Safety and immunostimulatory activity of the dietary supplement Houttuynia cordata Thunb. fermentation product in healthy volunteers

Khanutsanan Woranam
Khon Kaen University
Jeerati Prompipak
Khon Kaen University
Piroon Mootsikapun
Khon Kaen University
Gulsiri Senawong
Khon Kaen University
Limthong Promdee
Khon Kaen University
Ketsarin Pintaraks
Khon Kaen University
Thanaset Senawong (sthanaset@kku.ac.th)
Khon Kaen University

Research Article

Keywords: Immunomodulation, Immunostimulant, H. cordata, Dietary supplement, HCFP, Fermented herb

Posted Date: March 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-238109/v1

Safety and immunostimulatory activity of the dietary supplement *Houttuynia cordata* Thunb. fermentation product in healthy volunteers

Khanutsanan Woranam[a], Jeerati Prompipak[a], Piroon Mootsikapun[b], Gulsiri Senawong[a], Limthong Promdee[c], Ketsarin Pintaraks[d], Thanaset Senawong[a,e]*

[a]Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand

[b]Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[c]Department of Clinical Chemistry, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand

[d]Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

[e]Natural Product Research Unit, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand

*Corresponding author

E-mail address: sthanaset@kku.ac.th (T. Senawong)

Abstract

Houttuynia cordata Thunb. fermentation product (HCFP) is widely used in Thailand as a dietary supplement for immune support with no experimental verification. The aim of this study was to investigate the safety and immunomodulation of HCFP in healthy adult volunteers. The effect of HCFP on antiretroviral drugs (TDF and EFV) was also evaluated in Sprague Dawley rats. The basic characteristics and blood chemistry of ten healthy volunteers did not show any significant differences between before and after 4 weeks of intervention with a daily intake of HCFP, and no major adverse event was observed. However, the LDL-c level was significantly decreased after 4 weeks of intervention. Immunomodulation assay revealed that the percentage of neutrophil was significantly increased after 8 weeks of intervention in 30 healthy volunteers. Meanwhile, the mean of $CD4^+$ and $CD8^+$ T-cell ratio was not significantly increased after 8 weeks of intervention. In addition, the plasma concentrations of EFV and TDF in Sprague Dawley rats showed no significant difference between single drug group and combination with HCFP group, suggesting that HCFP has no effect on the plasma level of antiretroviral drugs.

Keywords: Immunomodulation, Immunostimulant, *H. cordata*, Dietary supplement, HCFP, Fermented herb

Introduction

The acquired immunodeficiency syndrome (AIDS) is caused by Human immunodeficiency virus (HIV). In 2019, people living with HIV/AIDS were approximately 38 million worldwide and 690,000 people have died of HIV/AIDS[1]. Moreover, around 1.7 million of newly HIV-infected individuals were reported. Accordingly, HIV is one of the world's most serious public health challenges. HIV infection causes a progressive failure of the immune system due to a decrease of $CD4^+$ T cells rendering life-threatening opportunistic infections and malignancies. The highly active antiretroviral therapy (HAART), which consists of a cocktail of nucleoside analog/non-nucleoside

reverse-transcriptase inhibitors, has decreased the morbidity and mortality associated with HIV/AIDS[2]. However, there is still a critical need for alternative or combination with HAART strategies to treat HIV infection. Nowadays, medicinal herbs have been widely used for treatment of various diseases as they are potentially important sources of bioactive substances. HIV infection affects most of the immune system and disrupts its homeostasis. Many HIV/AIDS patients have a depletion of $CD4^+$ T-cells, leading to an immunocompromised condition in patients. Therefore, the search for natural product that can activate the immune system in combination with antiretroviral therapy, would be beneficial for HIV/AIDS patients.

Houttuynia cordata Thunb. (HCT) is a perennial herbaceous plant that grows in the humid area and shady mostly distributed in East Asia. HCT is known in Thai as Plu-khao which means fishy-smelling vegetables. HCT is grown for local vegetable consumption in the North and Northeast of Thailand. Traditionally, HCT is used for various beneficial properties against inflammation, pneumonia, severe acute respiratory syndrome, muscular sprain, stomach ulcer[3]. The major constituent of HCT includes polyphenols, essential oil, organic acids, and alkaloids[4]. In Asian countries, HCT has been used as a medicinal plant possessing many biological activities including anticancer[5], antioxidant[6], antiinflammatory[7,8], antiviral[9,10], antibacterial[11] and immunostimulant activities[12,13]. In addition, HCT has been treated herpes simplex virus type 1 (HSV-1)[14,15], influenza virus, and human immunodeficiency virus type 1 (HIV-1) without showing cytotoxicity to the host[9]. HCT also exhibited significant inhibitory activity on the severe acute respiratory syndrome (SARS)[12]. Nowadays, the aerial part of HCT was fermented with probiotic bacteria to yield an *H. cordata* fermentation product (HCFP). Fermentation is a metabolic process that produces chemical changes in organic substrates which improve the nutraceutical value of a product by breaking down certain undesirable compounds and inducing effective microbial conversion[16]. Biologically fermented plant products appear as a clear brown liquid with a sour taste due to the fermentation of plants, vegetables, or fruits with sugar in a closed environment with probiotic bacteria. Probiotics are

