

# Preclinical and Clinical Safety and Efficacy of Faramir Treatment: A Novel Anti-Retroviral Drug

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**Abstract:** *Background:* Alternative treatment is generally used from the first reporting of HIV/AIDS. Introduction of new herbal drugs with antiviral properties may be a significant contribution towards treating HIV-positive patients. This survey was thus conducted in order to evaluate preclinical toxicology, clinical safety and efficacy of Faramir.

*Objective:* To evaluate preclinical and the clinical safety and efficacy of "Faramir"

*Methods:* In the first stage, carried out in 2011, toxicity tests were examined on three groups of six male rats administered different dosages of the drug. In the second stage, Phase II clinical trial with Faramir was conducted at the Voluntary Counseling and Testing (VCT) Center at Imam Khomeini Hospital, Tehran, Iran from February 2012, as a single arm clinical trial. After checking inclusion and exclusion criteria, seven HIV-positive patients received the treatment with Faramir tablets containing 330 mg three times a day for six months. The patients were evaluated for the treatment efficacy and the possible adverse effects. Patients were also followed for six months after the treatment duration. Laboratory tests and CD4 counts were checked each month and viral load was measured each three months. Adherence to treatment, clinical observations and adverse effects were registered each month.

*Results:* In the toxicity tests, gavages' administration up to 2 gm/kg showed no poisonous effect or mortality after 72 hours. Viral load decreased significantly after receiving the treatment duration ( $P=0.028$ ). The effect of Faramir on viral load showed a decrease of virus number in three patients as undetectable. Faramir did not show any serious adverse effects except mild skin rashes in three people (42.9%), distension in two people (28.6%), diarrhea in two people (28.6%), stomach burn in one person (14.3%), local dry skin in one person (14.3%) and itching in one person (14.3%) which were improved without discontinuation of the drug. The symptoms disappeared with necessary treatments.

*Conclusions:* In this stage, some evidence was found in support of the efficacy and safety of Faramir. Implementation of phase III clinical trial is recommended.

**Keywords:** HIV, Faramir, Viral load, CD4 count.

## INTRODUCTION

HIV/AIDS is one of the most important infectious diseases that affects health worldwide. HIV affects human immune system, rendering it too weak to defend the body against common infections such as tuberculosis. It also increases the risk of other opportunistic infections and tumors, through which human life is often threatened and lost. Three decades after the first description of AIDS, an estimated people 35 million [33.2 million– 37.2 million] live with HIV in 2013, with 2.1 million [1.9–2.4 million] new cases reported that year. In addition, 1.5 million [1.4–1.7 million] HIV-infected persons died [1-3].

There is no definite treatment or effective vaccine for HIV infection up to now. Anti-retroviral (ARV)

therapy is generally used as the most effective treating method for HIV infection. These medications can significantly reduce the morbidity and mortality of HIV infection although, some HIV strains are resistant to antiretroviral drugs. One major limitation for efficacy of antiretroviral drugs is multi-drug resistant strains [4-6]. Moreover, these drugs are expensive, with multiple adverse effects, and they also required strict adherence to the medications, especially in developing countries. It is reported that ARV medications have some serious adverse effects included hepatitis, lactic acidosis, cardiovascular side effects and drug reactions [7-9]. As a result, the cost and complications of ARV drugs encourage considering other treatments and medications. For example, it has been found that extracts of *Croton lechler* may reduce the frequency and severity of diarrhea in HIV positive patients [10]. Similarly calanolide, extracted from *Calophyllum lanigerun*, may bear antiretroviral properties [11]. Faramir is composed of a mixture of natural elements

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with probable antiretroviral properties. We conducted this study to evaluate preclinical toxicology in laboratory animals and the safety and efficacy of Faramir through phase IIA clinical trial among HIV-positive patients in Tehran.

## MATERIALS AND METHODS

### Study Design

In the first stage, preclinical toxicology was performed in Tehran University of Medical Sciences, 2011. Toxicity tests were examined on three groups of six male rats with mean weight of 160 gms, obtained from Karaj Breeding Center. These rats were placed for two weeks before the examination in a room with controlled temperature and light (a 12-hour light and dark cycle), and supplied water *ad libitum* one day before the test. All ethical points on using animals were considered and all the experiments were approved by the institutional review board. The rats were divided into three groups taking different dosages of the drug included 0.5, 1 and 2 g/kg. They were subsequently followed for 72 hours.

