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Therapeutic Efficacy of Isoflavone - Forte against HIV: A Case Series

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Abstract: ***Background: Despite the effectiveness of current HIV therapies, such as highly active antiretroviral therapy (HAART), eradicating latent viral reservoirs remains a challenge. Isoflavone-Forte, a formulation rich in viral replication blockers, anti-inflammatory, and anticancer agents, may offer a solution. This study investigates its efficacy in HIV-positive patients with a history of tuberculosis. Methods: Five patients were treated with Isoflavone-Forte derived from Polygonum cuspidatum resin, extracted via a chromatographic separation method. Results: All patients experienced significant reductions in viral load and improvements in immune parameters. No side effects were reported during or after treatment. Conclusions: These results suggest that Isoflavone-Forte effectively mitigates HIV infection, improving viral load and immune parameters, with no observed side effects. While these outcomes show promise for Isoflavone-Forte as a beneficial therapeutic strategy for HIV, further studies are necessary for validation.luable insights for improving individualized care in NSCLC.***

Keywords: Isoflavone-Forte, *Polygonum cuspidatum*,HIV treatment, SARS-CoV-2, patients with HIV and tuberculosis, Disease stabilization

1. Introduction

Acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) report in 2021, it was estimated that approximately 37.7 million people were living with HIV globally at the end of 2020. Since the start of the epidemic, more than 75.7 million people have become infected with HIV, and 32.7 million people have died from AIDS-related illnesses. Current therapies, such as highly active antiretroviral therapy (HAART), have substantially improved treatment outcomes but do not eradicate latent viral reservoirs, which are potentialcauses for viral rebound (Chun et al., 1997). Eradicating latent viral reservoirs in HIV patients necessitates a chronic, long-term treatment strategy. This approach imposes a substantial economic burden on both the patient and the healthcare system. Patients often struggle with the high costs of continuous medication, regular health check-ups, and associated supportive care. This financial burden can be particularly debilitating for patients who lack comprehensive health insurance or live in low-income conditions.

Moreover, the healthcare system is strained by the need to allocate substantial resources for the long-term care of these patients. This includes not only the direct costs related to antiretroviral medications but also indirect costs such as hospital admissions, diagnostic tests, healthcare personnel, and patient management infrastructure.

In developing countries, these issues are magnified. With often under-resourced healthcare systems and a high prevalence of HIV, the economic and medical burden can be overwhelming. Access to essential antiretroviral medication may be limited due to high costs, and the long-term nature of the treatment can strain already limited healthcare resources. Moreover, these countries may lack the infrastructure necessary for continuous patient monitoring and management, adding additional complexities to the treatment process. Therefore, finding cost-effective and efficient treatment strategies is critical to ease the economic and medical burden of HIV treatment on both patients and healthcare systems, particularly in developing nations.

Traditional medicinal preparations are increasingly being explored as viable alternatives to modern pharmaceutical treatments for conditions like HIV, cancer, and other diseases. Several reasons make these alternative therapies appealing:

Comparable Efficacy: Research has shown that some traditional medicines have comparable efficacy to modern treatments. For instance, certain plant-based compounds have been found to inhibit the replication of viruses or the growth of cancer cells effectively.

Fewer Side Effects: Traditional medicines are often associated with fewer side effects than their modern pharmaceutical counterparts. They are typically derived from natural sources, and as a result, they may be less toxic and better tolerated by patients. However, it's important to note that this is not universally true, and some traditional medicines can have severe side effects or interact negatively with other medications.

Cost-Effective: Traditional medicines can often be less expensive to produce and purchase than modern

pharmaceuticals. This is particularly significant in low-income or developing countries where the high cost of modern treatments can be prohibitive.

Availability: In many regions, particularly in developing countries, access to modern pharmaceuticals may be limited. Traditional medicines, often derived from locally available resources, can provide an accessible alternative.

Following this line of reasoning, we turned our attention towards traditional medicinal preparations. One promising candidate emerged from the flora known for its antiviral activities - Polygonum Cuspidatum, also known as snake-root, snakeweed, or Easter-ledges. This plant has been prominently featured in the traditional medicinal recipes of Japan, India, China, Australia, and New Zealand.

