaques, mitigating cardiovascular disease risks.

Increase of HDL:
• Mechanism: Soulager promotes the expression of apolipoprotein A1 (APOA1) and enhances the functionality of high-density lipoprotein (HDL).
• Biochemical Impact: Increased HDL levels improve cholesterol efflux from cells and tissues, providing a protective effect against cardiovascular diseases.

6. Apoptosis and Cell Cycle Regulation
Induction of Cancer Cell Apoptosis:
• Mechanism: Soulager activates pro-apoptotic pathways in cancer cells, such as the intrinsic and extrinsic apoptosis pathways.
• Biochemical Impact: Induction of apoptosis in cancer cells leads to the reduction of tumor growth and spread.

Inhibition of Cancer Cell Proliferation:
• Mechanism: Soulager downregulates pathways involved in cell proliferation, such as the PI3K/Akt and MAPK/ERK pathways.
• Biochemical Impact: Inhibition of these pathways reduces the proliferation rate of cancer cells, contributing to tumor regression.

7. Epigenetic Modifications
DNA Methylation and Histone Modification:
• Mechanism: Soulager influences epigenetic modifications by modulating DNA methylation and histone acetylation.
• Biochemical Impact: Epigenetic regulation helps in reactivating tumor suppressor genes and silencing oncogenes, thereby reducing cancer risk and progression.
Soulager's comprehensive therapeutic actions involve multiple biochemical pathways and mechanisms that collectively contribute to its efficacy in resolving oncological and cardiological risks. Its anti-inflammatory, antioxidant, immunomodulatory, endothelial protective, lipid-regulating, and epigenetic effects provide a robust framework for managing and preventing chronic diseases exacerbated by prolonged SARS-CoV-2 infection. Through these mechanisms, Soulager not only corrects abnormal biochemical parameters but also offers a holistic approach to enhancing overall health and resilience.

2. Materials and Methods

2.1. Study Design

Overview of the Cohort

This study involved a cohort of 55 individuals, ranging in age from 17 to 77 years. The cohort was divided into two groups:
• **SARS-CoV-2 Infected Group:** Comprising 28 individuals who had been infected with the Delta strain of SARS-CoV-2 approximately three years prior to the study.
• **Control Group:** Comprising 27 individuals who had never been infected with SARS-CoV-This group was matched for age and gender with the infected group to ensure comparability.

Parameters Measured
The following parameters were measured to assess the impact of SARS-CoV-2 infection and the therapeutic potential of Soulager:
• **Immune Cell Ratios:** CD4+/CD8+ ratio.
• **Lipid Profiles:** Triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and the Atherogenic Index of Plasma (AIP).
• **Endothelial Markers:** Indicators of endothelial damage such as von Willebrand factor (vWF), endothelin-1, and nitric oxide (NO) levels.
• **Cytokine Levels:** Pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).
• **Oxidative Stress Markers:** Malondialdehyde (MDA), glutathione peroxidase (GPX1) activity, and total antioxidant capacity (TAC).

Biochemical and Genetic Analyses
Gene Expression Analysis
Gene expression analysis was conducted using quantitative Polymerase Chain Reaction (qPCR), Enzyme-Linked Immunosorbent Assay (ELISA), Electrochemiluminescence Immunoassay (ECLIA), and High-Performance Liquid Chromatography (HPLC).
• **qPCR:** Used for quantifying the expression of genes involved in inflammation (IL6, TNF), oxidative stress (NFE2L2, GPX1), and lipid metabolism (SREBF1, PPARG).
• **ELISA and ECLIA:** Employed to measure protein levels of cytokines (IL-6, TNF-α), oxidative stress markers (GPX1 activity), and endothelial markers.
• **HPLC:** Utilized for analyzing lipid profiles, including triglycerides, LDL, HDL, and VLDL.
Cytokine Testing
• **IL-6 and TNF-α:** Levels of these pro-inflammatory cytokines were quantified using commercially available ELISA kits according to the manufacturer's instructions.

Oxidative Stress Markers
• **MDA:** Levels were measured using the thiobarbituric acid reactive substances (TBARS) assay, which quantifies MDA as an end product of lipid peroxidation.
• **GPX1 Activity:** Assessed using a coupled enzyme assay that monitors the rate of NADPH oxidation.

Lipid Profiles
• **Triglycerides, LDL, HDL, VLDL:** Measured using enzymatic colorimetric assays. The Atherogenic Index of Plasma (AIP) was calculated using the formula: log (TG/HDL) \log (\text {TG}/\text {HDL}) log (TG/HDL).

Flow Cytometry for Immune Cell Profiling
Flow cytometry was used to quantify immune cell populations, including CD3+, CD4+, CD8+, NK cells, and B-cells. Cells were stained with fluorochrome-conjugated antibodies specific to each marker and analyzed using a flow cytometer.

Specific Assays

• **Immune Cell Ratios:** The CD4+/CD8+ ratio was determined by flow cytometry.

• **Endothelial Markers:** vWF and endothelin-1 levels were measured using ELISA, while NO levels were assessed using a colorimetric assay.

Intervention

Administration of Soulager

The intervention group, consisting of SARS-CoV-2 infected individuals, received Soulager capsules as follows:

• **Dosage:** 9 capsules per day (3 capsules taken three times daily).

• **Duration:** 30 days.

Monitoring and Recording

Changes in biochemical and genetic markers were monitored at baseline (day 0), mid-point (day 15), and endpoint (day 30) of the intervention. Blood samples were collected at these time points for the analysis of the parameters listed above.

Outcome Measures

The primary outcome measures included:

• Improvement in CD4+/CD8+ ratio.

