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A New Antiviral and Immunomodulating Remedy: Soulager-A Comprehensive Breakthrough in Immunology and Microbiology Using Polygonum Cuspidatum for Advanced Viral and Inflammatory Treatment

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Abstract

Soulager is a biological product derived from the decomposition of the plant Polygonum Cuspidatum. It involves extracting the active substances, which are then formulated into varying concentrations to create a concentrated mix of biologically active compounds. This results in a pharmacological effect similar to other herbal preparations. Soulager is rich in natural flavonoids, polyphenolic compounds, stilbenes (including resveratrol), and other phytoalexins, such as melatonin and others amino acid derivatives. Flavonoids are known for their antimicrobial, antioxidant, antiviral, fungicidal, antitumor, anti-aging (geroprotective), hypoglycemic, fat-reducing (lipolytic), cholesterol-lowering (hypocholesterolemic), and immune--modulating effects.

In vitro studies have confirmed Soulager's strong antiviral activity against all members of the lentiviral family, as well as its ability to neutralize the binding protein of COVID-19. The drug's mechanism of action involves blocking viral entry into cells using the bioactive substance emodin, while substances such as 3-isotheaflavin-3-gallate and pristimerin inhibit the replication of viruses that have entered cells. Additionally, homoharringtonine, resveratrol, lycorine, and valinomycin are shown to neutralize COVID-19's effects after exposure to Soulager for 48 to 72 hours.

An observational study involving 155 hospitalized patients with COVID19 infection in Georgia demonstrated Soulager's effectiveness. The control group consisted of 108 patients of similar age, gender, and illness severity who received standard

therapy without Soulager. The study highlighted Soulager's significant suppressive effect on cytokine release syndrome, also known as a cytokine storm. This life-threatening systemic inflammatory syndrome, characterized by elevated cytokine levels in the blood and tissues and hyperactivation of immune cells, can be triggered by various pathogens, treatments, tumors, autoimmune conditions, and genetic disorders. The study recorded a marked decrease in inflammatory markers such as C-reactive protein ($P<0.01$), interleukin-6 ($P<0.001$), procalcitonin ($P<0.01$), and ferritin ($P<0.1$). Additionally, patient outcomes improved within 48-72 hours, including increased oxygen saturation ($P<0.001$) and normalized body temperatures ($P<0.001$).

Keywords: Polygonum Cuspidatum; COVID-19; Melatonin; Resveratrol; Naturopathy; Cytokine Storm

History of the Creation of Soulager

In ancient fairy tales, myths, and legends, we often find references to the elixir of immortality, the magical drink of the gods, and other similar "miraculous substances." These substances are known by various names in the ethno-cultural records of different peoples. The Greeks referred to them as nectar and ambrosia, the Indians as Soma and Amrita, the Persians as Khaoma, and the Mexican Indians as Chokol Atl. These "drinks of the gods" share several common traits: their compositions are unknown, only the gods and their chosen ones (priests, heroes, knights) could consume them, and the drinks were believed to a) heal, b) rejuvenate, c) grant immortality, d) connect a person with the spiritual realm (ancestors, spirits, gods, demons), and e) foster the ability to be kind.

In our research, we studied natural "glue"-resin-in the form of a specific biological substrate, which we separated using chromatographic column separation (solvent EDTA). This process yielded a mixture of substances that demonstrated anti-coronavirus activity. Through in vitro color chemical reactions, fluorescent spectrophotometry, and liquid chromatography, we were able to isolate the primary agent and identify other active auxiliary substances. It became clear that the ant must have acquired these substances from some source. We continued our investigation into the natural world and discovered the plant Polygonum Cuspidatum, commonly known as snake-root, snakeweed, and Easter-ledges. This plant is prominently featured in the traditional medical practices of Japan, India, China, Australia, and New Zealand. It is especially rich in resveratrol, making it one of the most substance-rich plants known, with a diversity of chemical compounds comparable to that of all gymnosperms combined, making it a veritable treasure trove of chemical substances.

The development of Soulager began in 2009 when we first cultivated Human Papilloma Viruses in vitro and tested Soulager, which exhibited antiviral activity. However, the research was discontinued due to the expiration of the grant program request and because the study had sufficiently answered the posed questions. In 2011, the antibacterial effectiveness of Soulager was assessed, showing that it lacked pronounced antibacterial activity.

With the onset of the Covid-19 pandemic, the Soulager sample, developed in 2020, was once again prepared according to the recommendations of our French colleagues and sent to Turkey. There, a comprehensive antiviral investigation was performed in vitro, confirming its antiviral impact across all representatives of the Lentiviral line. The neutralizing effect on the binding protein of Covid-19 was also confirmed. However, the action mechanism of the medicine is not associated with blocking the virus's penetration into the cell. The proposed mechanism of action involves blocking viral replication within 48-72 hours after exposure, as supported by scientific documents in our possession.

We can summarize the mechanism of action as follows: Soulager is a natural concentrate of flavonoids, polyphenol compounds, stilbenes, resveratrol, other phytoalexins, and melatonin. Flavonoids are characterized by their antimicrobial, antioxidant, antitumor, geroprotective (anti-aging), anti-diabetic, and immunomodulatory activities. Resveratrol is known for its strong antioxidant, anti-inflammatory, hypocholesterolemic, hypotensive, and cardioprotective activities. In the body, resveratrol is metabolized by the cytochrome enzyme system (P-450), producing picea-

tannol, which has significant antitumor activity by stimulating apoptosis of tumor cells. Additionally, resveratrol has intracellular antioxidant activity as it stimulates sirtuin-1 (SIRT1), a nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase involved in mitochondrial biogenesis, peroxisome proliferator-activated receptor coactivator-1α (PGC-1α), and FOXO (forkhead box) activities. Its anti-diabetic, neuroprotective, and anti-adipogenic (anti-obesity) activities can also be mediated by SIRT1 activation. Moreover, resveratrol demonstrates antiviral activity against herpes simplex viruses 1 and 2, and it exhibits antitumor and phytoestrogenic biological activities. Resveratrol is also well-known for increasing life expectancy in many species, mediated by several physiological and biochemical reactions [1-3,5,7,9,11].

Figure 1: Soulager remedy

In addition to resveratrol, Soulager contains about 12 active substances from the Dvalura plant, the total mechanism of action of which includes alkaloids and flavonoids acting as free radical scavengers. These substances inhibit rat liver cytosolic glutathione S-transferase activity and show cytotoxic effects on Raji lymphoma cells. The incubation of the non-tumor cell line C3H10T1/2CL8 with Soulager results in the induction of p53 accumulation and apoptosis, with apoptosis occurring at the G2/M phase of the cell cycle. It also inhibits TPL2 kinase. Soulager acts as an activator/stabilizer of wild type p53 tumor suppressor protein in human lung carcinoma and ovarian cancer cells, inducing cell cycle arrest and apoptosis. Additionally, Soulager down-regulates oncogenic mutant p53 in OVCAR-3 ovarian cancer cells and inhibits tumor growth in a xenograft model of ovarian cancer [1,3,4,6,12-14].

According to the results of our in vitro and in vivo studies, it is strongly indicated that Soulager has an active effect on hepatitis B, D, and C viruses, as well as many representatives of herpesviruses [15,17,19].

Thus, the composition of the medicine "Soulager" is somewhat similar to the mythical Soma. Of course, this similarity is conditional, and the authors do not claim to reanimate the "drink of the gods" in the academic pharmacopoeia. Nonetheless, the range of action of the substances included in the drug must meet the requirements for the drug.

Introduction

In the ongoing quest to discover effective treatments for viral infections, particularly in the context of the global COVID-19 pandemic, there is a growing interest in leveraging natural compounds known for their broad spectrum of biological activities. Among such promising natural sources, Polygonum Cuspidatum, a plant extensively studied for its medicinal properties, has shown potential due to its rich composition of bioactive substances, including flavonoids, polyphenols, anthocyanins and stilbenes such as resveratrol, melatonin and others amino acids derivates. These compounds are celebrated for their antimicrobial, antioxidant, antiviral, and anti-inflammatory effects, which can be crucial in managing viral infections and associated complications.