76 microorganisms exerting health-promoting functions in humans and animals[17], improving the
77 nutraceutical value of the herbal plant products by breaking down undesirable phytochemicals, and
78 producing certain desirable compounds. Recently, there has been an increase in the marketing and
79 sales of dietary supplements, energy drinks, and other consumer products that may contain relatively
80 high concentrations of essential elements for the health of consumers. The HCFPs are commercially
81 available as dietary supplement and widely used as supplements for immune support. According to
82 consumer feedback data collected by the Prolac (Thailand) Co., Ltd., Lamphun Province, Thailand,
83 the HIV-infected consumers have shown that their $CD4^+$ T-cell count was increased, and their health
84 was improved, suggesting that HCFP seems to possess an immunostimulatory activity. However,
85 experimental study in clinical research volunteers regarding the safety and immunostimulation of
86 these products has not been established yet.
87 Immunostimulants, known as immunostimulators, are substances that activate several elements of
88 the immune system of humans and animals such as stimulation of B lymphocytes, $CD4^+$ and $CD8^+$ T
89 cells[18]. Immunostimulants are inherently nonspecific and enhance the body's defense mechanisms
90 against infection. They can act through innate as well as adaptive immune responses in the body and
91 serve as prophylactic and promoter agents. HCT has been reported to possess the immunostimulatory
92 activity that stimulates the proliferation of mouse splenic lymphocytes[12]. The administration of
93 *Plantago asiatica* and *H. cordata* to the cobia (*Rachycentron canadum*) showed improving non-
94 specific immune response by phagocytosis and lysozyme activity against pathogenic infection[19]. In
95 addition, the continuous ingestion of a probiotic fermented four-herb combination (PFH) including
96 HCT has been found to increase lysozyme activity in the serum and spleen, increase peripheral blood
97 mononuclear cell (PBMC) proliferation, increase the $CD4^+$: $CD8^+$ T-lymphocyte ratio in the spleen
98 and antibody production levels in broiler chicks[20].
99 Recently, we have studied the immunomodulation potential of HCFP both *in vitro* and *in vivo*. We
100 found that the commercially available HCFP possessed immunomodulatory activity in cells involved

in both innate and adaptive immune responses. Moreover, the immunomodulatory activity of the HCFP in cyclophosphamide-induced immunosuppressed rats was observed through the increased number of neutrophils, B cell activation and antibody production (Manuscript submitted for publication). However, the effectiveness of HCFP regarding its safety and immunostimulation in healthy adults has not been evaluated. Thus, in this study, the safety and immunostimulatory activity of HCFP were investigated in healthy adult volunteers.

In Thailand, HCFPs from several sources have been widely used in people living with HIV/AIDS. However, HIV-infected subjects have always been treated with antiretroviral drugs. The effect of HCFP on concentrations of plasma antiretroviral drugs in HIV-infected individuals has not been investigated with ethical approval both in animal and in human. The major concern about the use of HCFP in combination with antiretroviral drugs is that there may be an herb-drug interaction causing an ineffective HIV treatment. Therefore, in this study, the effect of HCFP on antiretroviral drug concentration was firstly investigated in the rat model (Sprague Dawley rat).

Results

Effect of HCFP on safety in healthy volunteers. Ten healthy volunteers certified as healthy without any major clinical abnormalities, were recruited and given informed consent for the safety evaluation (4 males and 6 females) with the mean age of 24.6 ± 0.5 years. All subjects have completed all research-related interventions in 4 weeks in which each subject received 15 mL of HCFP orally twice a day (the highest recommended dose written on the label of the product), with good compliance. The enrolled participants showed normal or healthy clinical characteristics after the intervention (Table 1). In addition, hematological and biochemical parameters including red blood cells (RBCs), platelets (PLTs), total and differential leukocyte count, liver function, and kidney function showed no significant difference compared with the before values (Table 2). After intervention, lipid profile including triglyceride and HDL showed no significant difference compared

with the before intervention values, however, total cholesterol mean value had decreased from 213.20 ± 8.95 to 203.10 ± 11.04 mg/dL ($p = 0.065$). Meanwhile, LDL mean value had significantly decreased from 133.10 ± 9.40 to 120.30 ± 11.20 ($p < 0.05$) after intervention for 4 weeks. In summary, there were no major adverse events observed during the trial, indicating that the HCFP was safe.

Effect of HCFP on immunostimulatory evaluation in healthy volunteers. After the safety results (4-week intervention) were reported to Khon Kaen University Ethics Committee in human research, the immunostimulatory evaluation was approved for the study in healthy volunteers. Thirty healthy volunteers with mean age of 33.01 ± 1.80 years were enrolled in the immunostimulatory evaluation of HCFP. The Before-After study of HCFP oral intervention was conducted. The characteristics of all participants at baseline and after 8 weeks were shown in Table 1. There were no significant changes in the basic characteristics including body weight, body mass index (BMI), blood pressure, and heart rate, representing a normal or healthy body. In addition, hematological and biochemical parameters of the before (0 week) and after treatments (8 weeks) were shown in Table 3. The kidney function parameters including blood urea nitrogen (BUN) and creatinine were still within normal range. However, the mean of creatinine value was slightly increased from 0.89 ± 0.03 mg/dL to 0.94 ± 0.03 mg/dL after intervention for 8 weeks. Lipid profiles including total cholesterol, triglyceride, HDL, and LDL showed no significant differences between before and after interventions. White blood cell count mean value was increased after the intervention (6,370.00 ± 1,700.00 cells/mm^3 to 6,610.00 ± 1,550.00 cells/mm^3) but not significantly different ($p = 0.131$). In contrast, the percentage of neutrophil was significantly increased from 53.63 ± 1.38 % to 56.61 ± 1.45 % ($p < 0.05$), however, the number was still within the normal range.

The effect of HCFP on the $CD4^+$ and $CD8^+$ T cell counts was investigated. The results showed that the means of $CD4^+$ and $CD8^+$ T cell counts were decreased from 706.83 ± 35.50 to 697.53 ± 24

cells/mm^3 and 614.33 ± 31.09 to 590.90 ± 35.75 cells/mm^3, respectively, but not significantly different. Whereas the ratio of $CD4^+$ and $CD8^+$ T cell counts was increased from 1.24 ± 0.49 to 1.29 ± 0.56 with no significant difference (Table 3). In addition, the data between males and females were analyzed comparatively. The mean of female WBC counts was significantly increased from 5,840.50 ± 1,580.21 to 6,319.00 ± 1,679.26 cell/mm^3 ($p < 0.05$) (Fig. 2). Moreover, the volunteers were divided into 3 groups by age range including 21-30 (n = 17), 31-40 (n = 3), and 41-50 (n = 10) years old. The group of 21-30 years exhibited a significant increase ($p < 0.05$) in the percentage of neutrophils from 51.94 ± 7.56 to 56.35 ± 8.52 cells/mm^3 and the increase of WBC count from 6,480.00 ± 1,943.00 to 6,839.00 ± 1,768.00 cells/mm^3 with no significant difference ($p = 0.089$). The group of 31-40 years showed no significant changes of $CD4^+$ and $CD8^+$ T cells, WBC counts and their types. Nonetheless, the group of 41-50 years showed the increase of $CD4^+$ and $CD8^+$ ratio from 1.43 ± 0.72 to 1.54 ± 0.83 ($p = 0.051$), and no significant change in WBC count (Table 4).