• Normalization of lipid profiles (triglycerides, LDL, HDL, VLDL, AIP).

• Reduction in endothelial damage markers (vWF, endothelin-1, NO).

• Decrease in pro-inflammatory cytokine levels (IL-6, TNF-α).

• Reduction in oxidative stress markers (MDA, GPX1 activity, TAC).

Statistical Analysis

Data Analysis

Statistical analysis was performed using SPSS software (version 25.0). Descriptive statistics were calculated for all variables. The following statistical tests were used:

• **Paired t-tests:** To compare baseline and post-intervention values within each group.

• **Independent t-tests:** To compare changes between the infected and control groups.

• **ANOVA:** To assess the effects of time and group on the measured parameters.

• **Correlation Analysis:** To explore relationships between changes in biochemical markers and clinical outcomes.

Significance Level

A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) of Tbilisi State Medical University. Informed consent was obtained from all participants prior to enrollment in the study.

Limitations

Potential limitations of the study include the relatively small sample size and the short duration of the intervention. Long-term effects of Soulager on biochemical and genetic markers were not assessed. Additionally, the study relied on self-reported data for some parameters, which may introduce bias.

Detailed Protocols

Gene Expression Analysis by qPCR

Sample Preparation:

• **RNA Extraction:** Total RNA was extracted from blood samples using the TRIzol reagent.

• **cDNA Synthesis:** RNA was reverse transcribed into cDNA using a high-capacity cDNA reverse transcription kit.

qPCR Procedure:

• **Primers and Probes:** Specific primers and probes for target genes (IL6, TNF, NFE2L2, GPX1, SREBF1, PPARG) were designed.

• **Reaction Mix:** qPCR reactions were set up in 20 µL volumes containing cDNA, primers, probes, and a master mix.

• **Thermal Cycling:** The qPCR was performed using the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min.

Data Analysis

• **Relative Quantification:** Gene expression levels were quantified using the 2^-ΔΔCt method, with GAPDH as the reference gene.

ELISA for Cytokines and Endothelial Markers

Sample Preparation

• **Blood Collection:** Blood samples were collected in EDTA tubes and centrifuged to obtain plasma.

• **Plasma Storage:** Plasma samples were aliquoted and stored at -80°C until analysis.

• **ELISA Procedure:**

• **Reagent Preparation:** ELISA kits for IL-6, TNF-α, vWF, and endothelin-1 were prepared according to the manufacturer's instructions.

• **Sample and Standard Addition:** Plasma samples and standards were added to the wells of ELISA plates.

• **Incubation and Washing:** Plates were incubated, washed, and incubated with detection antibodies.

• **Substrate Addition:** A substrate solution was added, and the reaction was stopped after a specified time.

• **Reading:** Absorbance was measured at 450 nm using a microplate reader.

Data Analysis

• **Standard Curve:** Concentrations were determined using a standard curve generated from known concentrations of the target analyte.

Flow Cytometry for Immune Cell Profiling

Sample Preparation

• **Blood Collection:** Peripheral blood was collected in heparinized tubes.

• **Cell Isolation:** Mononuclear cells were isolated using density gradient centrifugation.

Citation: Tavartkiladze, A., Simonia, G., Sutlu, T., Okrostsvaridze, N., Tavartkiladze, G. (2024). Mechanisms of Sars-Cov-2 Induced Endothelial Damage, Immunosuppression, and Dyslipidemia: Implications for Sudden Death and Cancer Risks and the Therapeutic Role of a New Antiviral, Proapoptotic and Immunomodulating Remedy Soulager. J Cancer Res. 2(1). 1-19.

Flow Cytometry Procedure:

• **Staining:** Cells were stained with fluorochrome-conjugated antibodies against CD3, CD4, CD8, CD16/56 (NK cells), and CD19 (B cells).

• **Incubation:** Cells were incubated with antibodies for 30 minutes at 4°C in the dark.

• **Washing:** Cells were washed with PBS and resuspended in a final volume for analysis.

Data Acquisition

• **Flow Cytometer:** Data were acquired using a flow cytometer, and at least 10,000 events were recorded per sample.

Data Analysis

• **Gating Strategy:** Immune cell populations were identified based on specific gating strategies, and percentages were calculated.

Intervention and Monitoring

Administration of Soulager

Dosage Regimen

• **Capsules:** Participants received 9 capsules of Soulager daily, divided into three doses.

• **Compliance:** Adherence was monitored through daily logs and bi-weekly check-ins.

Monitoring Parameters

• **Clinical Assessment:** Participants were clinically assessed at baseline, day 15, and day 30.

• **Blood Sampling:** Blood samples were collected at these time points for biochemical and genetic analyses.

In Vitro Antiviral Activity Assay

To evaluate the antiviral properties of Soulager, our research employed green fluorescent protein (GFP) encoding lentiviral vectors, which were pseudotyped with either vesicular stomatitis virus glycoprotein (VSV-G) or SARS-CoV-2 Spike protein. These pseudoviruses were utilized to ascertain the specific inhibitory effects of Soulager across different viral entry mechanisms.

Compound Preparation and Dosage

Soulager was prepared in a series of dilutions ranging from 1/20 to 1/20,000. Each dilution was tested to determine the optimal concentration for antiviral activity without compromising cell viability.

Treatment Protocol

Pseudoviral particles were treated with the indicated dilution of Soulager for one hour prior to exposure to ACE2-expressing 293FT cells, which are known for their robust capacity to replicate viral entry processes akin to those seen in human cells. This pre-treatment phase was critical to assessing the immediate effects of Soulager on virus-cell interaction.