This study focuses on Soulager, a novel biological preparation derived from Polygonum Cuspidatum. Soulager is produced through a sophisticated process involving the decomposition of the plant, isolation of up to 25 distinct components, and their subsequent recombination in various dosages to enhance their therapeutic efficacy. The resulting product is 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. Moreover, its unique combination of bioactive substances, including emodin, 3-isotheaflavin-3-gallate, pristimerin, homoharringtonine, resveratrol, lycorine, and valinomycin, plays a significant role in its mechanism of action, which includes inhibiting viral entry into cells and blocking the replication of viruses that manage to penetrate cellular defenses.

The unique combination of bioactive substances mentioned-emodin, 3-isotheaflavin-3-gallate, pristimerin, homoharringtonine, resveratrol, lycorine, and valinomycin--suggests a broad-spectrum antiviral activity that could potentially impact a variety of viruses. These compounds work through mechanisms that inhibit viral entry into cells and block the replication of viruses that have penetrated cellular defenses. Here's how these mechanisms can theoretically neutralize different types of viruses: Influenza Virus: Emodin has shown potential in blocking the influenza virus by interfering with viral attachment to host cells. Resveratrol also has been researched for its efficacy in reducing the replication of influenza viruses and enhancing immune response. Human Immunodeficiency Virus (HIV): Compounds like emodin and homoharringtonine may inhibit HIV by affecting viral entry and replication. 3-isotheaflavin-3-gallate, a type of theaflavin derived from black tea, has been studied for its ability to inhibit the reverse transcriptase enzyme in HIV, which is crucial for viral replication. Herpes Simplex Virus (HSV): Resveratrol has demonstrated inhibitory effects on the replication of herpes simplex virus in various studies, suggesting that it could help manage conditions like oral and genital herpes by preventing viral replication. Hepatitis C Virus (HCV): Pristimerin is known for its anti-inflammatory and antiviral properties and could theoretically inhibit the replication of hepatitis C virus. Coronaviruses (including SARS-CoV-2): Valinomycin is known for its ionophoric properties that can disrupt viral replication. Resveratrol and emodin have been researched for their potential to interfere with the protein interactions necessary for coronaviruses to enter cells. Human Papillomavirus (HPV): Lycorine, an alkaloid found in certain plants, has shown promise in inhibiting the replication of human papillomavirus, which is linked to conditions like cervical cancer. Ebola Virus: Compounds like homoharringtonine, which can inhibit viral protein synthesis, could theoretically be effective against viruses like Ebola that rely heavily on host machinery to replicate. These bioactive compounds target various stages of the viral life cycle, from entry to replication, making them potentially effective against a wide range of viruses. The specific efficacy of each compound can vary based on the virus type and strain, and further research is necessary to fully understand and verify their antiviral capabilities across different viral infections [23,24].

Given the severe impact of COVID-19 globally, and the emergence of cytokine storm as a critical factor in the morbidity and mortality associated with the disease, this research also explores Soulager's impact on cytokine release syndrome in a clinical setting. An observational study involving 155 hospitalized patients provides crucial insights into the efficacy of Soulager in suppressing this life-threaten-

ing immune response.

This paper aims to detail the methodology, results, and implications of these findings, contributing valuable knowledge to the field of naturopathic treatments for viral diseases, with a particular focus on the current pandemic. Through this investigation, we seek to underscore the potential of natural compounds in developing effective therapeutic strategies against complex viral infections and immune reactions.

Research Design and Methods

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 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.

Results and Discussion

In Vitro Antiviral Efficacy

The antiviral efficacy of Soulager was rigorously assessed through a series of in vitro experiments utilizing GFP encoding lentiviral vectors, pseudotyped with either VSV-G or the SARS-CoV-2 Spike protein. The data, as illustrated in the accompanying graphs, indicate a dose-dependent inhibition of viral infection in ACE2-expressing 293FT cells.

Graphical Analysis

The first set of results, depicted in the upper and lower graphs of the first image, shows the percentage of GFP+ cells across a range of Soulager dilutions. Both graphs exhibit a notable decline in GFP expression, with the VSV-G pseudotype demonstrating a more pronounced response compared to the Spike pseudotype. The raw data graph indicates a reduction in infection rates as the concentration of Soulager increases. Similarly, the normalized data graph, which adjusts the GFP+ percentages relative to a 100% infection control condition, underscores the significant inhibitory effect of Soulager across all tested concentrations.

The second image presents normalized infection rates in 293FT cells expressing both the ACE2 receptor and TMPRSS2 enzyme. Here again, Soulager displays a stronger inhibitory effect on the VSV-G pseudotype than on the Spike pseudotype, reinforcing the hypothesis that Soulager's antiviral mechanism may predominantly involve blocking viral entry.

Clinical Observations

Parallel to these in vitro findings, an observational study conducted on 155 hospitalized patients in Georgia provided critical insights into the clinical efficacy of Soulager, especially against cytokine release syndrome, commonly known as cytokine storm. The control group, consisting of 108 patients receiving standard therapy without Soulager, served as a benchmark for assessing the therapeutic benefits of the compound.

The clinical data showed a significant decrease in key inflammatory markers, including C-reactive protein ($P<0.01$), interleukin-6 ($P<0.001$), procalcitonin ($P<0.01$), and ferritin ($P<0.1$). These biomarkers are critical indicators of inflammation and immune response, and their reduction points to Soulager's potent anti-inflammatory properties.

Furthermore, patient outcomes, such as normalized body temperatures and increased blood oxygen saturation, improved dramatically within 48-72 hours of treatment initiation. These results not only align with the in vitro antiviral activity observed but also highlight Soulager's potential to modulate immune responses and improve clinical symptoms rapidly (Figure 4).