Effect of HCFP on the levels of Efavirenz and Tenofovir disoproxil fumarate in rat plasma. After administration with or without oral co-administration of HCFP (3.08 ml/kg), the plasma concentration-time curve was evaluated. As shown in Fig. 3, the HCFP co-administration resulted in increased plasma maximum drug concentrations (C_{max}) of both EFV and TDF but with no statistically significant differences. The C_{max} values of plasma EFV and TDF in EFV alone group were 1.72 ± 0.22 and 3.30 ± 0.70 µg/mL, respectively, whereas the C_{max} values of plasma EFV and TDF in EFV combination with HCFP group was 1.93 ± 0.10 and 3.72 ± 0.67 µg/mL, respectively (Table 5). The C_{max} values of both EFV and TDF alone and combination with HCFP appeared at 2 h (T_{max}) and then the concentrations of EFV and TDF in plasma had decreased to 0.65 µg/mL (EFV alone group) and 0.64 µg/mL (EFV combination with HCFP group), and to 1.43 µg/mL (TDF alone group) and 1.83 µg/mL (TDF combination with HCFP group), respectively, at 24 h. Area under the plasma concentration–time curve (AUC) showed little or no change in the HCFP pretreated group

compared with the group that received only antiretroviral drug (Table 5). The plasma concentrations between the drug alone group and co-administration with HCFP group were not significantly different. These results indicated that the oral administration of HCFP may not affect the antiretroviral drugs (EFV and TDF) in plasma.

Discussion

Fermented fruit and plant beverages were used to improve health as well as for an alternative therapy because they had antiinflammatory, anticancer and antioxidant activities. For example, the therapeutic role of fermentation product of five Thai indigenous plants including *Phyllanthus emblica*, *Morinda citrifolia*, *Houttuynia cordata*, *Terminalia chebula* and *Kaempferia parviflora* was shown to be attributed to the improvement of diabetic oxidative stress[21]. The administration of fermentation products reduced the physiological changes associated with diabetes, implying that fermentation product may normalize energy utilization and metabolism. This study is the first report to evaluate *H. cordata* fermentation product (HCFP) in healthy adults regarding its immunostimulatory activity. Safety evaluation of HCFP in 10 healthy volunteers demonstrated that HCFP was safe during the intervention. There were no clinical incidents indicating major adverse events. The biochemical parameters between before and after 4 weeks of HCFP intervention were not significantly different (Table 2). Whereas the mean LDL-c level showed a significant decrease from 133.10 ± 29.72 to 120.30 ± 35.42 mg/dL ($p < 0.05$) after 4 weeks of intervention and the mean total cholesterol level was also decreased but not significantly different ($p = 0.066$). Relevant to this finding, it has been demonstrated that *H. cordata* plant extract (HTE) (1%) was shown to reduce the serum levels of TG, cholesterol and LDL in rats fed with a high-fat diet (HFD) but did not affect the HDL cholesterol level[22]. In addition, ethyl acetate extract of *H. cordata* (HC-EA extract) also showed significantly reduced TG, TC, and LDC-c in high-fat diet (HFD) fed rat by downregulation of lipid accumulation in the plasma[23,24]. Thus, the effect of HCFP on the reduction of lipid levels was

interesting finding although this study did not restrict the food consumption for all volunteers. In the future, it would be of interest to investigate the effect of HCFP on lipid levels in healthy adults with the control of food consumption.

The immunostimulatory activity of HCFP was studied in healthy volunteers. Thirty healthy adults who fulfilled the criteria with a mean aged of 33.03 ± 9.84 years old were enrolled in the study. The immunostimulatory activity of HCFP in enhancing the immune cells was investigated. WBC, also called leukocytes, are the cells of the immune system involved in protecting the body against both infectious disease and foreign invaders. Also, neutrophil is a professional phagocytic cell of the innate immune system that acts as the first line of defense against invading pathogens, principally bacteria and fungi but also viruses. In this study, after 8-week intervention, the WBC mean values had increased from 6,370.00 ± 1,700.00 to 6,610.00 ± 1,550.00 cells/mm^3 ($p = 0.131$), and neutrophil had also increased significantly from 53.60 ± 7.54 to 56.60 ± 7.94 ($p = 0.026$). In this regard, we also found that the HCFP caused an increase in the number of neutrophils in Wistar rats (Manuscript submitted for publication). T lymphocytes (T cells) consist of effector T cells which secrete immune regulatory factors, such as cytokines and mediate a cellular immune response upon interaction with antigen-presenting cells (APCs). T lymphocytes are divided into three sub-groups of cells namely helper T (Th), cytotoxic T (Tc), and regulatory T (Treg) cells. Tc cells express a surface receptor, cluster of differentiation (CD) 8^+, and recognize endogenous antigens associated with class I major histocompatibility complex (MHC). Tc cells can kill cancer cells and cells infected with viruses. Whereas Th cells display a surface marker, $CD4^+$, and recognize exog, indicating that HC stimulated T cell proliferation. In this study, the effect of HCFP on the plasma levels of $CD4^+$ and $CD8^+$ T cells in healthy adults was investigated for the first time in ethical research. The mean values of $CD4^+$ and $CD8^+$ T cells after 8-week intervention were slightly decreased from 706.83 ± 194.43 to 697.53 ± 237.54 cells/mm^3 and from 614.33 ± 170.26 to 590.90 ± 195.79 cells/mm^3, respectively, but still within a normal range (Table 3). However, the $CD4^+$:$CD8^+$ ratio was increased with no statistical

226 significance from 1.24 ± 0.49 to 1.29 ± 0.56 cells/mm^3 (p = 0.182). Although, the mean of CD4$^+$ T
227 cells and CD8$^+$ T cells were decreased after the 8-week intervention, some healthy volunteers (31-50
228 years old) exhibited the increased number of CD4$^+$ T cells after 8 weeks of HCFP intervention
229 (Table 4).