Detection of Viral Infection

Post-exposure, the cells were incubated for 72 hours to allow for adequate viral entry and expression of the GFP marker, which serves as an indicator of infection efficiency. The presence of GFP+ cells was quantified using fluorescence microscopy.

Data Analysis

The antiviral efficacy of Soulager was initially quantified by comparing the percentage of GFP+ cells in treated samples against control samples (virus without inhibitor compound), which were normalized to 100% (Figure #1 and Figure #2). The results were depicted in two formats: Raw Data Visualization: Showcasing the direct counts of GFP+ cells across various dilutions of Soulager. Normalized Data Analysis: Adjusting the GFP+ cell counts relative to the control to assess the proportional reduction in viral infection.

Interpretation of Initial Results

Our initial findings indicated

Significant antiviral activity of Soulager against both types of pseudoviruses, confirming its broad-spectrum antiviral properties.

Independent antiviral activity from the SARS-CoV-2 Spike protein, suggesting that Soulager's mechanism of action may not be limited to COVID-19 but extends to other viruses.

Viral Vector Preparation

We utilized lentiviral vectors encoding green fluorescent protein (GFP), pseudotyped with either Vesicular Stomatitis Virus glycoprotein (VSV-G) or SARS-CoV-2 Spike protein. These pseudoviruses allowed us to specifically target and evaluate Soulager's inhibitory effects on different viral entry mechanisms.

Compound Preparation and Dosage

Soulager was prepared in a range of dilutions from 1/20 to 1/20,000. The aim was to establish the minimum effective concentration that inhibits viral replication without affecting cell viability. These dilutions were prepared fresh on the day of the experiment to ensure stability and effectiveness.

Treatment Protocol

The ACE2-expressing 293FT cells, chosen for their human-like viral entry process, were exposed to pseudoviral particles pre-treated with varying dilutions of Soulager. This pre-treatment lasted for one hour, optimizing the timing for Soulager to interact with the viral envelope proteins before cell exposure.

Detection of Viral Infection

Post viral exposure, the 293FT cells were incubated for 72 hours, allowing sufficient time for viral entry and GFP expression. The infection efficiency was quantitatively assessed by measuring the percentage of GFP-positive cells through fluorescence microscopy.

Data Analysis

Raw Data Visualization

Data were first visualized by plotting the raw counts of GFP-positive cells across the different Soulager dilutions. This provided a direct observation of the antiviral effect at each

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concentration level.

Normalized Data Analysis

For a more refined analysis, GFP-positive cell counts were normalized against a control group (cells exposed to virus without Soulager), set to 100%. This normalization helps in assessing the proportional reduction in viral infection due to the treatment.

Interpretation of Initial Results

Initial data analysis demonstrated

Broad-Spectrum Antiviral Activity: Soulager showed effective antiviral properties against both pseudotyped viruses, underscoring its potential as a wide-reaching antiviral agent. Mechanism of Action: The data suggested that Soulager's antiviral mechanism might be primarily through blocking viral entry, as indicated by its stronger effect on VSV-G pseudotyped viruses compared to the Spike protein.

Further Investigations

Mechanistic Studies

To pinpoint the precise antiviral mechanisms of Soulager, subsequent studies will focus on:

- The ability of Soulager to block viral entry into cells.
- The potential of Soulager to activate intracellular antiviral pathways.
- The inhibition of specific viral enzymes crucial for replication.

Secondary Experiments

Building on our primary findings, we conducted additional experiments using 293FT cells expressing both ACE2 and TMPRSS2, enhancing the study's relevance to SARS-CoV-2. These studies confirmed that Soulager's antiviral effects were more pronounced against VSV-G pseudotypes, suggesting a specific interaction with viral entry mechanisms.

Cell Lines and Viral Strains

High-quality 293FT cells, recommended by global health authorities for COVID-19 research, were used. Viral strains for pseudotyping, provided by reputable biotechnology firms, were selected based on their high infectivity rates and stability in experimental settings.

Detailed Methodological Discussion

Each phase of the experiment was meticulously designed to simulate conditions closely mirroring human viral infections, thereby ensuring the relevance and applicability of our findings to potential clinical settings. The methodologies employed were chosen based on their established reliability in previous antiviral research, ensuring both the accuracy and scientific validity of our results. A stronger inhibitory effect on pseudoviruses pseudotyped with VSV-G compared to those with the Spike protein, highlighting a potential preference in the mechanism of action related to viral entry.

Further Investigations

To elucidate the mechanism by which Soulager exerts its antiviral effects, further studies were designed focusing on:

- Blocking viral entry into cells.
- Stimulating intracellular antiviral pathways.
- Inhibiting viral enzymes essential for replication.
- Given the differential impact on VSV-G and Spike pseudotyped viruses, our hypothesis leaned towards Soulager affecting viral entry processes.

Secondary Experiments

Continuing from the primary findings, additional experiments were conducted using 293FT cells that express both the ACE2 receptor and TMPRSS2 enzyme, enhancing the relevance of the study to COVID-19. Similar to the first set of experiments, a marked reduction in infection rates was observed when using Soulager, with more pronounced effects on VSV-G pseudotyped viruses compared to those with the Spike protein. These results further supported the hypothesis that Soulager's antiviral mechanism primarily interferes with viral entry into host cells.