For the second stage of the study, carried out from February 2012 to February 2013, a phase II clinical trial was initiated at the Voluntary Counseling and Testing (VCT) center in Imam Khomeini Hospital, a referral treatment center affiliated with the Tehran University of Medical Sciences. There was only one arm and Faramir was used in all interventions. HIV infection was confirmed by two positive ELISA and one positive Western blot test. After taking written consent and checking inclusion and exclusion criteria, seven HIV positive patients received the treatment with Faramir as a 330 mg/tablet administered orally, three times a day for six months. The patients were followed for six months after the treatment duration and were visited regularly to assess their clinical, biochemical, immunological, and virological conditions.

At enrollment time, a medical history and comprehensive physical examination were obtained and registered in one structured questionnaire and a physical assessment form. Patients' clinical conditions were evaluated by carrying out a comprehensive physical examination at baseline and each month after treatment start. Adherence to treatment and adverse effects were registered each month. Effects or possible side effects were observed six months after the treatment and any adverse events were recorded. Pregnancy tests were performed at the baseline and

whenever pregnancy was suspected. Biochemical assays, including Complete Blood Count (CBC), platelets, Fasting Blood Sugar (FBS), triglyceride, cholesterol, serum electrolytes, amylase, blood urea nitrogen (BUN), serum creatinine, uric acid, serum bilirubin and liver enzymes, prothrombin time, thyroid function test and urinalysis were assessed at baseline, followed by the treatment. CD4 counts were determined by flow cytometry at the baseline and each month, viral loads were measured by a central laboratory using quantitative ultrasensitive polymerase chain reaction (PCR) at the baseline and each three months.

The PARTEC kit and whole blood was used as a sample for CD4 counting. 20  $\mu$ L of sample was mixed with 20  $\mu$ L of antibody and put it in a dark place for 15 minutes. Then, 800  $\mu$ L of buffer was added to the mixture before flow cytometry was started.

For the viral load assay, QIAGEN kit was used to extract the RNA from the plasma of HIV positive patients. The buffers used in the test were: AVL buffer contains RNA carrier and, AW1 and AW2 buffer for third and fourth passes and AVE buffer for the last pass in centrifuge.

### Patients and Criteria

The inclusion criteria were as follow: HIV infected adults aged between 18-65 years, asymptomatic HIV positive patients, patients naïve to any antiretroviral or immuno-modulatory therapy, plasma HIV RNA of  $\geq 1000$  copies/mL, CD4+ T cell count  $\geq 200$ , absolute neutrophil count  $\geq 1000/\mu$ L, platelets  $\geq 50 \times 10^3/\mu$ L, hemoglobin  $\geq 8.0$  g/dL, transaminases  $\leq 3 \times$  upper limit of normal, serum creatinine  $< 1.5$  mg/dL and written informed consent.

Patients were excluded if they had the following conditions: pregnancy or breast-feeding for female patients, active substance abuse or alcohol consumption, hepatitis B surface antigen and hepatitis C antibody, receiving therapy for an opportunistic infection, taking investigational or immune-modulatory drugs and growth hormone within 180 and 30 days respectively before entering the study, concurrent herpes simplex virus (HSV) infection, patients with CD4  $< 200$  cells/mm<sup>3</sup>, creatinine clearance less than 50 ml/min tested by Cockcroft-Gault equation, using human growth hormone 30 days before entering to the study, starting testosterone or anabolic steroids 30 days before entering to the study, chronic treatment with

immuno-suppressant drugs, cytotoxic chemotherapy, interferon treatment, or radiation therapy within the preceding three weeks and current AIDS defining indicator disease including opportunistic infections, AIDS dementia, AIDS wasting syndrome and AIDS associated malignancy.