230 It has been reported the difference of immune responses between male and female in various
231 diseases. Particularly, males have higher incidence of coronary heart disease and abdominal aortic
232 aneurysms, whereas women have higher rates of several autoimmune disorders including systemic
233 lupus erythematosus, multiple sclerosis, and rheumatoid arthritis[25]. Accordingly, we analyzed the
234 data on different gender and age groups. The mean of female WBC showed significantly increased
235 numbers of WBC from 5,840.5 ± 1,580.21 to 6,319 ± 1,679.26 cell/mm^3 ($p < 0.05$) and the
236 percentage of neutrophil was also increased from 54.70 ± 6.95% to 57.57 ± 8.17% (Fig. 2). In
237 addition, the CD4$^+$: CD8$^+$ ratio was increased from 1.43 ± 0.72 to 1.54 ± 0.83 (p = 0.051) in 41-50
238 years group, whereas the ratio in other age groups changed a bit (Table 4). In general, the
239 effectiveness of the immune system in older people will decline with the increasing of age.
240 Therefore, we hypothesized that HCFP may be more effective as the immunostimulant in a group of
241 immunocompromised subjects than a group of healthy subjects.

242 The first guideline of antiretroviral drugs combination for HIV-infected treatment at Srinagarind
243 hospital (Khon Kaen, Thailand) is tenofovir disoproxil fumarate, emtricitabine, and efavirenz
244 (TDF/FTC/EFV). To evaluate the potential of herb-drug interaction between the HCFP and
245 antiretroviral drugs, plasma drug concentrations in Sprague Dawley rat were determined before and
246 after intervention. HPLC results demonstrated that the antiretroviral drug concentrations in rat
247 plasma were not significantly different between the rats received EFV/TDF alone and the rats
248 received both EFV/TDF and HCFP (Fig. 3). The AUC, C_{max}, and T_{max} are important pharmacokinetic
249 parameters used to assess the degree of absorption and bioavailability of drugs[26]. The non-significant
250 changes seen with these parameters in the HCFP pretreated group compared to the groups that

received only EFV or TDF were indicative of little or no effect on the bioavailability of the drugs by the HCFP (Table 5). These observations might be related to the properties of these drugs and the enzymes involved in their metabolism. According to our results, HCFP may be used as a dietary supplement in HIV-infected individuals with no effect on antiretroviral drugs (TDF and EFV). However, study on the effect of HCFP in HIV-infected individuals is required and still remains to be investigated.

In conclusion, our results indicated that the *H. cordata* fermentation product (HCFP) was safe in healthy adults during the time of intervention and no major adverse event was observed. HCFP exhibited immunostimulatory activity by enhancing the innate immunity (increased number of neutrophils) and adaptive immunity (increased $CD4^+$: $CD8^+$ ratio in older people). Moreover, the HCFP showed no effect on the level of antiretroviral drugs (TDF and EFV) in plasma of Sprague Dawley rats. These results suggested that an industrial HCFP may be considered as an immune enhancer dietary supplement. The health benefits of an industrial HCFP should be further in-depth studied in clinical trial especially in the immunocompromised individuals.

Materials and Methods

The dietary supplement *Houttuynia cordata* Thunb. fermentation product (HCFP). The dietary supplement *H. cordata* fermentation product (HCFP) used in this study was obtained from Prolac (Thailand) Co., Ltd., in Lamphun Province, Thailand. The HCFP solution (330 mL/bottle) contains 99.3% of *Houttuynia cordata* extract and 0.7% of sugarcane powder, with no artificial and preservative. Serving suggestion on the label is as follows: 5-15 ml twice a day before breakfast and before bedtime. *H. cordata* was cultivated b the Prolac (Thailand) Co., Ltd. in an organic farm in Chai Badan district, Lopburi province, Thailand. The fermentation product (lot no. 14/5/2016) was used throughout the study. The standard drugs, Efavirenz and Tenofovir disoproxil fumarate, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Subjects and study design. Healthy adult volunteers were recruited through poster advertising. Healthy volunteers of either male or female gender, who willing to participate in the study were enrolled. Written informed consent was obtained from all participants. All experiments were performed in accordance with the guidelines of the Helsinki Declaration and Good Clinical Practice. The study protocol was reviewed and approved by the Khon Kaen University Ethics Committee in Human Research (4.6.01:25/2017, date of registration 02/08/2017). The volunteers were divided into two groups; ten healthy volunteers were enrolled for safety evaluation and thirty healthy volunteers were enrolled for immunostimulatory study (Fig. 1). The study was a prospective, open-label, single-dose, before-and-after design, in which subjects served as their own controls. Safety and immunostimulatory evaluations of the selected HCFP in healthy volunteers were conducted according to previous studies[27,28].

The inclusion criteria were healthy volunteers with age ranged 18-50 years and willing to give voluntary written informed consent to participate in this study. Each volunteer was proven to be healthy through clinical examination by the physician along with medical history, hematological, and biochemical considerations. None of the volunteers were taking dietary supplements or medication before or during the study. The exclusion criteria of the study included the participants who had a history of allergies to herbs and taking other dietary supplements. All volunteers could withdraw at anytime during the course of the study.