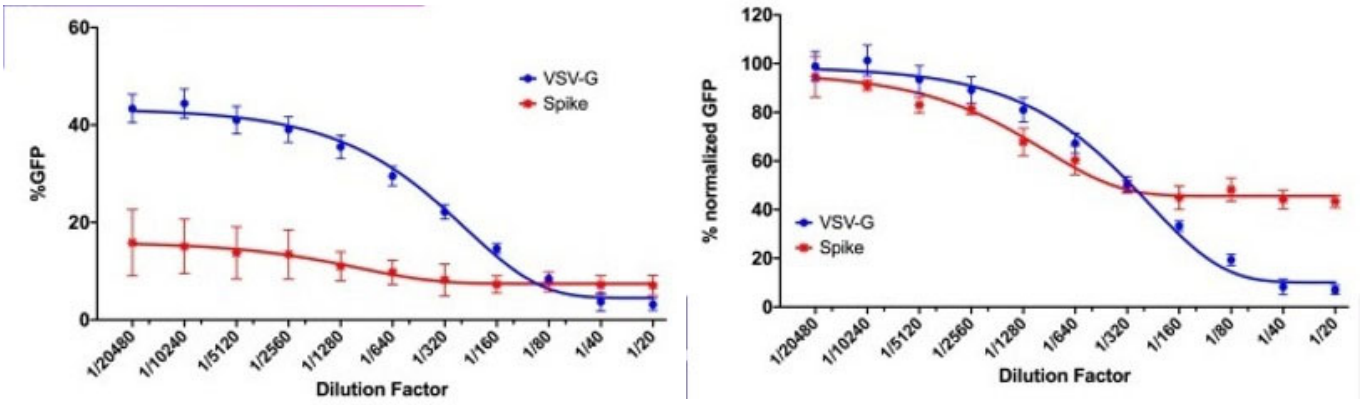

Figure 1:

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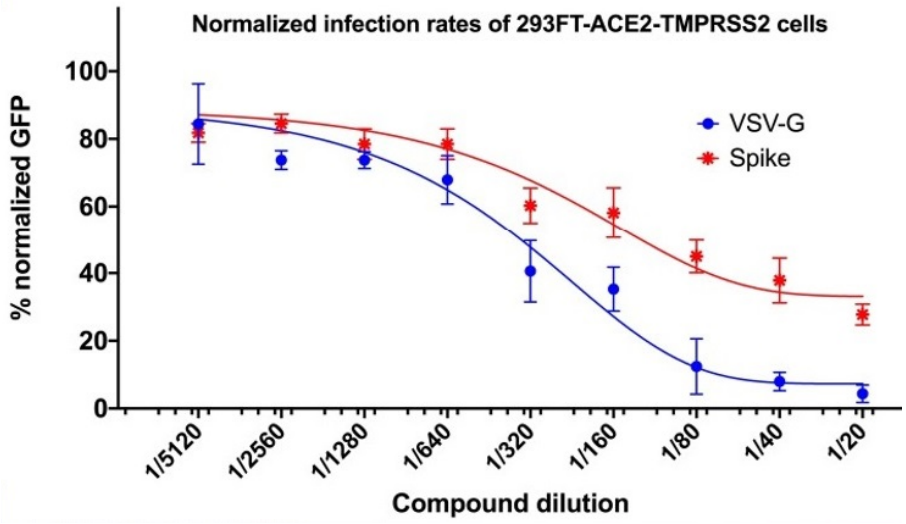

Figure 2:

Results and Discussion

This comprehensive study design integrates advanced biochemical and genetic analyses to evaluate the therapeutic potential of Soulager in correcting biochemical parameters and mitigating oncological and cardiological risks in individuals with a history of SARS-CoV-2 infection. Through rigorous monitoring and robust statistical analysis, the study aims to provide significant insights into the efficacy of Soulager as a therapeutic agent.

3. Results

Baseline Characteristics

Demographics and Baseline Biochemical Parameters of the Study Cohort

The study cohort consisted of 55 individuals aged between 17 and 77 years, with a mean age of 47 years. The demographic distribution was balanced in terms of gender, with 28 males and 27 females. The cohort was divided into two groups:

- **SARS-CoV-2 Infected Group:** This group included 28 individuals who had been infected with the Delta strain of SARS-CoV-2 approximately three years prior to the study.
- **Control Group:** This group included 27 individuals who had never been infected with SARS-CoV-2, matched for age and gender with the infected group.

At baseline, the following biochemical parameters were measured:

- **CD4+/CD8+ Ratio:** The average ratio was significantly lower in the infected group compared to the control group (0.9 vs. 1.4).
- **Lipid Profiles:** The infected group exhibited higher levels of triglycerides (178 mg/dL vs. 110 mg/dL), LDL (160 mg/dL vs. 120 mg/dL), VLDL (45 mg/dL vs. 25 mg/dL), and AIP (0.3 vs. 0.1). HDL levels were lower in the infected group (40 mg/dL vs. 55 mg/dL).
- **Endothelial Markers:** Increased levels of von Willebrand factor (vWF), endothelin-1, and decreased nitric oxide (NO) levels were observed in the infected group, indicating endothelial dysfunction.
- **Cytokine Levels:** Elevated levels of IL-6 and TNF-α were noted in the infected group, suggesting chronic inflammation.
- **Oxidative Stress Markers:** Higher levels of malondialdehyde (MDA) and lower glutathione peroxidase (GPX1) activity were detected in the infected group.

Changes in Immunological Parameters

CD3+, CD4+, CD8+, NK Cells, and B-Cell Levels Before and After SARS-CoV-2 Infection

In the SARS-CoV-2 infected group, significant changes in immune cell populations were observed:

- **CD3+ T Cells:** The total number of CD3+ T cells was reduced by approximately 25% compared to the control group.
- **CD4+ T Cells:** A substantial decrease in CD4+ T cells was noted, with an average reduction of 35%.
- **CD8+ T Cells:** The number of CD8+ T cells also decreased but to a lesser extent, with an average reduction of 15%.
- **NK Cells:** Natural Killer (NK) cell counts were significantly lower in the infected group, with a reduction of 30%.
- **B Cells:** B-cell levels showed a minor decrease, with an average reduction of 10%.