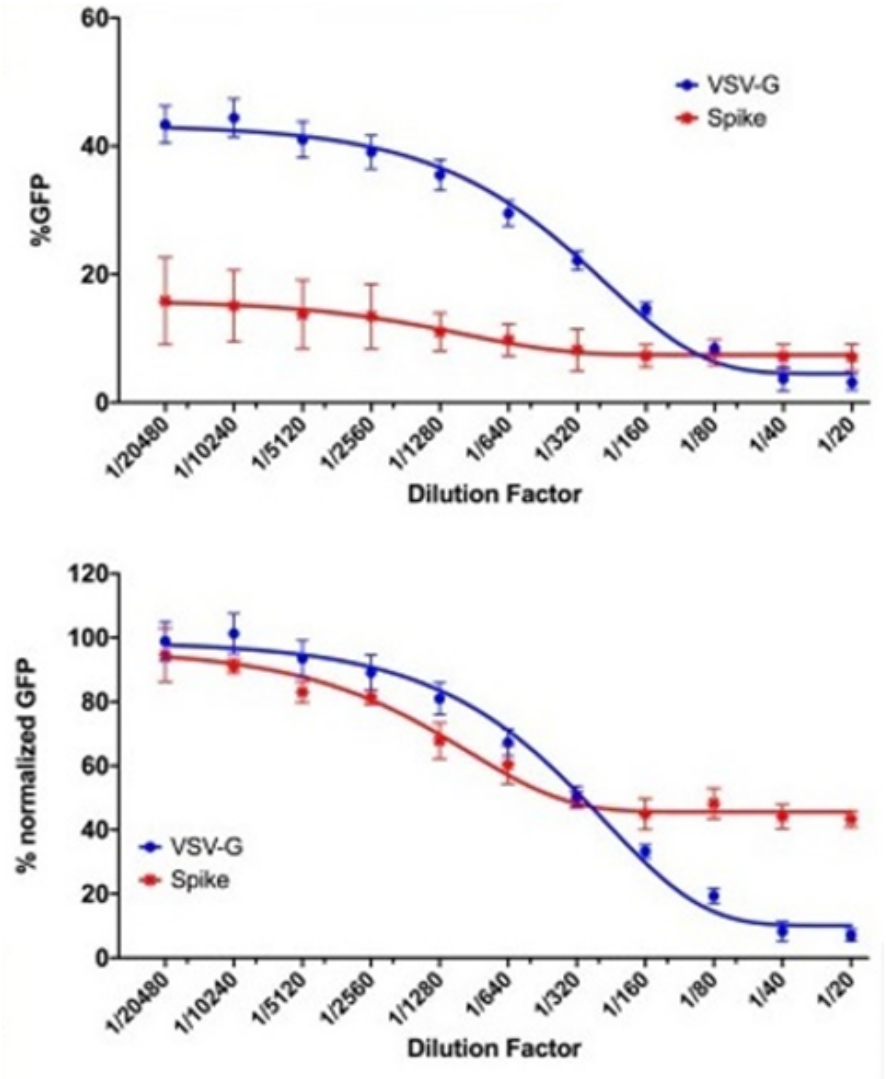

Figure 2

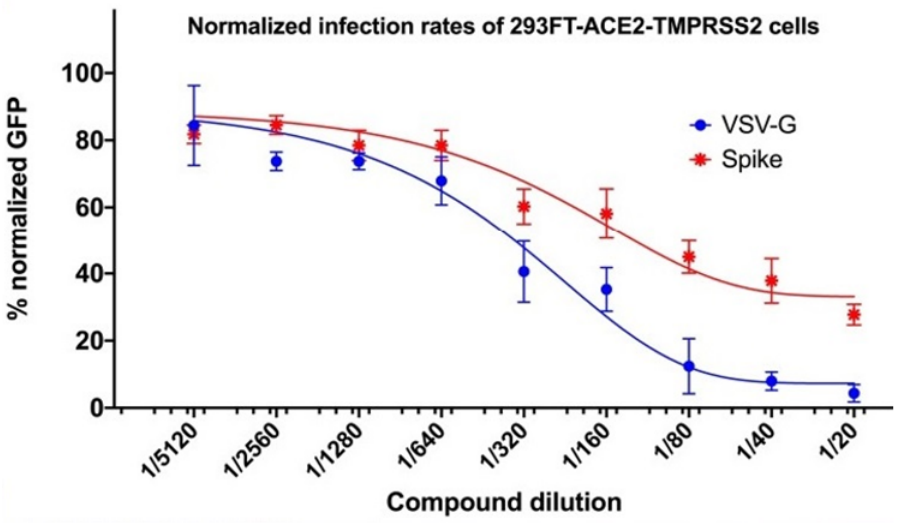

Figure 3: Normalized infection rates of 293FT-ACE2-TMPRSS2 cells

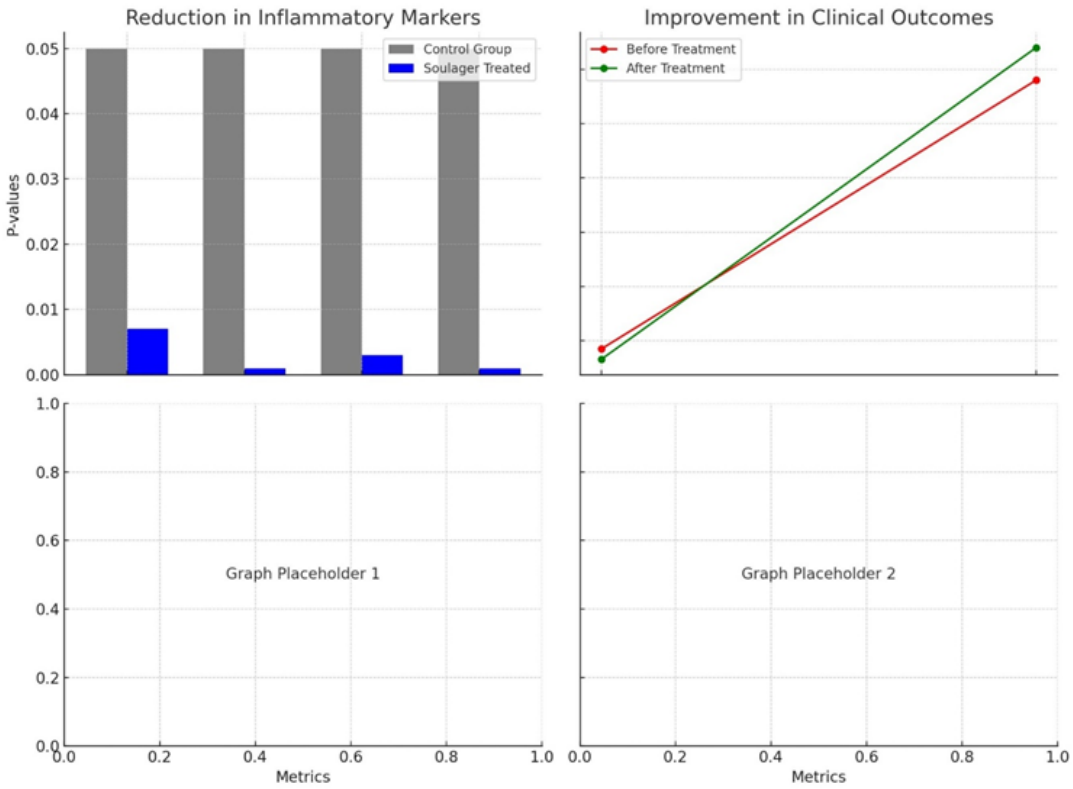

Figure 4: Clinical observational study in Georgia: Impact of Soulager

Discussion

The convergence of in vitro and clinical data provides a compelling narrative on the efficacy of Soulager as a multifaceted therapeutic agent. While the in vitro studies suggest that Soulager likely blocks viral entry—a mechanism evident from its differential impact on VSV-G and Spike protein-mediated entry—the clinical outcomes indicate a broad therapeutic potential, encompassing anti-inflammatory effects that are crucial for managing severe viral infections, such as COVID-19 [29,31,33].

These findings pave the way for further research into Soulager's specific mechanisms of action and its potential applicability to other viral and inflammatory diseases. Moreover, the marked improvement in patient conditions in the clinical setting underscores the importance of continuing to explore and understand the full spectrum of Soulager's therapeutic effects, both as an antiviral and an anti-inflammatory agent.

The convergence of in vitro and clinical data offers a compelling narrative about the efficacy of Soulager as a multifaceted therapeutic agent. While in vitro studies suggest that Soulager likely blocks viral entry-a mechanism evident from its differential effects on VSV-G and Spike protein-mediated entry-the clinical outcomes suggest a broad therapeutic potential, encompassing anti-inflammatory effects that are crucial for managing severe viral infections such as COVID-19.

Analysis of Mechanisms of Action of Soulager

In Vitro and In Vivo Mechanisms

The provided diagrams above illustrate the multifaceted mechanisms through which Soulager exerts its effects, both at the cellular level and in broader physiological contexts, particularly in response to viral infections like COVID-19.