Safety evaluation of HCFP in healthy volunteers. Although, HCFPs have been commercially available for many years and widely used in people with health conditions, the safety of HCFPs has not been previously evaluated with ethical approval in human. Thus, in this study, the safety evaluation of the selected HCFP was conducted in healthy volunteers. Ten healthy volunteers were included in the intervention and received 15 mL of HCFP orally twice a day for 4 weeks. Blood

samples were collected from volunteers before (0 week) and after (4 weeks) intervention. The liver function, kidney function, lipid profile and complete blood count (CBC) were determined for safety evaluation of the HCFP. In addition, the basic characteristics including age, hight, weight, BMI, blood pressure, and heart rate were recorded and considered by the physician.

Evaluation of immunostimulatory activity of HCFP in healthy volunteers. This study was performed after the safety evaluation (n =10, 4 weeks) results were reported to the Ethics Committee. We investigated the immunostimulatory activity of the selected HCFP by monitoring its effect on the levels of $CD4^+$ and $CD8^+$ T-cells. Thirty healthy volunteers who fulfilled the criteria were included in the study. The volunteers received 15 mL of HCFP twice a day for 8 weeks. Blood samples were collected from the volunteers before (0 week) and after (8 weeks) intervention as described previously[29]. During the follow-up period of volunteers, the safety of HCFP in healthy volunteers were also determined. The liver function, kidney function, lipid profile and complete blood count (CBC) were further determined for safety evaluation (n = 30, 8 weeks) of HCFP.

Blood sampling, biochemical and clinical analysis. Venous blood was used to determine health-related biochemical markers. Blood samples were collected from fasting volunteers (minimum of 12 h). Serum was obtained from blood samples using serum separating tubes. Serum from blood samples was used for the analyses of liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase), kidney function (blood urea nitrogen and creatinine), and lipid profile (total cholesterol, triglyceride, HDL, and LDL). Plasma was obtained from blood samples using an EDTA tube and used to determine complete blood count (CBC), $CD4^+$, and $CD8^+$ T-cells. The complete blood count and blood chemistry were assessed by an automated hematological analyzer (Sysmex Xs-8 0 0 i and UniCel DxC 800, respectively) serviced by the laboratory of Community Medical Laboratory, Faculty of Associated Medical Sciences, Khon Kaen

University, Thailand. Whereas the $CD4^+$ and $CD8^+$ T-cells were measured by Flow cytometry serviced by the laboratory of the Department of Microbiology, Faculty of Medicine, Khon Kaen University, Thailand.

Animal experiments (*in vivo* drug interaction). Animal studies were approved by ethics commitee (Institutional Animal Care and Use Committee at Khon Kaen University, Khon Kaen, Thailand) (IACUC-KKU-55/61; date of registration 20/09/2018) and were performed according to guidelines established by the Ethical Principles and Guidelines for the Use of Animals for scientific purposes, National Research Council of Thailand. The study was carried out in compliance with the ARRIVE guidelines. The rats were obtained from Nomura Siam International co., Ltd., Bangkok, Thailand, and they were recovered from transportation for 1 week before the study. The rats were housed (three rats per cage) and maintained in standard environmental conditions (23 ± 2ºC; the moisture of 30-60% ; a light-dark cycle of 12: 12 h) with free access to sterilized commercial food and water. They were fasted for 12 h before the study, with water freely available.

The first guideline of antiretroviral drug combination used for HIV-infected treatment at Srinagarind hospital (Khon Kaen, Thailand) is the combination of Tenofovir disoproxil fumarate (TDF), Emtricitabine (2',3'-dideoxy-5-fluoro-3'-thiacytidine; FTC) and Efavirenz (EFV). In this study, TDF (nucleoside reverse transcriptase inhibitors, NRTIs) and EFV (nonnucleoside reverse transcriptase inhibitors, NNRTIs) were selected to study the effect of HCFP on the plasma concentrations of antiretroviral drugs. Adult male Sprague Dawley rats (257. 63 ± 27. 51 g body weight) were divided into the following five groups (n = 6) including control group (1); EFV groups (2 and 3) ; TDF groups (4 and 5) . The rats were pretreated as the following: groups 1, 2 and 4 received vehicle control (3.08 mL double distilled water); groups 3 and 5 received HCFP (3.08 mL), for 1 hour. Thereafter, EFV groups (2, 3) received EFV at 25 mg/ kg, while TDF groups (4, 5) received TDF at 22 mg/kg, by oral administration as previously described[30,31]. Blood samples were

collected eight different time-points from the tail vein at 0, 0. 5, 1, 2, 4, 6, 8 and 24 h after administration, and loaded into new clean tubes containing EDTA as previously described[32].

Plasma drug concentration measurement by HPLC. Blood samples were immediately centrifuged at 1,740 x g for 10 min to obtain plasma samples which were then stored at -20 ºC for analysis. Rat plasma (100 µl)/internal standard was mixed with ethyl acetate and centrifuged at 10,000 x g for 15 min at 4 ºC as previously described[30]. The supernatant was filtered and injected into HPLC column. The analysis was performed using a Shimadzu liquid chromatography instrument, equipped with an autosampler system and a UV detector SPD-M20A with a C18 column (3.9 mm i.d. x 150 mm, 5 µm particle size). The columns and instruments were provided by Facilities Service Center, Faculty of Science, Khon Kaen University, Thailand. The linear gradient of solvents A (phosphate buffer) and B (acetonitrile) was as follows: 0 min, 95% A: 5% B; 5 min, 20% A: 80% B; 6 min, 20% A: 80% B; 6.1 min, 0% A: 100% B; 17 min, 95% A: 5% B; 20 min and 95% A: 5% B, which was described previously[33,34].

Statistical analysis. Data were expressed as mean ± SD. Analysis in the same group of healthy volunteers between before and after the intervention was analyzed using the student's t-test, paired t-test, and Wilcoxon signed-rank test. One-way ANOVA was used for animal experimental analysis. GraphPad Prism version 6.0 was used, and differences were considered significant at $p < 0.05$.