Polygonum Cuspidatum is notably rich in resveratrol, a flavonoid that is renowned for its potential health benefits. Besides resveratrol, the plant contains several other bioactive compounds that are commonly found in a diverse range of species, making it a substantial "repository" of bioactive substances. The richness and diversity of these bioactive compounds make Polygonum Cuspidatum an intriguing focus for our exploration into alternative HIV treatments.

We studied a natural "glue" - resin (in the form of a specific biological substrate), which we decomposed on a column by the method of chromatographic separation (solvent EDTA). A mixture of the substances that possessed anti-coronavirus activity was obtained in the experiment - by using In Vitro color chemical reactions, fluorescent spectrophotometry and liquid chromatography, we were able to separate the substance that was the main agent and other substances - which were auxiliary but active substances. The ant had to get these substances from somewhere: we moved on and started searching the fauna and came to the plant Polygonum Cuspidatum, often called snake-root, snakeweed and Easter-ledges. The plant with the highest percentage of modern and ancient traditional: Japanese, Indian, Chinese, Australian and New Zealand medical recipes. It is rich in resveratrol, the richest plant substance known to all, and in addition contains about as many species of substances as all gymnosperms combined, that is, it is considered to be the most abundant "repository" of chemical substances.Therefore, weexplored fauna with reported anti-viral activities and selected *Polygonum cuspidatum*, also known as snake-root, snakeweed.This plant is the richest known source of resveratrol (a flavonoid)and contains several other compounds that are commonly found in gymnosperms, which are considered the most abundant "repository" of bioactive components(Ke Jia., et al 2023).

At this stage, Isoflavone-Forte is registered in Georgia as a biologically active supplement. The active ingredient is encapsulated in gelatin capsules that dissolve in the intestine, as gastric juice can alter the pharmacological properties of Isoflavone-Forte. Isoflavone-Forte is an herbal formulation that contains flavonoids, polyphenolic compounds, stilbenes, resveratrol, phytoalexins, and melatonin, all of which are known to exhibit beneficial pharmacological effects.

For instance, resveratrol has strong antioxidant, anti-inflammatory, and anticancer properties and can trigger tumor cell apoptosis (Ke Jia., et al 2023). In addition, the Isoflavone-Forte also contains approximately 12 active compounds obtained from the Polygonum *cuspidatum*, including alkaloids and flavonoids that act as free-radical scavengers and inhibit specific harmful reactions within the body.

Isoflavone-Forte was first tested *in vitro* on human papillomavirusin 2009 andyielded some promising results.In 2011, it was found to not have significant antibacterial activity. However, in 2020, during the coronavirus disease 2019 (COVID-19) pandemic, Isoflavone-Forte, we preparedagainIsoflavone-Forte and we was sent to Turkey for antiviral investigation, andthe new research confirmed its efficacy against a lentiviral line and its neutralizing effect on the binding protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19.

The proposed mechanism (Ke Jia., et al 2023) of Isoflavone-Forte's antiviral actioninvolves blocking viral replication within 48–72 hours post-exposure in our in vitro researches.

Here, we report five clinical cases wherein Isoflavone-Forte was successfully used for anti-HIV therapy, and our preliminary results demonstrate the potential of this formulation as a therapeutic agent for HIV infection.

2. Materials and Methods

We initiated our investigation by examining a specific biological substrate: a natural "resin" or "glue". Our primary objective was to isolate the active compounds within this resin.

To achieve this, we employed a method known as column chromatography, which is a widely used technique to separate chemical substances based on their varying degrees of absorption to a stationary phase (in this case, the column) and their solubility in a solvent (in this case, ethylenediaminetetraacetic acid, or EDTA).

The process started by packing a column with a stationary phase. The resin was then dissolved in the EDTA and introduced into the column. As the solution traveled down the column due to gravitational force, the different compounds within the resin began to separate. This separation occurs because different compounds have different affinities for the stationary phase and the solvent, causing them to move at different speeds down the column.

After the compounds were separated within the column, we collected them fractionally. Each fraction contained a different compound, or a group of compounds with similar properties. After the fractions were collected, we then proceeded to identify and analyze them to determine their specific properties and potential antiviral activities.