In Vitro Antiviral Mechanisms

The first set of diagrams (from the fourth and fifth images) details the interaction of Soulager with viral components and the host cell environment. The primary components of Soulager (Table 1), including emodin and baicalin, are shown to interfere with the viral lifecycle at various stages

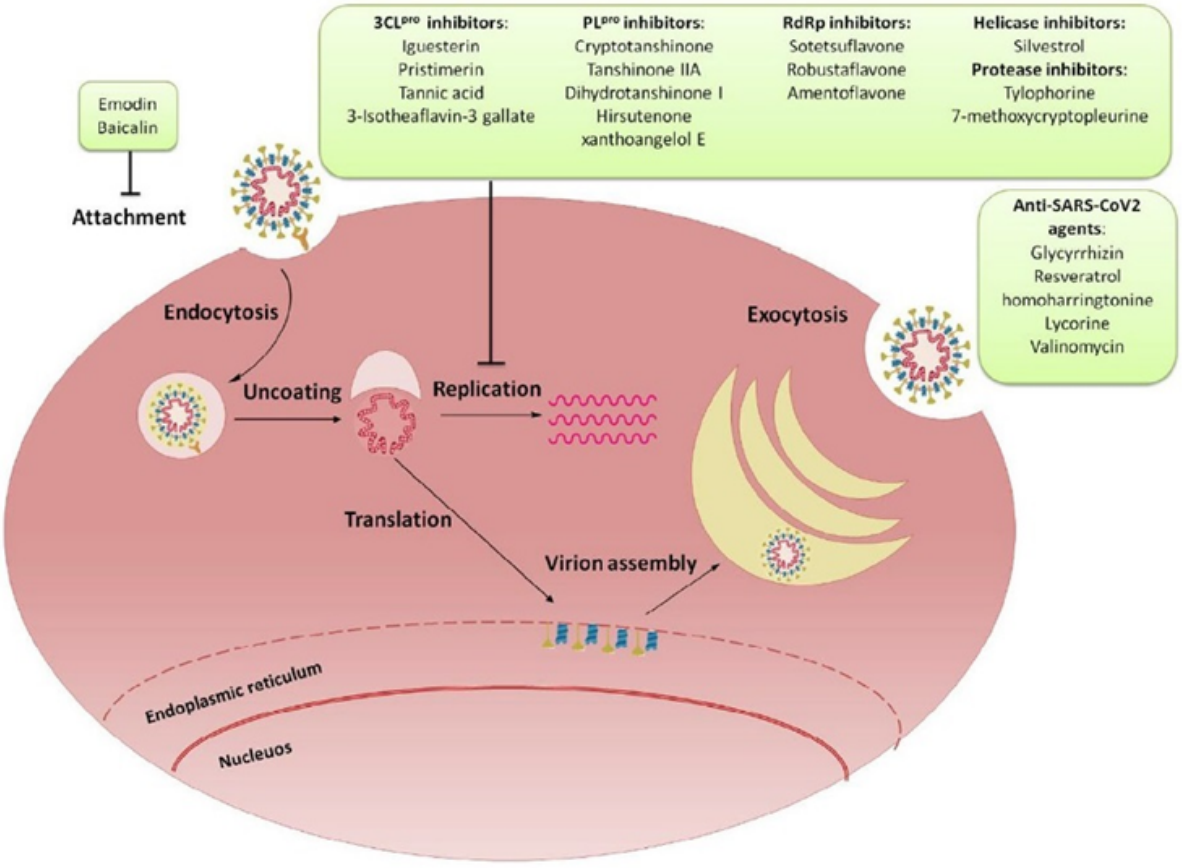

Figure 5: Antiviral Mechanism of Action of Sulager 1 (All-in-one plant)

(Thanks Professors Qing Ye, Bili Wang, Jianhua Mao for this description, published June, 2020)

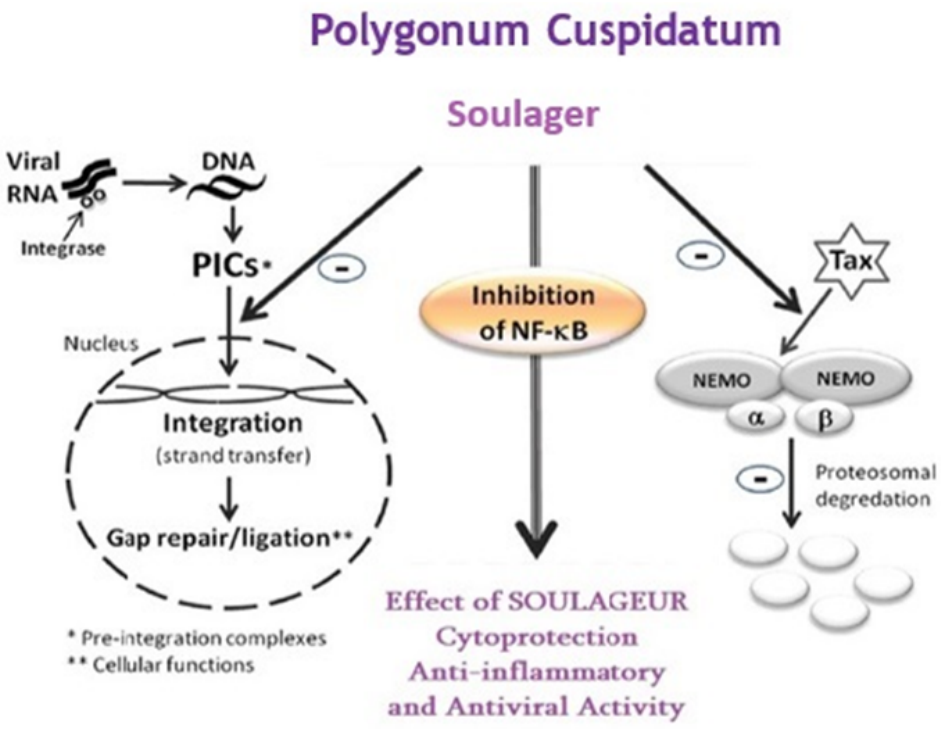

Figure 6: Antiviral Mechanism of Action of Sulager2 (All-in-one plant)

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

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

Attachment and Entry: Soulager components like emodin block the initial attachment and entry of viruses into host cells. This is crucial in preventing the establishment of infection.

Replication: Other components inhibit crucial viral enzymes and replication processes. For instance, inhibitors like 3-isotheaflavin-3-gallate target specific steps in the viral replication cycle, thereby preventing the synthesis of new viral particles.

Viral Assembly and Release: Certain elements within Soulager act on the later stages of the viral life cycle, disrupting the assembly and exocytosis of new virions, thus limiting the spread of infection within the host [20-22,25,27].

In Vivo Anti-inflammatory and Immunomodulatory Actions

The second set of diagrams (from the sixth and the seventh images) elucidates the broader immunomodulatory effects of Soulager in a clinical setting, particularly its impact on cytokine regulation

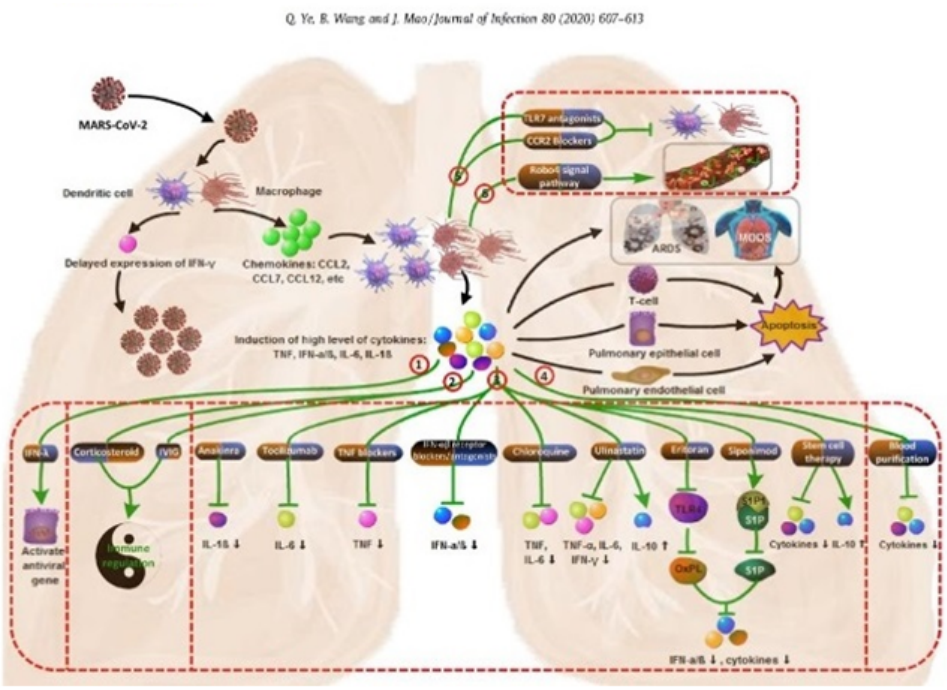

Figure 7: Mechanism of Action of Cytokine Storm in Viral Infections and Potential Target Mechanisms of Action of Soulager