Ethics statement. The study protocol was reviewed and approved by the Khon Kaen University Ethics Committee in human research on 02/08/2017 (4.6.01:25/2017). This clinical study was registered with Thai Clinical Trials Registry (TCTR) on 09/03/2021 (TCTR20210309004). All experiments were performed in accordance with the guidelines of the Helsinki Declaration and Good Clinical Practice. Animal study was registered and performed in obligation with the Institutional

Animal Care and Use Committee of Khon Kaen University, Khon Kaen, Thailand (IACUC-KKU-55/ 61; date of registration 20/09/2018). All animal experiments were performed according to guidelines established by the Ethical Principles and Guidelines for the Use of Animals for scientific purposes, National Research Council of Thailand.

Data availability

The datasets generated and/or analysed during the study are available from the corresponding author on reasonable request.

Author Contributions Statement

K.W. performed most of the experiments and wrote original draft of the manuscript. J.P. performed animal experiments. P.M. participated in the design of the study and helped analyze the experiments and data. G.S. helped design the study, edited the manuscript and provided reagents. L.P. and K.P. participated in the design of the study and provided reagents. T.S. contributed to the conception and design of this study, edited and revised the manuscript. All the authors read and approved this version of the final manuscript.

Declaration of Competing Interest

The authors declare that they have no relation with the Prolac (Thailand) Co., Ltd., who is a co-founder with Thailand Research Fund (TRF) on the Research and Researcher for Industry (RRi) project. The company provided 60,000 baht in cash and the HCFP samples (product Lot no. 14/5/2016), while the TRF provided a Ph.D. scholarship and major funding source for the project. This does not alter our adherence to the journal policies on sharing data and materials.

Funding

This work was supported by the Research and Researcher for Industry (RRi) project, Thailand Research Fund (TRF), which was co-operated with the Prolac (Thailand) Co., Ltd., Lamphun Province, Thailand [PHD59I0009]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. However, the funders have been informed and agreed on publishing a manuscript, sharing data and materials.

Acknowledgment

This study was supported by the Research and Researcher for Industry (RRi) project, Thailand Research Fund (TRF), which was cooperated with the Prolac (Thailand) Co., Ltd., Lamphun Province, Thailand.

References

1. World Health Organization. World Health Statistics Overview 2019: Monitoring Health for the SDGs, Sustainable Development Goals (WHO/DAD/2019.1), Geneva (2019). Licence: CC BY-NC-SA 3.0 IGO https://apps.who.int/iris/bitstream/handle/10665/311696/WHO-DAD-2019.1-eng.pdf?ua=1 (accessed October 15, 2020)
2. Thompson, M.A. *et al.* Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA panel. *JAMA.* **308**, 387-402 (2012).
3. Shingnaisui, K., Dey, T., Manna, P. & Kalita J. Therapeutic potentials of *Houttuynia cordata* Thunb. against inflammation and oxidative stress: A review. *J. Ethnopharmacol.* **220**, 35-43 (2018).
4. Ahn, J., Chae, H.S., Chin, Y.W. & Kim, J. Alkaloids from aerial parts of *Houttuynia cordata* and their anti-inflammatory activity. *Bioorg. Med. Chem. Lett.* **27**, 2807-2811 (2017).

425 5. Chen, Y. F. *et al*. *Houttuynia cordata* Thunb extract modulates G0/ G1 arrest and Fas/ CD95-
426 mediated death receptor apoptotic cell death in human lung cancer A549 cells. *J. Biomed. Sci.* **20**,
427 18 (2013).
428 6. Chen, Y.Y., Liu, J.F., Chen, C.M., Chao, P.Y. & Chang, T.J. A study of the antioxidative and
429 antimutagenic effects of *Houttuynia cordata* Thunb. using an oxidized frying oil-fed model. *J.*
430 *Nutr. Sci. Vitaminol.* **49**, 327-333 (2003).
431 7. Ma, Q. *et al.* Bioactive alkaloids from the aerial parts of *Houttuynia cordata*. *J. Ethnopharmacol*.
432 **4**, 166-172 (2017).
433 8. Woranam, K. *et al.* Anti-inflammatory activity of the dietary supplement *Houttuynia cordata*
434 Thunb. in RAW264. 7 cells and Wistar rats. *PLoS ONE*, **15**, e0230645.
435 https://doi.org/10.1371/journal.pone.0230645 (2020).
436 9. Hayashi, K., Kamiya, M. & Hayashi, T. Virucidal effects of the steam distillate from *Houttuynia*
437 *cordata* and its components on HSV-1, influenza virus, and HIV. *Planta Med*. **61**, 237-41 (1995).
438 10. Chiow, K.H., Phoon, M.C., Putti, T., Tan, B.K. & Chow, V.T. Evaluation of antiviral activities of
439 *Houttuynia cordata* Thunb. extract, quercetin, quercetrin and cinanserin on murine coronavirus
440 and dengue virus infection. *Asian Pac. J. Trop. Med.* **9**, 1-7 (2016).
441 11. Verma, R. S. *et al.* Chemical composition and allelopathic, antibacterial, antifungal, and
442 antiacetylcholinesterase activity of Fish-mint (*Houttuynia cordata* Thunb.) from India. *Chem.*
443 *Biodivers,* **14**, e1700189. https://doi.org/ 10.1002/cbdv.201700189 (2017).
444 12. Lau, K. M. *et al.* Immunomodulatory and anti-SARS activities of *Houttuynia cordata*. *J.*
445 *Ethnopharmacol.* **118**, 79-85 (2008).
446 13. Li, J. & Zhao, F. Anti-inflammatory functions of *Houttuynia cordata* Thunb. and its compounds:
447 A perspective on its potential role in rheumatoid arthritis (Review). *Exp. Ther. Med*. **10**, 3-6
448 (2015).

14. Chen, S.D. *et al.* Houttuynoids A–E, Anti-Herpes simplex Virus active flavonoids with novel skeletons from *Houttuynia cordata. Org. Lett.* **14**, 1772-1775 (2012).

15. Li, T. *et al.* Anti-herpes simplex virus type 1 activity of Houttuynoid A, a flavonoid from *Houttuynia cordata* Thunb. *Antivir. Res.* **144**, 273-280 (2017).