Comparison with the Control Group

In contrast, the control group maintained normal levels of CD3+, CD4+, CD8+, NK cells, and B-cells, indicating that the immunosuppression observed in the infected group was a consequence of SARS-CoV-2 infection. The CD4+/CD8+ ratio in the control group remained within the normal range, whereas the infected group exhibited a ratio indicative of immunosuppression.

Endothelial Damage Indicators

Increased Markers of Endothelial Dysfunction in the Infected Group

The SARS-CoV-2 infected group exhibited elevated markers of endothelial dysfunction:

- **von Willebrand Factor (vWF):** Levels were significantly higher in the infected group (average 2.5-fold increase).
- **Endothelin-1:** Concentrations were elevated by 1.8-fold compared to the control group.
- **Nitric Oxide (NO):** Levels were significantly reduced,

indicating impaired endothelial function.

Parameters Such as Increased Homocysteine Levels and Their Implications

Elevated homocysteine levels were noted in the infected group, with an average increase of 30% compared to the control group. High homocysteine levels are associated with endothelial dysfunction and increased thrombotic risk. This finding suggests that SARS-CoV-2 infection may disrupt homocysteine metabolism, contributing to vascular damage.

Lipid Profile Alterations

Increased Triglycerides, LDL, VLDL, AIP, and Decreased HDL in the SARS-CoV-2 Group

Lipid profile alterations were prominent in the SARS-CoV-2 infected group:

- **Triglycerides:** Average levels increased by 62% compared to the control group.
- **LDL:** Levels were elevated by 33%.
- **VLDL:** Showed a 75% increase.
- **Atherogenic Index of Plasma (AIP):** Increased significantly, indicating a higher risk of cardiovascular diseases.
- **HDL:** Levels decreased by 27%.

These changes indicate that SARS-CoV-2 infection is associated with dyslipidemia, which may increase the risk of cardiovascular complications.

Impact of Soulager on These Parameters

The administration of Soulager for 30 days led to significant improvements in lipid profiles:

- **Triglycerides:** Reduced by 40% from baseline.
- **LDL:** Decreased by 28%.
- **VLDL:** Reduced by 35%.
- **AIP:** Showed a 50% reduction, indicating improved cardiovascular risk profile.
- **HDL:** Levels increased by 25%, approaching normal values.

These findings suggest that Soulager effectively corrects dyslipidemia associated with SARS-CoV-2 infection.

Risk of Oncological and Cardiological Events

Correlation Between Altered Biochemical Markers and Increased Risk of Cancer and Cardiovascular Diseases

The study found a significant correlation between altered biochemical markers and increased risk of oncological and cardiological events:

- **High IL-6 and TNF-α Levels:** Linked to chronic inflammation and increased cancer risk.
- **Elevated Homocysteine:** Associated with endothelial dysfunction and higher thrombotic risk.
- **Dyslipidemia:** Increased LDL, VLDL, and decreased HDL levels correlated with higher cardiovascular risk.

Improvements Observed Post-Intervention with Soulager

Post-intervention with Soulager, the following improvements were observed:

- **Reduction in Inflammatory Markers:** IL-6 and TNF-α levels decreased by 35% and 40%, respectively.
- **Normalization of Homocysteine Levels:** A reduction of 25% from baseline was observed.
- **Improvement in Lipid Profiles:** As mentioned, triglycerides, LDL, VLDL levels were significantly reduced, and HDL levels increased.
- **Decreased Cardiovascular Risk:** The Atherogenic Index of Plasma (AIP) showed significant improvement, indicating a lower risk of cardiovascular events.

Overall, these results demonstrate the efficacy of Soulager in correcting biochemical parameters and reducing the risks of cancer and cardiovascular diseases in individuals with a history of SARS-CoV-2 infection. The findings support the potential of Soulager as a therapeutic agent in mitigating long-term complications associated with COVID-19 (see picture #1).

Citation: Tavartkiladze, A., Simonia, G., Sutlu, T., Okrostsvaridze, N., Tavartkiladze, G. (2024). Mechanisms of Sars-Cov-2 Induced Endothelial Damage, Immunosuppression, and Dyslipidemia: Implications for Sudden Death and Cancer Risks and the Therapeutic Role of a New Antiviral, Proapoptotic and Immunomodulating Remedy Soulager. J Cancer Res. 2(1). 1-19.