### Ethical Considerations

Seven HIV positive patients recruited in the study and were given written informed consent prior to the enrollment. Patients were informed totally and made aware of the treatment and procedure, then asked to sign a consent form. Participation was completely voluntary and all patients were notified about the study objectives, probable side effects of Faramir and their rights as to leaving the project. Patients were notified that any probable side effects resulting from the project will be covered by the study sponsor. Confidentiality was carefully followed during this study and access to information was permitted to few authorized researchers only. The protocol for this study was developed according to the principles of the Declaration of Helsinki and was approved by the institutional review board (IRB) of Tehran University of Medical Sciences (TUMS).

### Statistical Analysis

Data was analyzed using SPSS software Version 16. The results were shown as the mean  $\pm$  SD. Non-parametric tests of Wilcoxon Signed Rank Test was also carried out to examine CD4 count and the viral load before and after the intervention. P values of  $<0.05$  were considered statistically significant.

## RESULTS

### Preclinical Toxicology

In the toxicity tests performed on the three groups of six male rats, gavages' administration up to 2 gm/kg showed no poisonous effect or mortality after 72 hours. Toxicological studies on laboratory animals revealed no adverse effects on neither allergic nor immunologic effects in the experimental doses.

### Clinical Safety and Efficacy

Five patients were male and two were female. The mean age for the patients was 30.71 years (SD=3.82) with the minimum of 24 and maximum of 35 years. Table 1 presents the demographic characteristics of the patients.

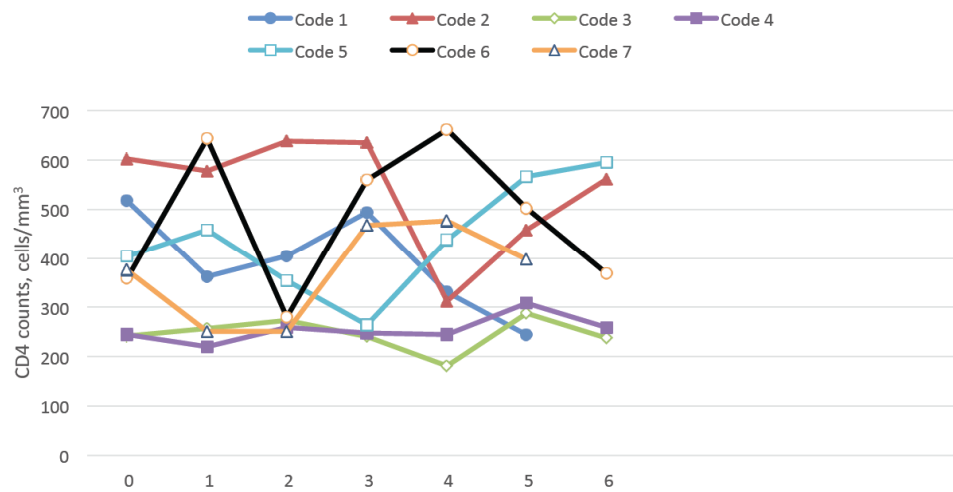
**Table 1: Demographic Characteristics of the Patients Attending in the Study**

Variable	Categories	N (%)
Gender	Male	5 (71.4)
	Female	2 (28.6)
Age (years)	24-29	2 (28.6)
	30-35	5 (71.4)
Marital Status	Married	4 (57.1)
	Single	3 (42.9)
Occupation	Employed	6 (85.7)
	Housewife	1 (14.3)
Educational Level	Junior high school	1 (14.3)
	Senior high school	2 (28.6)
	University	4 (57.1)
Route of Transmission	Injection drug use	1 (14.3)
	Sexual contact	5 (71.4)
	Unknown	1 (14.3)