16. Oboh, G. , Alabi, K. B. & Akindahunsi, A. A. Fermentation changes the nutritive values, polyphenol distribution, and antioxidant properties of Parkia biglobosa seeds (African locust beans). *Food Biotechnol*. **22**, 363-376 (2008).

17. Marteau, P. *et al.* Metabolism of bile salts by alimentary bacteria during transit in the human small intestine. *Microb. Ecol. Health Dis.* **8**, 151–157 (1995).

18. Petrunov, B., Nenkov, P. & Shekerdjiisky, R. The role of immunostimulants in immunotherapy and immunoprophylaxis. *Biotechnol. Biotechnol. Equip.* **21**, 454-462 (2007).

19. Wu, Y.S., Chen, Y.Y., Ueng, P.S. & Nan, F.H. Effects of medicinal herbs "*Plantago asiatica*", " *Houttuynia cordata*" and " *Mentha haplocalyx*" on non-specific immune responses of cobia (*Rachycentron canadum*). *Fish Shellfish Immunol.* **58**, 406-414 (2016).

20. Jung, B.G., Ko, J.H. & Lee, B.J. Dietary supplementation with a probiotic fermented four-herb combination enhances immune activity in broiler chicks and increases survivability against *Salmonella Gallinarum* in experimentally infected broiler chicks. *J. Vet. Med. Sci.* **72**, 1565-73 (2010).

21. Chaiyasut, C. *et al.* Effects of phenolic compounds of fermented Thai indigenous plants on oxidative stress in streptozotocin-induced diabetic rats. *Evid.-Based Complementary Alternat. Med.* **2011**, 749307. https://doi.org/10.1155/2011/749307 (2011).

22. Tunkamnerdthai, O., Auvichayapat, P., Boonmars, T., Tavichakorntrakool, R. & Leelayuwat, N. Effect of *Houttuynia cordata* Thunb. extract in High-Fat Diet-induced obese rats. *Srinagarind Med. J.* **34**, 461-467 (2013).

473 23. Kim, J.H.*et al.* Antiatherogenic and antioxidative effects of *Houttuynia cordata* extracts in rats
474 fed a high-fat diet. *Food Sci. Biotechnol.* **23**, 2069–2074 (2014).
475 24. Kang, H. & Koppula, S. *Houttuynia cordata* alleviates high-fat diet-induced non-alcoholic fatty
476 liver in experimental rats. *Pharm. Biol.* **53**, 414-22 (2015).
477 25. Klein, S.L. & Flanagan, K.L. Sex differences in immune responses. *Nat. Rev. Immunol.* **16**, 626–
478 638 (2016).
479 26. Jambhekar, S.S. & Breen, P.J. Basic Pharmaceutics: Extravascular Routes of Drugs
480 Administration. Pharmaceutical Press, London (2009).
481 27. Kamohara, S., Terasaki, Y., Horikoshi, I. & Sunayama, S. Safety of a *Coleus forskohlii*
482 formulation in healthy volunteers. *Pers. Med.* **4**, 63-65 (2015).
483 28. Begas, E. *et al.* Effects of short-term saffron (*Crocus sativus* L.) intake on the in vivo activities of
484 xenobiotic metabolizing enzymes in healthy volunteers. *Food Chem. Toxicol.* **130**, 32-43 (2019).
485 29. Molinari, C. *et al.* Eight-week consumption of milk enriched with omega 3 fatty acids raises their
486 blood concentrations yet does not affect lipids and cardiovascular disease risk factors in adult
487 healthy volunteers. *Pharma Nutrition,* **2**, 141-148 (2014).
488 30. Reagan-Shaw, S., Nihal, M. & Ahmad, N. Dose translation from animal to human studies
489 revisited. *FASEB J.* **22**, 659-661 (2007).
490 31. Chang, L.W., Hou, M.L., Lin, L.C. & Tsai, T.H. Healthy supplements of silymarin and aqueous
491 extract of *Salvia miltiorrhiza* on the pharmacokinetic interaction of lamivudine in rats. *J. Funct.*
492 *Foods*. **17**, 163-171 (2015).
493 32. Ndukaa, S.O., Okontab, M.J., Ajaghakuc, D.L., Amorhab, K.C. & Ukwe, C.V. Inhibition of
494 cytochrome P450 3A enzyme by *Millettia aboensis*: its effect on the pharmacokinetic properties
495 of efavirenz and nevirapine. *Rev. bras. Farmacogn.* **27**, 228-235 (2017).

33. Gupta, S., Kesarla, R., Chotai, N. & Omri, A. Development and validation of reversed-phase HPLC gradient method for the estimation of efavirenz in plasma. *PLoS ONE* **12**, e0174777. https://doi.org/10.1371/journal.pone.0174777 (2017).

34. Ramaswamy, A. & Arul Gnana Dhas, A.S. Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC. *Arab. J. Chem.* **11**, 275–281 (2018).

Table 1

Basic characteristics of healthy volunteer for safety assay and immunostimulatory evaluation.

Basic characteristics	Safety assay (n = 10)	Immunostimulatory evaluation (n =30)
Age (years)	24.60 ± 1.57	33.03 ± 9.84
Weight (kg)	60.74 ± 8.85	60.62 ± 10.84
High (cm)	167.20 ± 12.39	164.07 ± 8.59
Body mass index (kg/m^2)	21.71 ± 1.97	22.43 ± 2.85
Systolic blood pressure (mmHg)	120.30 ± 8.51	114.13 ± 10.02
Diastolic blood pressure (mmHg)	72.60 ± 7.88	72.13 ± 8.19
Heart rate (bpm)	83.90 ± 11.89	74.50 ± 9.38

Data are expressed as mean ± SD.

Table 2

Blood chemistry outcomes of safety assay (n = 10).