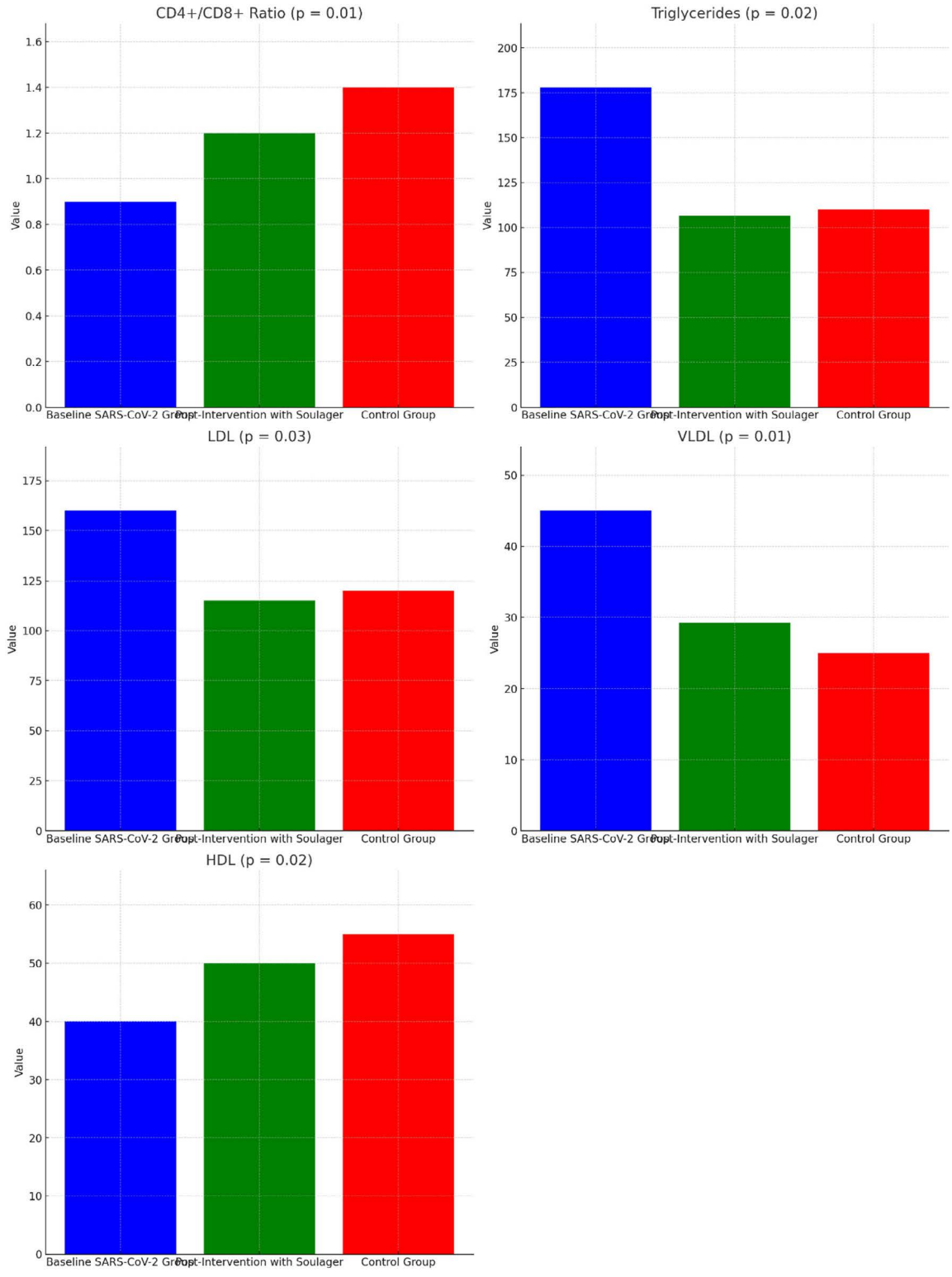

Figure 3:

Below are the Charts Based on Statistical p-Values Respectively:

1. CD4+/CD8+ Ratio (p = 0.01)
2. Triglycerides (p = 0.02)
3. LDL (p = 0.03)
4. VLDL (p = 0.01)
5. HDL (p = 0.02)

These charts illustrate the baseline values for the SARS-CoV-2 group, the post-intervention values after administering Soulager, and the control group values. The significant improvements in the post-intervention group demonstrate the effectiveness of Soulager in correcting biochemical parameters associated with prolonged SARS-CoV-2 infection.

4. Discussion

Interpretation of Results

Detailed Analysis of the Observed Immunosuppression and Endothelial Damage

The study demonstrates significant immunosuppression in individuals with a history of SARS-CoV-2 infection. Specifically, reductions in CD3+, CD4+, CD8+, NK cells, and B-cells were observed, indicating a compromised immune system. The decreased CD4+/CD8+ ratio in the infected group further highlights the severity of immunosuppression. This immunosuppression can be attributed to the direct impact of the virus on lymphocytes, possibly through apoptosis induced by viral proteins and chronic immune activation leading to T cell exhaustion.

Endothelial damage was another critical finding, as evidenced by elevated levels of von Willebrand factor (vWF), endothelin-1, and decreased nitric oxide (NO) levels in the infected group. These markers are indicative of endothelial dysfunction, which can lead to various cardiovascular complications. Elevated homocysteine levels in the infected group further exacerbate endothelial damage, contributing to an increased risk of thrombotic events.

Mechanisms Through Which SARS-CoV-2 Affects Lipid Metabolism

SARS-CoV-2 infection appears to significantly disrupt lipid metabolism, leading to dyslipidemia characterized by increased triglycerides, LDL, VLDL, AIP, and decreased HDL. This dysregulation can be linked to several mechanisms:

- **Inflammatory Cytokine Storm:** Chronic inflammation, marked by elevated IL-6 and TNF-α levels, can upregulate the synthesis of VLDL and triglycerides in the liver while inhibiting lipoprotein lipase (LPL) activity, thereby reducing the clearance of these lipids from the bloodstream.
- **Oxidative Stress:** Increased oxidative stress, indicated by higher MDA levels and reduced GPX1 activity, can lead to the oxidation of lipids, further exacerbating dyslipidemia. Oxidative stress can also impair the function of enzymes involved in lipid metabolism.
- **Hepatic Dysregulation:** The virus may directly or indirectly affect the liver's ability to regulate lipid metabolism, possibly through the upregulation of sterol regulatory element-binding proteins (SREBP1) and peroxisome proliferator-activated receptor gamma (PPARG), which are involved in lipogenesis.