It's worth noting that column chromatography, such as the method we used, is a crucial tool in biochemistry and molecular biology, used extensively in the isolation,

purification, and analysis of complex organic mixtures.We studied a natural "glue" - (resin (in the form of a specific biological substrate), which we by decomposing it ed on a column by the and method of using chromatographic separation (solvent: ethylenediaminetetraacetic acidEDTA) to isolate the active compounds. A mixture of the substances that possessed compounds possessing anti-coronavirus activity was obtained in the experiment - by using In Vitro in vitro color chemical reactions, fluorescent spectrophotometry, and liquid chromatography. , we were able to We then separated the primary active compound substance that was the main agent fromand other substances - which were auxiliary but active substances.

Patienthistory and treatment

Five male patients, Born in: 1970, 1973, 1975, 1978, 1980, HIV-positive patients with a history of tuberculosiswere included in this study, the duration of which was 1.3 years, of which 7 months were treatment and the rest were follow-up. All patients had elected to discontinue antiretroviral therapy by early 2022 owing to severe side effects. At the beginning of the study in April 2022, the average viral load in each patient'sblood and the immune indicators were recorded. The patients presented with general weakness, appetite loss, anemia, and an average weight of 45 kg.

All five patients were treated with Isoflavone-Forte (300 mg, 2-2 capsules three times a day after meals) starting mid-April 2022. After one month, the average viral load was tested in Germany Lab - MVZ LABOR DR.LIMBACH (Georgian Branch)and confirmed by a second test in GeorgiaThereafter, bimonthly tests were conducted to monitor the viral load by RT-PCR method during 1.3 years and also all other tests we made in Georgia in the lab for Infectious Diseases Hospital (Tbilisi) and Institute for Personalized Medicine (Tbilisi), the tests was: CBC, LFTs, Creatinine, Blood Coagulation, Lipid Profile, Immunoglobulins (G, A, M – total) by ELISA method and Cellular Immune System's parameters (CD3+, CD4+, CD8+, B-Lymphocytes and NK-cells in the blood) by method flow Cytometer.

In March 2023, owing to a less-than-expected increase in the $CD4^+/CD8^+$ ratio, additional tests were performed to investigate potential bacterial infections. Follow-up control test (monitor the viral load) were carried out from April 2023.

3. Results

At the beginning of the study in April 2022, the average viral load in all patients blood was recorded to be 2,100,000 copies/ml, and the average values of immune indicators were observed to be significantly below the normal range ($CD4^+$ = 105, $CD8^+$ = 350, $CD4^+/CD8^+$ = 0.34).After one month of treatment with Isoflavone-Forte, the patients reported improvement in their general condition evaluations (body weight gain increased energy levels, improved appetite, andcorrespondingly increased food intake) however, laboratory tests showed an increased average viral load of 3,000,000 copies/ml. During the treatment period, the patients did not receive any other additional medications. A second confirmatory test in Germany revealed an average load of 3,820,000 copies/ml. Subsequent bimonthly tests indicated a two-fold decrease in the viral load.

By February 2023, average values of immune indicators showed improvement ($CD4^+$ = 398, $CD8^+$ = 860, and $CD4^+/CD8^+$ = 0.46) and the average viral load decreased to 300 copies/ml. The patients reported significantly improved health, no general weakness, average weight gains up to 65 kg, and improved digestive function (improved appetite, andcorrespondingly increased food intake). The follow-up control (monitor the viral load, CBC, CD3+, CD4+, CD8+, B-Lymphocytes and NK-cells in the blood) in April 2023 showed a further reduction in the average viral load to 150 copies/ml, which further decreased to 75 copies/ml by July 2023. The average values for immune indicators at this stage were as follows: $CD4^+$ = 415, $CD8^+$ = 680, and $CD4^+/CD8^+$ = 0.63.

Additional tests conducted in March 2023 revealed that all five patients had active syphilis infections, indicating a possible alternative infection source. The patients were consulted by a venereologist and appropriate antibiotic therapy was prescribed.At the start of Ani-HIV treatment, the total average HIV viral load was 3,820,000 copies/ml (May 2022), which reduced to 75 copies/ml by July 2023. Similarly, $CD4^+$ levels improved from 105 (May 2022) to 415 (July 2023); the $CD4^+/CD8^+$ ratio increased from 0.34 (May 2022) to 0.63 (July 2023). These results were indicative of disease stabilization, and the treatmentwith modifications to manage syphiliswas continued until complete HIV load neutralization and eradication of syphilis were achieved (29 June 2023).We conducted replication studies to determine how patients' viral load and immune status changed after the diagnosis of syphilis.