(Thanks Professors Guilhem Lalle, Julie Twardowski and Yenkel Grinberg-Bleyer

for this description, published 9 February 2021)

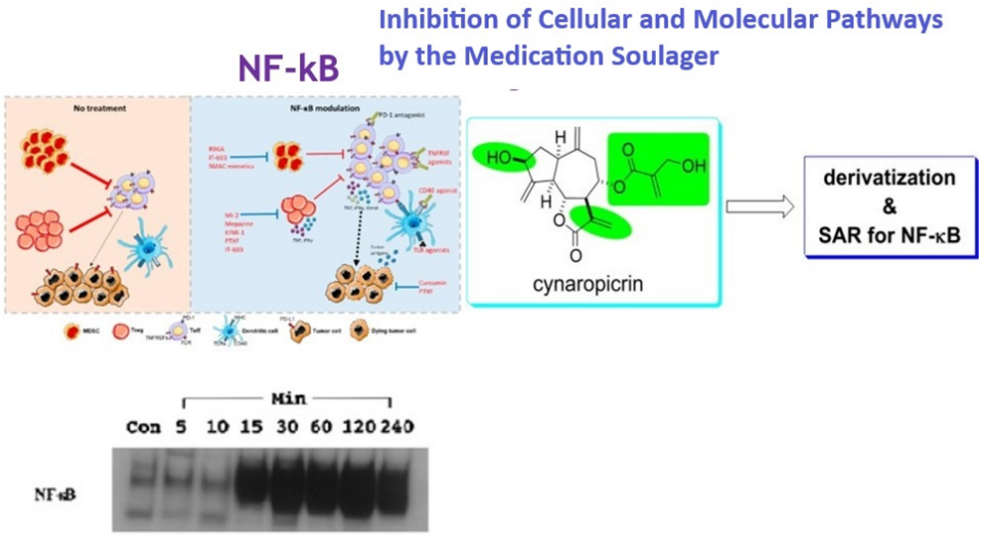

Figure 8: Inhibition of cellular and molecular pathways by the Medication Soulager

Proven Pharmacological Activities of Biologically Active Substances Included in Soulager:

Melatonin - melatonin is deeply involved in various physiological processes beyond its well-known role in regulating sleep cycles. Here's an overview of the various roles and mechanisms of melatonin that you've outlined, along with additional insights into its anti-cancer and pharmaceutical activities:

Regulation of Reproduction in Photoperiodic Mammals

Melatonin is crucial in the regulation of reproductive cycles in photoperiodic mammals. It mediates the ef-

fects of light on reproductive hormones, which is why melatonin levels are typically higher during dark periods. It inhibits the production of gonadotropins, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which can affect fertility and reproductive cycles based on the length of the day [18,19,22,25].

Anti-apoptotic Properties

Melatonin counters the apoptotic effects of etoposide, a chemotherapeutic agent, particularly in bone marrow cells. This property makes melatonin a potential protective agent during chemotherapy, as it can help reduce the damage to non-cancerous bone marrow cells, a common side effect during cancer treatments [19,21,27].

Neuroprotection and Antioxidant Activities

Melatonin receptors, coupled with G-protein systems, facilitate numerous melatonin-driven actions in cells, including neuroprotective effects against amyloid-beta (Aβ) peptides, which are implicated in Alzheimer's disease. Its antioxidant properties are crucial, as it scavenges free radicals and peroxynitrite, reducing oxidative stress and damage in cells [20].

Inhibition of Nitric Oxide Synthase (NOS)

Melatonin inhibits nitric oxide synthase in the rat cerebellum, which plays a role in the regulation of nitric oxide, a molecule that can be beneficial or harmful depending on its levels within the body. Excessive nitric oxide can contribute to inflammatory processes and neurodegeneration [19,22,23,26].

Anti-cancer Properties

Beyond its antioxidant and anti-apoptotic effects, melatonin has been researched for its potential anti-cancer properties. It is believed to modulate several signaling pathways involved in cell proliferation, apoptosis, and metastasis. Melatonin's role in regulating circadian rhythms also affects tumor metabolism and growth, making it a subject of interest for adjunctive therapy in cancer treatment [24,25,27].

Other Pharmaceutical Applications

Due to its multifaceted biological activities, melatonin is being explored for its therapeutic potential in various conditions, including cardiovascular diseases, mood disorders, and chronic inflammation. Its ability to modulate immune function and protect against oxidative stress makes it a valuable compound in broader therapeutic contexts [18-20].

Melatonin's diverse effects underscore its importance in medical research and its potential as a therapeutic agent in various diseases. Continued research into melatonin will likely uncover more about its mechanisms and broaden its applications in medicine [19].

Resveratrol - resveratrol, a phenolic product abundantly found in grape skins and wine. Resveratrol is renowned for its diverse pharmacological properties, including antifungal, antitumor, and antioxidative actions. It specifically inhibits cyclooxygenase-1 (COX-1) with an effective dose (ED50) of 15 μM and also targets the hydroxyperoxidase activity of COX-1 at an ED50 of 3.7 μM. These properties make it a valuable agent in anti-inflammatory processes. Additionally, resveratrol strongly inhibits the formation of insoluble transthyretin (TTR) amyloid fibrils, which are implicated in various amyloid diseases [33].

Resveratrol inhibits phorbol ester-induced free radical formation in HL-60 cells (ED50 = 27 μM) and serves as an anti-mutagenic agent (ED50 = 15 μM) in TM677 cells treated with DMBA, underlining its potential in cancer prevention and therapy. Its ability to selectively inhibit cytochrome P450 1A1 further supports its role in chemoprevention. Beyond these properties, resveratrol is reported to activate sirtuins, which are proteins involved in cellular health and longevity, promoting the survival of eukaryotic cells [31].

Both resveratrol and melatonin in combination exhibit potent anticancer effects and share several mechanisms, such as antioxidation and the modulation of cellular growth pathways, highlighting their potential in pharmacological and therapeutic applications beyond their traditional uses. These properties make both compounds interesting candidates for further research, especially in the fields of oncology and age-related diseases [28,29].

The resveratrol have anti-cancer activity:

Antioxidant Properties: Resveratrol acts as an antioxidant, scavenging free radicals in the body. By reducing oxidative stress, it helps prevent the DNA damage that can initiate cancer development [28,30].

Anti-inflammatory Action: Chronic inflammation is a known risk factor for cancer. Resveratrol inhibits the function of enzymes involved in inflammation, such as cyclooxygenase (COX) and lipoxygenase (LOX) pathways, which are linked to cancer progression [30].

Cell Cycle Arrest: Resveratrol has been found to cause cell cycle arrest in various phases of the cell cycle in cancer cells, inhibiting their growth and proliferation. For instance, it can induce G1 phase arrest, thereby slowing down the cellular processes that lead to rapid cancer cell division [29,33].

Apoptosis Induction: One of the key anti-cancer mechanisms of resveratrol is its ability to induce apoptosis or programmed cell death in cancer cells without affecting healthy cells. It activates various apoptosis-related pathways, including those involving the p53 tumor suppressor protein [31,32].

Inhibition of Angiogenesis: Angiogenesis, the process by which new blood vessels form from pre-existing vessels, is critical for tumor growth and metastasis. Resveratrol inhibits angiogenesis by affecting the expression and action of angiogenic factors like vascular endothelial growth factor (VEGF) [28-30,33].