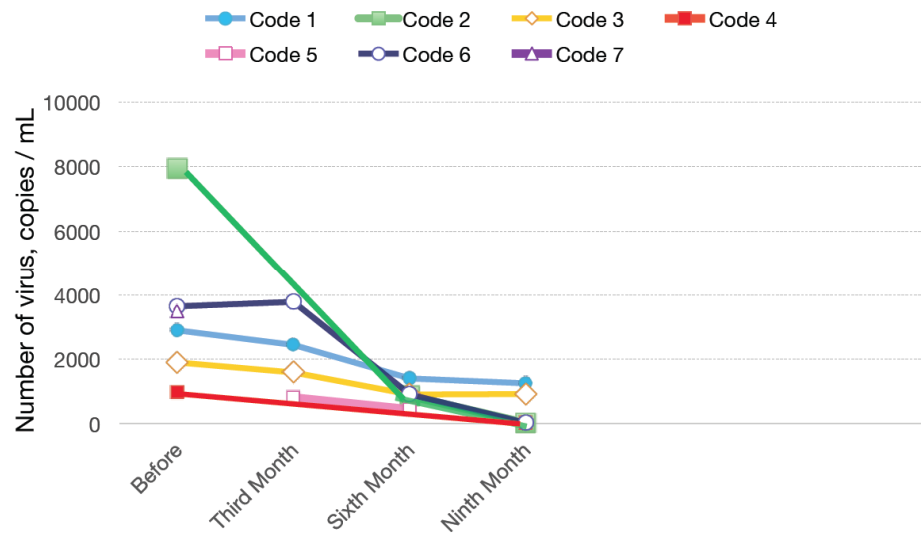
Mean CD4 count and median viral load at the baseline was 392.43/ $\mu$ l (SD=132.91) and 3195 copies/ml, respectively. The patients had near complete adherence to Faramir during the treatment time.

The mean CD4 count was increased gradually in the third and sixth months; however, compared to the baseline level, the increase was not statistically significant during six months of the treatment, and the CD4 counts showed fluctuation within six months of the therapy (Figure 1). Viral load decreased significantly after the end of follow up period ( $P=0.028$ ) compared to the baseline. The effect of Faramir on viral load showed a decrease in virus number in three patients as undetectable ( $\leq 20$  copies/mL, Figure 2).

Faramir did not show any serious adverse effects except mild skin rashes in three people (42.9%), distension in two people (28.6%), diarrhea in two people (28.6%), stomach burn in one person (14.3%), local dry skin in one person (14.3%) and itching in one person (14.3%) which were improved without discontinuation of the drug. The symptoms disappeared with necessary treatments. Additionally, no sex-based differences were identified in the adverse effects of Faramir. Most importantly, the clinical examinations of various organ systems including cardiovascular, gastrointestinal, hepatic, central nervous system (CNS), urological, psychiatric, hematological, respiratory, ophthalmic, cutaneous and musculoskeletal were normal. Furthermore, no laboratory test abnormalities were observed in this study.



**Figure 1:** CD4 count changes in seven HIV positive patients during 6 months of taking Faramir.



**Figure 2:** HIV viral load changes in seven HIV positive patients during 9 months.

## DISCUSSION

In this study the preclinical and clinical safety and efficacy of Faramir were investigated. There was no control group in this stage and the study was initiated non-randomized on a small number of HIV positive patients. This is due to the fact that it was required to confirm the safety of Faramir in the patients before examination in the control group. Furthermore, design of phase 1 clinical trial on herbal medications is not a necessary part of the procedure of making a new herbal medication. Many herbal drugs are widely used on the market, with possible and recognized side effects.

In the toxicity tests performed on the three groups of six male rats, oral administration of Faramir showed no poisonous effect or mortality in a short time window.

The literature review about herbal medicine for treatment of HIV<sup>+</sup> patients showed that some of herbal drugs had positive effects on enhancement of immune system, specifically, decreasing viral load or relieving symptoms of HIV-positive patients [12-15].

Faramir had no effect on CD4 counts during the study. It seems that this medication has no direct effect on immune system in the short term, and the CD4 counts showed fluctuation within six months of the treatment. Consequently, long-term use of this drug may increase CD4 count.

A study on IMOD<sup>TM</sup>, another natural immunomodulator that contains *Tanacetum vulgare* (tansy), *Rosa canina* and *Urtica dioica* (nettle), selenium, flavonoids and carotene, found that this drug can cause increases in CD4 levels and decrease the dissemination of HIV through the host body [12].

The viral load became undetectable in three patients. The IMOD study demonstrates that this drug had no significant effect on viral load [13].