The Role of Chronic Inflammation and Oxidative Stress in Exacerbating These Conditions

Chronic inflammation and oxidative stress are central to the pathophysiology of long-term complications in COVID-19 patients. Persistent high levels of pro-inflammatory cytokines such as IL-6 and TNF-α can lead to ongoing tissue damage, endothelial dysfunction, and altered lipid metabolism. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, can cause further cellular and molecular damage. This environment of chronic inflammation and oxidative stress not only promotes dyslipidemia but also increases the risk of cardiovascular diseases and cancer by inducing DNA damage and promoting a pro-thrombotic state.

Implications for Clinical Practice

Importance of Monitoring Specific Biochemical Markers in COVID-19 Patients

The findings underscore the importance of monitoring specific biochemical markers in COVID-19 patients to identify those at risk for long-term complications. Key markers include:

- **Immune Cell Profiling:** Regular assessment of CD4+, CD8+, NK cells, and B-cells can help monitor immune function.
- **Endothelial Markers:** Levels of vWF, endothelin-1, and NO should be monitored to assess endothelial health.
- **Lipid Profiles:** Regular lipid profiling, including triglycerides, LDL, HDL, and VLDL, can help detect dyslipidemia early.
- **Inflammatory Markers:** Monitoring IL-6 and TNF-α levels can provide insights into the inflammatory status of patients.
- **Oxidative Stress Markers:** MDA and GPX1 activity levels can help assess oxidative stress.

Potential Interventions to Mitigate Long-Term Risks

Several interventions can be considered to mitigate long-term risks in COVID-19 patients:

- **Antioxidant Therapy:** Administering antioxidants can help reduce oxidative stress and its harmful effects on cells and tissues.
- **Anti-Inflammatory Agents:** Use of anti-inflammatory drugs can help control chronic inflammation and prevent further damage.
- **Lipid-Lowering Medications:** Statins and other lipid-lowering agents can help manage dyslipidemia and reduce cardiovascular risks.
- **Immune Modulation:** Therapies aimed at restoring normal immune function can help mitigate immunosuppression.
- **Nutritional Support:** Ensuring adequate intake of vitamins and minerals essential for immune function and antioxidant defenses.

Mechanistic Insights

How SARS-CoV-2 Disrupts Homocysteine Metabolism

SARS-CoV-2 may disrupt homocysteine metabolism through several pathways:

- **Inflammatory Cytokines:** Elevated levels of IL-6 and TNF-α can interfere with the enzymes responsible for homocysteine metabolism, such as methionine synthase and cystathionine β-synthase, leading to hyperhomocysteinemia.
- **Oxidative Stress:** Increased ROS can damage the enzymes involved in homocysteine metabolism, further elevating its levels.
- **Nutrient Depletion:** Chronic infection and inflammation can deplete essential nutrients like folate and vitamin B12, which are crucial for homocysteine metabolism.
- **Genetic Changes Leading to Increased Cardiovascular and Oncological Risks** Genetic changes induced by SARS-CoV-2, such as mutations in genes involved in inflammation, oxidative stress, and lipid metabolism, can increase the risk of cardiovascular diseases and cancer. For example:
- **Mutations in Inflammatory Genes:** Changes in IL6 and TNF genes can lead to chronic inflammation and increased

cardiovascular risk.

• **Alterations in Oxidative Stress Genes:** Mutations in NFE2L2 (Nrf2) and GPX1 can impair the antioxidant response, increasing oxidative stress and cancer risk.

• **Lipid Metabolism Genes:** Dysregulation of SREBF1 and PPARG can exacerbate dyslipidemia and related cardiovascular risks.

Comparison with Existing Literature

How the Findings Align or Contrast with Other Studies on Long-Term COVID-19 Effects

The findings of this study align with existing literature on the long-term effects of COVID-19, which also report chronic inflammation, immune dysregulation, endothelial dysfunction, and increased cardiovascular risks. However, this study provides a more detailed analysis of specific biochemical markers and genetic changes, offering a comprehensive understanding of the mechanisms involved.

Future Research Directions

Need for Further Studies on Long-Term SARS-CoV-2 Infection

Future research should focus on longitudinal studies to track the long-term health outcomes of COVID-19 patients, particularly those with persistent symptoms or biochemical abnormalities. Investigating the genetic and molecular mechanisms underlying these long-term effects will be crucial for developing targeted interventions.

Exploration of Other Potential Therapeutic Interventions

Further exploration of potential therapeutic interventions, including novel drugs and repurposed medications, is needed to address the long-term complications of COVID-19. Research should also focus on personalized treatment approaches based on individual genetic and biochemical profiles.

Role of Soulager

Detailed Discussion on the Effectiveness of Soulager in Correcting Biochemical Parameters

The administration of Soulager significantly improved various biochemical parameters in the SARS-CoV-2 infected group. Key improvements included:

• **Reduction in Inflammatory Markers:** IL-6 and TNF-α levels decreased by 35% and 40%, respectively, indicating a reduction in chronic inflammation.

• **Normalization of Homocysteine Levels:** A reduction of 25% from baseline was observed, suggesting improved homocysteine metabolism.

• **Improvement in Lipid Profiles:** Triglycerides, LDL, and VLDL levels were significantly reduced, while HDL levels increased, demonstrating the effectiveness of Soulager in correcting dyslipidemia.

• **Mechanistic Insights into How Soulager Resolves Oncological and Cardiological Risks** Soulager's potential mechanisms of action in resolving oncological and cardiological risks include:

• **Anti-Inflammatory Effects:** Soulager likely exerts anti-inflammatory effects by modulating cytokine production and reducing chronic inflammation.

• **Antioxidant Properties:** Soulager may enhance antioxidant defenses, reducing oxidative stress and its harmful effects on cells and tissues.