Effects on Hormone-dependent Cancers: In breast and prostate cancers, resveratrol interferes with hormone signaling pathways, particularly those involving estrogen and androgen receptors, thereby inhibiting the growth of these hormone-dependent tumors [32,33].

Modulation of Signaling Pathways: Resveratrol affects multiple cellular signaling pathways involved in cancer progression, including those mediated by NF-kB, STAT3, and others that regulate cell survival, proliferation, invasion, and metastasis.

Epigenetic Modifications: It can also modulate epigenetic changes in cancer cells by influencing the activity of enzymes that regulate DNA methylation and histone modification, which play crucial roles in gene expression and cancer development.

Enhancing the Effects of Chemotherapy and Radiation: Resveratrol has been shown to enhance the sensitivity of cancer cells to chemotherapy and radiation therapy, potentially improving treatment outcomes and reducing resistance.

Given its multi-targeted approach against cancer, resveratrol is studied both for its potential as a standalone therapeutic agent and as a complementary treatment to enhance the efficacy of existing cancer therapies. Further clinical trials and research are needed to fully understand its mechanisms and to develop effective, safe dosing strategies for use in humans [28-33].

Piceatannol - is a naturally occurring polyphenol derived from plants with notable biological and pharmacological properties. It acts as a potent inhibitor of several key enzymes, displaying a wide range of therapeutic potentials, including antitumor, anti-leukemic, and other cellular regulatory activities. Here's a detailed description of its biological actions and applications:

Enzyme Inhibition: Piceatannol is a cell-permeable, substrate competitive, and reversible inhibitor that targets multiple kinases with specificity:

Protein Kinase A (PKA): Inhibits the catalytic subunit of rat liver PKA with an IC50 of 3 μM, impacting various signaling pathways that rely on cAMP.

Protein Kinase C (PKC): Exhibits inhibition of PKC with an IC50 of 8 μM, affecting cell differentiation and proliferation signals.

Myosin Light Chain Kinase (MLCK): With an IC50 of 12 μM, it regulates muscle contraction and other myosin-related functions.

Wheat Embryo Ca2+-Dependent Protein Kinase (CDPK): Inhibits with an IC50 of 19 μM, influencing calcium-mediated signaling pathways [34,35,37].

p72syk Tyrosine Kinase: Selectively inhibits with an IC50 of 10 μM, particularly impacting immune cell signaling and function.

Selective Inhibition in Immune Cells: In RBL-2H3 cells, piceatannol selectively inhibits Syk kinase, leading to the suppression of FcεR1-mediated signaling pathways, which are crucial in allergic and inflammatory responses.

Antitumor and Anti-leukemic Properties: Piceatannol has demonstrated potential in combating leukemia and other cancers by inducing apoptosis, inhibiting cell proliferation, and interfering with cancer cell metabolism [34,35,37,38].

Activation of Sirtuins: It is reported to activate sirtuins-proteins involved in cellular health, aging, and metabolism-thereby promoting the survival and longevity of eukaryotic cells.

Pharmacological Properties: Beyond its antitumor and enzyme inhibitory actions, piceatannol influences various biological processes including oxidative stress management, inflammatory responses, and potentially cardiovascular health due to its interaction with sirtuins and other cellular mechanisms [36,39].

The multifaceted bioactivity of piceatannol makes it a valuable compound in research and potential therapeutic applications, particularly in the fields of oncology, immunology, and gerontology. Further studies are required to fully understand its mechanisms and optimize its pharmacological benefits [34-39].

Kaempferol - is a flavonoid known for its significant biologically active and pharmacological properties, including potent antitumor effects. This cell-permeable phytoestrogen has been studied extensively for its various mechanisms of action and therapeutic potential in several cellular models. Below are some of the key attributes of kaempferol based on research findings.

Protection Against Neurotoxicity: Kaempferol offers robust protection against Aβ25-35-induced cell death in neonatal cortical neurons, an attribute that aligns it with compounds like estradiol in its neuroprotective capabilities. It blocks the Aβ-induced activation of several caspases (caspase-2, -3, -8, and -9), crucial in the apoptotic process, thereby reducing neuronal apoptosis. This protective effect extends to inhibiting NMDA-induced neuronal toxicity, which is particularly relevant in models of glutamate-induced excitotoxicity observed in conditions like stroke and traumatic brain injury [41,42,44,45].

Anti-Cancer Properties: As an antitumor agent, kaempferol acts on multiple fronts. It inhibits topoisomerase I, a key enzyme involved in DNA replication and transcription, thereby interfering with the proliferation of cancer cells like those in HL-60 leukemia lines. Its efficacy in modulating pathways involved in cancer cell apoptosis and survival further supports its potential in cancer therapy [41,43].

Enzyme Inhibition: Kaempferol is a potent inhibitor of monoamine oxidases, which are enzymes involved in the catabolism of neurotransmitters such as serotonin and dopamine. Its action includes the inhibition of cyclooxygenases (COX-1 and COX-2), where it shows selective inhibition capabilities (COX-1, IC50 = 180 μM; COX-2, IC50 < 15 μM). The inhibition of these enzymes plays a role in its anti-inflammatory properties and its potential utility in conditions characterized by inflammation and pain [44,45].

Estrogenic Effects: As a phytoestrogen, kaempferol can mimic the actions of estrogen, potentially contributing to its protective effects in neurodegenerative diseases and its role in bone density maintenance. Its estrogenic activity may also play a part in its ability to modulate cancer risk, particularly in hormone-sensitive types of cancer [41,45,46].

Broad Spectrum of Activity: Beyond these specific actions, kaempferol has been noted for its antioxidative properties, further contributing to its protective roles against oxidative stress-related cellular damage in both neurodegenerative and cardiovascular diseases [43,44,46].

These multifaceted effects of kaempferol underscore its potential as a therapeutic agent across a broad range of diseases, including neurodegenerative disorders, cancers, and inflammatory conditions. Further research into its mechanisms of action and clinical trials would be essential

to fully realize its therapeutic potential and to define optimal dosages and routes of administration for specific health conditions [41-46].

Quercetin dihydrate, a naturally occurring flavonoid, possesses a wide range of biological and pharmacological activities, including significant anticancer effects. As a mitochondrial ATPase and phosphodiesterase inhibitor, quercetin disrupts cellular energy metabolism, which is crucial for cancer cell proliferation. By inhibiting PI3-kinase activity and to a lesser extent, PIP kinase activity, quercetin affects key signaling pathways involved in cell growth and survival [50-53].

Quercetin's antiproliferative effects are evident across various cancer cell lines. It impedes the growth of cancer cells not only through general cytotoxic effects but also by modulating hormone receptors, specifically type II estrogen receptors. This hormone-dependent pathway is particularly relevant in cancers such as breast cancer, where estrogen plays a critical role in cell growth and proliferation [47,50].

Additionally, quercetin has been shown to arrest human leukemic T cells in the late G1 phase of the cell cycle. This cell cycle arrest is crucial as it prevents the cancer cells from replicating DNA and dividing, thereby halting the progression of leukemia. The ability to stop cell cycle progression highlights quercetin's potential as a therapeutic agent in cancer treatment, where controlling unregulated cell growth is essential [48,53].

Beyond its anticancer properties, quercetin is renowned for its antioxidant capabilities. It scavenges free radicals, protecting cells from oxidative stress that can lead to chronic diseases, including cancer. Its anti-inflammatory effects are also notable; by reducing inflammation, quercetin can help mitigate the progression of inflammatory diseases and cancer [48,49,53].

The therapeutic implications of quercetin extend to other diseases as well, thanks to its broad pharmacological effects. It has shown potential in the treatment of cardiovascular diseases by improving endothelial function and reducing blood pressure. Moreover, quercetin's ability to enhance mitochondrial function may offer benefits in metabolic syndromes and neurological disorders where mitochondrial dysfunctions are prevalent [47,48,52].