4. Discussion

Isoflavone-Forte, a natural concentrate of flavonoids, polyphenols, stilbenes, resveratrol, phytoalexins, and melatonin, exhibits potent biological activities, including anti-inflammatory, immunomodulatory, antioxidant, antibacterial, and anticancer properties (Fresco et al., 2006). It inhibits nuclear factor kappa B (NF-κB), and this activity could be the mechanism underlying its anti-inflammatory and anticancer effects (Karin & Ben-Neriah, 2000). Isoflavone-Forteexerts strong anti-inflammatory activity by decreasing prostaglandin and leukotriene synthesis (Rainsford, 2007).

Isoflavone-Forte's potential antiviral efficacy against HIV-1, hepatitis C virus (HCV), and HTLV-1 has also been reported (Barnhart et al., 1999). Mechanistically, its disruptiveeffects on multiple growth points in the HIV-1 lifecycle are more potent thanthe effects on the initial cleavage step (Boonyaratanakornkit et al., 2010). It suppresses HCV replication and dose-dependently decreases viral proteases at the protein level (Kanda et al., 2013). Finally, it prevents HTLV-1 tax protein binding to inhibitors of κBα, a key factor in HTLV-1 pathogenicity (Darnell & Baltimore, 1997).Isoflavone-Forte's compounds can induce cell cycle arrest and apoptosis, particularly in human lung carcinoma and ovarian cancer cells, and downregulate the oncogenic mutant p53 in ovarian cancer cells. The drug also inhibits

tumor growth in ovarian cancer xenograft models and demonstrates efficacy against hepatitis B, D, and C viruses as well as herpesviruses.

Isoflavone-Forteactivates protein kinase C and NF-κBthrough the activation of the IKB kinases- The IκB kinase (IkappaB kinase or IKK) is an enzyme complex that is involved in propagating the cellular response to inflammation, specifically the regulation of lymphocytes (Carrasco & Merida, 2004). *In vitro*, it inducedHIV expression in latently infected cell lines and primary cells, thereby antagonizing HIV latency (Richman et al., 2009). Additionally, it inhibits HIV entry into target cells by downregulating the CD4 and CXCR4 receptors (Murooka et al., 2012).

In experiments using GFP ((Enhanced green fluorescent protein (eGFP))-encoding lentiviral vectors pseudo-typed with VSV-G (Vesicular stomatitis virus G) or SARS-CoV-2 spike protein, Isoflavone-Forte demonstrated antiviral activity against HIV-1-derived lentiviral vectors. Moreover, its antiviral effect was stronger against the VSV-G pseudo-type, suggesting that itsprimary mechanism of action may be to block viral entry (Melikyan, 2008).

5. Study Implications

These findings suggest Isoflavone-Forte could be a significant player in the treatment of various health conditions, including inflammatory diseases, cancer, and viral infections such as HIV and Hepatitis C. Its multifaceted action, which includes anti-inflammatory, immunomodulatory, antioxidant, antibacterial, and anticancer properties, and its efficacy against a range of viruses, places Isoflavone-Forte in a potentially unique position within the therapeutic landscape.

6. Conclusions

While more research is needed to fully understand the implications of these findings, Isoflavone-Forte could represent a critical advancement in natural therapeutics. Its broad-spectrum activity may offer a novel, multi-target approach to disease treatment, providing the medical community with a powerful tool against inflammation, cancer, and various viral infections. However, further studies are required to confirm these results, define optimal dosing, and determine potential side effects and contraindications.

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Informed Consent Statement:

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: A. Tavartkiladze conceived and designed the experiment; A. Tavartkiladzeand G. Simoniaperformed the experiments, analyzed the data, and wrote the manuscript; A. Tavartkiladze, G.Tavartkiladze and N.Okrostsvaridze contributed to data collection and manuscript revision; A. Tavartkiladze and G.Tavartkiladze provided technical support and assisted with the experimental design.All authors contributed to manuscript revision and have readand approved the submitted version.

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