A study in Africa assessed effectiveness of herbal drugs (without mentioning the name of the drug) on 33 HIV-positive patients, found significant reduction in viral load (85.4%,  $p = 0.0015$ ) and increase in CD4 count (226%,  $p < 0.0001$ ) after four and eight months and recommendations were made as to the use of the drug as an alternative or supplementary drug for HIV-positive patients [14].

A recent study in Ghana about the effect of combining two herbal drugs, *Betula alba* (BA) and *Sutherlandia frutescens* (SF) for HIV treatment showed increase in white blood cell and lymphocyte counts in immunosuppressed mice and decrease in viral load in HIV positive patients [15].

Another study on a compound of Chinese herbs (IGM-1) showed no positive effect, whether on viral load and CD4 count or symptoms of HIV positive patients after the treatment [16].

The effect of Faramir on viral load showed a decreased number of HIV in the patients without serious side effects. Furthermore, Faramir had minimal side effects in the experiment period, suggesting that Faramir can be classified as a non-poisonous medication. The absence of changes in the laboratory profiles revealed that Faramir did not show any adverse effects on the blood, liver, kidney or pancreas. Some patients presented mild skin rash, distension, diarrhea, stomach burn, local dry skin and itching, easily was controlled through symptomatic therapy. The study on IMOD<sup>TM</sup> also showed no adverse effect in phases I to III of clinical trials but had some side effects in 1-2 % of patients, such as nausea, headache and vertigo, gastritis, phlebitis and mild rash [12].

Alternative treatment is generally used from the first reporting of HIV/AIDS. The cost and complications of ARV drugs encourages seeking out other treatments and medications. The efficacy of alternative therapies has not been discovered unequivocally, although it is used common among HIV positive patients [12, 17, 18]. A study in China found that combination of a herbal drug, namely the Chinese herbal compound SH, and antiretroviral (ARV) drugs, increased the effect of ARV drugs in comparison with taking ARV drugs alone [19]. Antiretroviral treatment decreases the disease progression and restores a normal life expectancy.

Although antiretroviral therapy can reduce the chance of death and complications from the infectious diseases, these medications are expensive and have many adverse effects. The following are the main classes of ARV drugs used in treatment of HIV/AIDS patients: Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs) (commonly used in combination with NRTIs to inhibit multiplying of the virus), and Protease inhibitors (PIs), which suppress the virus replication at a later stage in its life cycle, preventing cells from producing new viruses. Some common side effects of these drugs include fatigue, anemia, diarrhea, nausea or vomiting, dizziness or headaches, insomnia, pain and nerve problems, skin rash, injection site reactions, dry mouth, weight loss and vivid dreams [20-22]. Furthermore, given the limitations of ARV drugs which can result in mutations in HIV, introduction of new herbal drugs such as Faramir with antiviral characteristics may be a great help towards treating HIV positive people. The results of this study show a new effective treatment for HIV positive patients with few adverse effects.

In conclusion, Faramir as a natural drug with minimal side effects, and the antiretroviral property can be a promising agent to treat patients with HIV/AIDS. In the phase II clinical trial, we found some positive evidence regarding the therapeutic effect and safety of Faramir. Implementation of phase III clinical trial is recommended.

## LIST OF ABBREVIATIONS

HIV	= human immunodeficiency virus
AIDS	= acquired immunodeficiency syndrome
ARV	= anti retro-viral
VCT	= voluntary counseling and testing
ELISA	= enzyme-linked immunosorbent assay
Mg	= milligram
Kg	= kilogram
CBC	= Complete blood count
FBS	= Fasting blood sugar
BUN	= blood urea nitrogen
PCR	= polymerase chain reaction

RNA = ribonucleic acid

HSV = Herpes simplex virus

IRB = institutional review board

TUMS = Tehran University of Medical Sciences

SPSS = Statistical Package for the Social Sciences

SD = standard deviation

CNS = central nervous system

NRTI = nucleoside reverse transcriptase inhibitors

NNRTI = Non-nucleoside reverse transcriptase inhibitors

PI = Protease inhibitors

### CLINICAL TRIAL REGISTRATION STATEMENT

This study is registered under the Iranian Registry of Clinical Trials (IRCT) available at <http://www.irct.ir/>. The registration identification number is IRC201112124076N6.

### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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