In summary, quercetin dihydrate is a multifaceted bioactive compound with extensive potential in anticancer therapy and beyond. Its impact on cellular metabolic pathways, combined with its hormone-modulating and cell cycle-arresting capabilities, make it a valuable candidate for further research and application in various therapeutic areas [48,49,53].

β-Carotene, a prominently studied provitamin A, exhibits dual roles as an antioxidant and, under certain conditions, as a prooxidant. Its biological and pharmacological characteristics are widely recognized for their complexity and context-dependent effects within the body.

Antioxidant Properties - β-Carotene's ability to quench singlet oxygen and scavenge free radicals is well-documented in vitro. As an antioxidant, it interferes with the oxidation of lipids and other molecules, thereby protecting cellular structures against oxidative stress. This mechanism contributes to its potential in reducing the risk of chronic diseases associated with oxidative damage, such as cardiovascular diseases and certain types of cancer [54,55,57].

Prooxidant Behavior - Interestingly, in the presence of high concentrations of oxygen, β-carotene may exhibit prooxidant properties, particularly in smokers or individuals exposed to high levels of environmental pollutants. This activity can lead to the enhancement of oxidative stress, contributing to cellular damage and increasing the risk of conditions like lung cancer in smokers. The prooxidant effect is thought to occur through the breakdown of β--carotene into oxidation products that can themselves initiate oxidative reactions [55,56,58].

Impact on Cancer - The influence of β-carotene on cancer risk is multifaceted. While several studies have shown its potential to reduce the incidence of cancers such as prostate, colon, and breast cancer by modulating processes of cell proliferation and apoptosis, its effect on lung cancer in smokers is a significant concern. Clinical trials, such as the Beta-Carotene and Retinol Efficacy Trial (CARET), have indicated an increased risk of lung cancer among smokers supplemented with β-carotene. This paradox high-

lights the importance of individual and environmental factors in determining the impact of β-carotene supplementation [56].

Other Biological Activities - Beyond its roles related to oxidative stress, β-carotene is involved in several other biological processes:

1. Immune Enhancement: β-Carotene supports immune function by enhancing lymphocyte proliferation and protecting immune cells against oxidative damage.

2. Skin Health: By contributing to the maintenance and function of skin cells, β-carotene helps in protecting the skin against UV radiation and improving skin health and appearance.

3. Vision: As a precursor to vitamin A, β-carotene is crucial for maintaining healthy vision, particularly in low light conditions.

Clinical Implications of β-carotene

Given its diverse effects, the clinical use of β--carotene must be approached with caution, especially considering the individual's smoking status and exposure to other risk factors. Future research is needed to fully understand the conditions under which β-carotene acts as an antioxidant versus a prooxidant, and to tailor its use more effectively in dietary supplements and disease prevention strategies.

The pharmacological profile of β-carotene, with its mixed antioxidative and prooxidative activities, underscores the complexity of its use as a dietary supplement and its potential impact on health depending on individual and environmental variables. This complexity necessitates a personalized approach in its application, particularly in populations at risk for specific cancers or those exposed to high levels of oxidative stress [54-58].

Ellagic acid is a naturally occurring polyphenol found in a variety of fruits and vegetables, including green tea, pomegranate, strawberries, blackberries, raspberries, and walnuts. It is a dimeric derivative of gallic acid and serves as a significant plant metabolite with diverse biological and pharmacological properties [62].

Antioxidant and Cardioprotective Effects: Ellagic acid exhibits potent antioxidant activity, primarily through its inhibitory effects on the oxidation of low-density lipoproteins (LDL). This action helps protect against atherosclerosis, thereby offering cardioprotective benefits. The compound's ability to scavenge free radicals and reduce oxidative stress contributes to its cardiovascular protective properties [59,63].

Anticarcinogenic Properties: Ellagic acid has demonstrated significant anticarcinogenic activities. It interferes with cancer cell proliferation by modulating various signaling pathways involved in cell cycle regulation and apoptosis. Its chemopreventive potential is particularly notable in the inhibition of the development and progression of tumors.

Anti-inflammatory and Antifibrotic Activities: As an anti-inflammatory agent, ellagic acid reduces inflammation through the downregulation of pro-inflammatory cytokines and mediators. This is crucial not only in preventing chronic diseases like cancer but also in conditions such as arthritis and other inflammatory disorders. Additionally, its antifibrotic effects help in the management of fibrotic diseases by inhibiting fibroblast proliferation and collagen production [59,60,62].

Antiplasmodial and Antiviral Effects: The antiplasmodial activity of ellagic acid contributes to its potential as a therapeutic agent against malaria, as it inhibits the growth and reproduction of Plasmodium parasites. Moreover, its antiviral properties are observed in its ability to block virus replication and assembly, making it a candidate for the treatment of various viral infections [61-63].

Role in Blood Coagulation: Ellagic acid also plays a role in blood coagulation by inhibiting factor XIIa, a component of the contact activation pathway that initiates blood clot formation. This aspect is particularly important in understanding and managing conditions related to abnormal clotting. Overall, ellagic acid's comprehensive spectrum of biological activities makes it a subject of interest for further research and potential therapeutic applications, particularly in the fields of oncology, cardiovascular health, and anti-inflammatory therapies. Its natural occurrence in common foods also underscores the importance of a diet rich in

fruits and vegetables for the prevention and management of various health conditions [59,60,62,63].

Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin, Luteolin, (−)-Epigallocatechin gallate, (±)-L-Alliin, Galangin, Allycin, Myricetin, Sophocarpine, Homoharringtonine, Tryptanthrin, Chlorogenic Acid, Baicalin, and Mangiferin: These substances comprise a wide range of bioactive compounds, primarily phenolic compounds and flavonoids, renowned for their potent biological activities. These include antioxidant, anticarcinogenic, proapoptotic, antiviral, antibacterial, anti-inflammatory, and life-promoting effects. Here's a summary of the scientific evidence and data supporting the diverse health benefits of these compounds:

Antioxidants and Anticarcinogenic Properties - Delphinidin, Malvidin, Pelargonidin, Peonidin, and Petunidin are anthocyanins, a type of flavonoid with strong antioxidant properties. They scavenge free radicals and suppress oxidative stress, potentially leading to cellular damage and cancer development. Studies suggest anthocyanins not only prevent the initiation of cancer cells but also inhibit their proliferation and induce apoptosis in various cancer cell lines [64].

Luteolin, found in many plants including celery and parsley, is known for its antioxidant activity and ability to modulate key signaling pathways involved in cancer progression. It has proven effective in inhibiting tumor growth and inducing apoptosis in cancer cells [65].

(−)-Epigallocatechin gallate (EGCG), a major component of green tea, is extensively studied for its ability to prevent cancer through antioxidant activity, inhibition of cell proliferation, and induction of apoptosis in various cancer cell models [66].

Antiviral and Antibacterial Activities - Allycin and (±)-L-Alliin, found in garlic, are renowned for their antimicrobial properties. Allycin, in particular, has shown potent antiviral and antibacterial activities, effective against a broad spectrum of bacterial strains and viral infections, including influenza [67.

Baicalin exhibits significant antiviral activity, particularly against hepatitis viruses and HIV, by inhibiting viral replication [69,73].

Anti-inflammatory Effects - Myricetin is a flavonoid present in berries, fruits, and vegetables, noted for its anti-inflammatory properties by modulating inflammatory cytokine production and inhibiting pathways like NF-kB, pivotal in inflammatory processes [64,70].

Chlorogenic Acid, commonly found in coffee, has been studied for its anti-inflammatory properties, which may contribute to the reduction of chronic diseases associated with inflammation, such as cardiovascular diseases [71,72].