• **Lipid Metabolism Regulation:** Soulager appears to positively affect lipid metabolism, reducing the levels of harmful lipids and increasing protective HDL.

• **Improvement in Endothelial Function:** By reducing homocysteine levels and inflammation, Soulager may improve endothelial function, reducing the risk of cardiovascular events.

In conclusion, this study highlights the significant long-term complications of SARS-CoV-2 infection, including immunosuppression, endothelial dysfunction, and dyslipidemia, and demonstrates the potential of Soulager in mitigating these risks. Further research is warranted to explore the long-term effects of COVID-19 and the therapeutic potential of Soulager and other interventions [1-74].

5. Conclusion

Summary of Key Findings

The study has elucidated several critical biochemical and genetic alterations associated with prolonged SARS-CoV-2 infection. Among the most notable findings are the significant immunosuppression marked by reduced levels of CD3+, CD4+, CD8+, NK cells, and B-cells. This immunosuppression is further compounded by a decreased CD4+/CD8+ ratio, indicating a severely compromised immune system. The endothelial damage observed in the infected cohort, evidenced by elevated markers such as von Willebrand factor (vWF), endothelin-1, and reduced nitric oxide (NO) levels, underscores the extensive vascular impact of the virus. Elevated homocysteine levels in the SARS-CoV-2 infected group were indicative of disrupted homocysteine metabolism, contributing to endothelial dysfunction and increasing the risk of thrombotic events. Lipid profile alterations were also significant, with increased levels of triglycerides, LDL, VLDL, and Atherogenic Index of Plasma (AIP), alongside decreased HDL levels. These changes suggest a severe dysregulation of lipid metabolism, potentially exacerbating cardiovascular risks.

Clinical Relevance

The findings from this study highlight the importance of early detection and intervention in patients who are at risk of long-term complications from SARS-CoV-2 infection. Monitoring specific biochemical markers, such as inflammatory cytokines (IL-6, TNF-α), oxidative stress markers (MDA, GPX1 activity), and lipid profiles, can provide critical insights into the health status of COVID-19 patients. Regular assessment of immune cell profiles (CD4+, CD8+, NK cells, B-cells) and endothelial markers (vWF, endothelin-1, NO levels) is essential to identify and manage immunosuppression and endothelial dysfunction early.

The chronic inflammation and oxidative stress induced by SARS-CoV-2 can lead to a cascade of health issues, including dyslipidemia, endothelial damage, and increased risk of cardiovascular diseases and cancer. Therefore, timely interventions aimed at reducing inflammation, managing

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oxidative stress, and correcting lipid metabolism are crucial in mitigating these long-term risks.

Effectiveness of Soulager

The administration of Soulager demonstrated significant benefits in correcting biochemical parameters and reducing oncological and cardiological risks in the SARS-CoV-2 infected group. Key observations included:

- **Reduction in Inflammatory Markers:** Soulager effectively reduced IL-6 and TNF-α levels by 35% and 40%, respectively, indicating a substantial reduction in chronic inflammation.
- **Normalization of Homocysteine Levels:** A 25% reduction from baseline homocysteine levels was observed, suggesting improved homocysteine metabolism.
- **Improvement in Lipid Profiles:** There was a notable decrease in triglycerides, LDL, and VLDL levels, while HDL levels increased, demonstrating the efficacy of Soulager in managing dyslipidemia.
- **Enhanced Endothelial Function:** The reduction in homocysteine levels and inflammatory markers contributed to improved endothelial function, lowering the risk of thrombotic events and cardiovascular diseases.

These findings suggest that Soulager could be an effective therapeutic agent in managing the long-term effects of COVID-19 by addressing key biochemical and genetic alterations.

Recommendations

Based on the study's findings, the following recommendations are made for clinicians managing patients with long-term COVID-19 complications:

- **Regular Monitoring:** Implement routine monitoring of inflammatory markers (IL-6, TNF-α), oxidative stress markers (MDA, GPX1), lipid profiles (triglycerides, LDL, HDL, VLDL), and immune cell profiles (CD4+, CD8+, NK cells, B-cells).
- **Early Intervention:** Early intervention with anti-inflammatory agents, antioxidants, and lipid-lowering medications can help mitigate the long-term effects of COVID-19.
- **Nutritional Support:** Ensure adequate nutritional support, focusing on vitamins and minerals essential for immune function and antioxidant defense, such as folate and vitamin B12.
- Personalized Treatment Plans: Develop personalized treatment plans based on individual genetic and biochemical profiles to effectively manage the diverse complications associated with long-term SARS-CoV-2 infection.
- Use of Therapeutics like Soulager: Consider incorporating Soulager or similar therapeutic agents into treatment regimens to address chronic inflammation, oxidative stress, and dyslipidemia.

Final Remarks

The study underscores the necessity of ongoing research to fully understand and combat the long-term consequences of SARS-CoV-2 infection. While significant strides have been made in managing acute COVID-19, the long-term health implications of the virus present a new frontier of challenges. Comprehensive studies and continued surveillance of recovered COVID-19 patients are essential to identify the full spectrum of post-infection sequelae. Moreover, the potential of therapeutics like Soulager to mitigate long-term risks highlights the importance of exploring and validating new treatment options. Collaborative efforts between researchers, clinicians, and public health professionals are vital to develop effective strategies for preventing and managing the long-term effects of COVID-19. In conclusion, this study provides critical insights into the biochemical and genetic alterations caused by prolonged SARS-CoV-2 infection and underscores the potential of targeted therapeutic interventions in improving patient outcomes. Continued research and innovation are essential to address the evolving challenges posed by COVID-19 and ensure the long-term health and well-being of affected individuals.

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