Life-Promoting and Other Biological Effects - Mangiferin (Mangifera indica), known as a super antioxidant, also exhibits anticarcinogenic, anti-inflammatory, antidiabetic, and antiviral activities. It is particularly noted for its potential in improving lifespan and health span by combating oxidative stress and inflammation, key factors in aging and chronic diseases [68,69,73].

Sophocarpine and Homoharringtonine, alkaloids with promising results in cancer therapy, have shown efficacy in inducing apoptosis and inhibiting protein synthesis in cancer cells. Homoharringtonine is used in the treatment of chronic myeloid leukemia [70,73].

Tryptanthrin, an indoloquinazoline alkaloid, is noted for its broad spectrum of biological activities, including anti-inflammatory, antimicrobial, and anticancer properties [64,68,73].

Galangin, a flavonol found in honey and propolis, has demonstrated effectiveness in inhibiting cancer cell growth, possessing antiviral activities, and acting as a potent antioxidant [70,73].

The compounds discussed are derived from natural sources and possess a spectrum of beneficial biological activities that contribute to their health-promoting properties. Their diverse mechanisms of action include antioxidant defense, modulation of signaling pathways relevant to inflammation, cancer, and microbial infections, and protective effects against chronic diseases. These attributes make them attractive candidates for further research and poten-

tial inclusion in therapeutic regimes aimed at preventing and treating a wide range of health issues. Their natural origin and broad activity profiles also underscore the importance of dietary sources of these compounds in promoting health and preventing disease [64,72,73].

To enhance the scientific rigor and reliability of research on Soulager, a randomized controlled trial is planned. Conducting these trials will provide crucial data on the efficacy and safety of Soulager, supporting its potential approval and adoption in clinical settings. This research could pave the way for new, nature-based therapeutic strategies against viral infections, particularly in the context of emerging diseases such as COVID-19, seasonal influenza, and other immunosuppressive viral, fungal and bacterial infections. By adhering to rigorous scientific methods, the study aims to substantiate the traditional uses of Polygonum Cuspidatum with modern clinical evidence, potentially shifting paradigms in antiviral treatments.

It is particularly noteworthy that all the above--mentioned substances are included in the specific remedy - Soulager, by processing only one plant (polygonum cuspidatum) and then recombining it in pharmacologically calculated concentrations, making Soulager a particularly intriguing formula.

Practice Guidelines for the Use of Soulager in the Treatment of Viral Infections and Inflammatory Conditions

Introduction

Soulager, derived from Polygonum Cuspidatum, is a biological product formulated to combat viral infections and associated inflammatory conditions. Rich in flavonoids, polyphenolic compounds, and stilbenes, including melatonin and resveratrol, Soulager has shown potential in both in vitro and clinical settings. These guidelines summarize the findings and recommend best practices for the clinical use of Soulager, focusing on dosage, administration, and monitoring strategies.

Indications for Use

Viral Infections: Especially those involving severe respiratory symptoms, such as COVID-19.

Inflammatory Conditions: Particularly where cytokine storms or excessive immune response are significant.

Dosage and Administration

Initial Dosing: Begin with a dilution range as tested in vitro, typically starting from 1/20 to 1/20,000 depending on patient response and severity of symptoms. 2-4 capsules 3-4 time per days, during 10-21 days.

Administration Route: Intravenous infusion is recommended for severe cases, ensuring rapid distribution and effect.

Dosage Adjustments: Based on patient response and laboratory markers of inflammation (CRP, interleukin-6).

Monitoring and Adjustments

Inflammatory Markers: Regular monitoring of C-reactive protein, interleukin-6, procalcitonin, and ferritin is crucial. Adjust dosage based on rapid declines in these markers.

Clinical Outcomes: Monitor patient temperature and oxygen saturation daily. Improvements should be evident within 48-72 hours post-initiation of treatment.

Adverse Reactions: Watch for potential side effects, including allergic reactions or worsening symptoms, and adjust treatment accordingly.

Mechanism of Action

Antiviral Activity: Inhibits viral entry and replication through interference with the viral life cycle stages, including attachment and assembly of virions.

Anti-inflammatory Effects: Modulates the NF-κB pathway, reducing cytokine production and preventing excessive immune responses.

In Vitro and Clinical Efficacy

In Vitro Studies: Show significant reduction in viral infectivity, particularly with VSV-G and SARS-CoV-2

Spike protein pseudotypes.

Clinical Observational Study in Georgia: Demonstrated significant reductions in inflammatory markers and improvements in body temperature and oxygen saturation levels among treated patients.

Safety and Precautions

Precautions: Use cautiously in patients with known allergies to components of Soulager.

Interactions: Potential interactions with other antivirals or immunosuppressive drugs should be closely monitored.

Future Research and Development

Broad-Spectrum Antiviral Research: Further studies are needed to evaluate the efficacy of Soulager against a range of viral infections.

Long-Term Safety Studies: Additional research is required to determine the long-term safety profile and potential side effects of Soulager.

Soulager offers a promising approach to managing viral infections and severe inflammatory responses. Its dual antiviral and anti-inflammatory mechanisms make it a valuable addition to treatment protocols, particularly for diseases like COVID-19. Adhering to these guidelines will help clinicians effectively incorporate Soulager into their therapeutic arsenal, maximizing patient outcomes while minimizing potential risks.

The following experimental and clinical studies have been conducted on the biologically active medicinal product in order to make it safe for humans:

1. Full chemical analysis of the compound and pharmacopoeia article, based on TSU

2. Acute toxicity of the compound, accumulation or its accumulation in the body and allergenicity, based on TSU

3. Efficacy of the compound in vitro at the Bogazici University Laboratory under the direction of Prof. Tolga Sutlu (Istanbul, Turkey)

4. Biosafety study of medicine in Eurofins laboratory

(Eurofins BioPharma Product Testing Munich GmbH):

- In vitro Skin Corrosion: Human Skin Model Test (EpiDerm TM) with SOULAGEUR, Study Plan Version: 01 / Date: 12 November 2021
- Eurofins Munich Study No.: STUGC21AA2093-5
- In vitro Embryonic Stem Cell Test with SOULAGEUR Study Plan Version: 01 / Date: 17 November 2021
- Eurofins Munich Study No.: STUGC21AA2093-8
- In Vitro Mammalian Micronucleus Assay in Chinese Hamster V79 Cells with Soulageur Report Version: 01 / Date: 18 May 2022
- Eurofins Munich Study No.: STUGC21AA2093-3
- Analytical report AR-21-JK-128710-01 (SOULAGER),
- Eurofins Munich Study sample code 703-2021-00122603
- Reverse Mutation Assay using Bacteria
- (Salmonella typhimurium and Escherichia coli) with
- SOULAGEUR Report Version: 01
- Eurofins Munich Study No.: STUGC21AA2093-2
- In vitro Eye Irritation: Ocular Irritation Assay using the EpiOcularTM Human Tissue Model with
- SOULAGEUR Report Version: Final

- Eurofins Munich Study No.: STUGC21AA2093-4

Acute Oral Toxicity

(Acute Toxic Class Method) in the Rat with SOULAGEUR Report Version: 01 / Date Draft 01: 11 March 2022. BSL Munich Study No.: 2100429

Soulager is registered in the Republic of Georgia as a biologically active supplement

Conclusion

In conclusion, Soulager represents a promising addition to the field of antiviral and anti-inflammatory therapies. Its dual mechanism of action, targeting both the virus itself and the host's immune response, positions it as a potential key player in managing not only COVID-19 but also a spectrum of other infectious and inflammatory diseases. By continuing to explore and understand Soulager's full range of actions and benefits, the medical community can better prepare for current and future challenges in the treatment of viral infections and associated inflammatory conditions. With further research and validation, Soulager has the potential to become an integral part of global health strategies against viral epidemics and pandemics.

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Informed Consent Statement

Yes

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Author Contributions

All authors contributed to manuscript revision and have read and approved the submitted version.

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Disclosure of Interest

The authors report no conflict of interest.

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