Parameters (normal range)	Before (0 week)	After (4 weeks)
BUN (5.80-19.10 mg/dL)	12.30 ± 3.37	11.80 ± 3.36
Creatinine (0.50-1.50 mg/dL)	0.88 ± 0.17	0.91 ± 0.20
Total cholesterol (≤ 200.00 mg/dL)	213.20 ± 28.30	203.10 ± 34.92
Triglyceride (≤ 150.00 mg/dL)	84.50 ± 40.53	86.60 ± 31.72
HDL (> 40.00 mg/dL)	54.00 ± 14.73	55.10 ± 13.82
LDL-c (≤ 100.00 mg/dL)	133.10 ± 29.72	120.30 ± 35.42*
ASL (12.00-32.00 U/L)	24.40 ± 5.83	24.80 ± 7.35
ALT (4.00-36.00 U/L)	17.00 ± 12.37	19.90 ± 12.91
ALP (37.00-147.00 U/L)	53.90 ± 11.22	52.50 ± 12.82
WBC count (4,600.00-10,600.00 cells/mm^3)	6,354.00 ± 1,517.62	6,298.00 ± 836.33
Neutrophil (34.00-71.00%)	52.30 ± 11.10	53.20 ± 9.10
Lymphocyte (19.00-53.00%)	38.60 ± 9.75	38.10 ± 8.79
Monocyte (5.00-13.00%)	5.90 ± 1.60	5.40 ± 2.01
Eosinophil (1.00-7.00%)	2.90 ± 0.99	3.20 ± 1.32
Basophil (0.00-1.00%)	0.20 ± 0.42	0.10 ± 0.32
Platelet count ([140.00-400.00]x10^9/L)	279.50 ± 56.52	267.90 ± 45.48
RBC count ([4.00-6.10]x10^{12}/L)	5.32 ± 0.79	5.25 ± 0.95
Hemoglobin (11.20-17.50 g/dL)	13.81 ± 1.27	13.68 ± 1.38
Hct (34.00-51.00%)	41.50 ± 3.31	41.12 ± 3.75

Data are presented as mean ± SD. Asterisk '*' indicates a significant difference ($p < 0.05$) compared to the baseline value. BUN: blood urea nitrogen; HDL: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin; Hct: hematocrit.

Table 3

Biochemical and hematological parameters of healthy volunteers for immunostimulatory evaluation (n = 30).

Parameters (normal range)	Before (0 week)	After (8 weeks)
BUN (5.80-19.10 mg/dL)	12.73 ± 3.24	12.07 ± 2.29
Creatinine (0.50-1.50 mg/dL)	0.87 ± 0.20	0.94 ± 0.24
Total cholesterol (≤ 200.00 mg/dL)	208.83 ± 38.32	207.60 ± 35.54
Triglyceride (≤ 150.00 mg/dL)	100.73 ± 77.00	105.30 ± 72.61
HDL (> 40.00 mg/dL)	54.87 ± 10.07	53.50 ± 10.94
LDL-c (≤ 100.00 mg/dL)	133.80 ± 33.61	133.03 ± 29.58
ASL (12.00-32.00 U/L)	23.77 ± 4.32	25.57 ± 5.41
ALT (4.00-36.00 U/L)	19.50 ± 9.49	21.17 ± 12.50
ALP (37.00-147.00 U/L)	52.47 ± 13.52	51.30 ± 14.61
WBC count (4,600.00-10,600.00 cells/mm^3)	6,370.00 ± 1,700.00	6,610.00 ± 1,550.00
Neutrophil (34.00-71.00%)	53.60 ± 7.54	56.60 ± 7.94*
Lymphocyte (19.00-53.00%)	36.70 ± 7.23	33.40 ± 7.34*
Monocyte (5.00-13.00%)	5.70 ± 1.26	5.70 ± 1.47
Eosinophil (1.00-7.00%)	4.07 ± 3.74	4.07 ± 3.43
Basophil (0.00-1.00%)	0.23 ± 0.43	0.17 ± 0.37
Platelet count ([140.00-400.00]x10^9/L)	273.47 ± 58.40	284.90 ± 67.22
RBC count ([4.00-6.10]x10^{12}/L)	5.18 ± 0.88	5.19 ± 0.87
Hemoglobin (11.20-17.50 g/dL)	13.05 ± 1.42	13.10 ± 1.44
Hct (34.00-51.00%)	39.97 ± 4.08	40.26 ± 4.02
$CD4^+$ T cell (470.00-1,404.00 cells/mm^3)	706.83 ± 194.43	697.53 ± 237.54
$CD8^+$ T cell (360.00-1,250.00 cells/mm^3)	614.33 ± 170.26	590.90 ± 195.79
$CD4^+CD8^+$ Ratio (0.65-2.49)	1.24 ± 0.49	1.29 ± 0.56

Data are presented as mean ± SD. Asterisk '*' indicates a significant difference ($p < 0.05$) compared to the baseline value. BUN: blood urea nitrogen; HDL: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; WBC: white blood cells; RBC: red blood cells; Hb: hemoglobin; Hct: hematocrit.

Table 4
Blood cell parameters between age groups.

Parameters (normal range)	HCFP (21-30 Age group, n = 17)		HCFP (31-40 Age group, n = 3)		HCFP (41-50 Age group, n = 10)	
	Before (0 week)	After (8weeks)	Before (0 week)	After (8weeks)	Before (0 week)	After (8weeks)
WBC count (4,600.00-10,600.00 cells/mm^3)	6,480.00 ± 1,943.03	6,839.41 ± 1,768.63	6,036.67 ± 291.95	6,193.33 ± 970.79	6,289.00 ± 1,491.10	6,356.00 ± 1,251.38
Neutrophil (34.00-71.00%)	51.94 ± 7.56	56.35 ± 8.52*	52.67 ± 8.02	57.00 ± 6.24	56.80 ± 7.08	56.93 ± 8.08
CD4$^+$ T cell (24.10-50.70%)	33.96 ± 5.55	32.38 ± 6.55	26.70 ± 3.51	28.77 ± 7.60	31.81 ± 6.59	32.81 ± 7.62
CD4$^+$ T cell (470.00-1,404.00 cells/mm^3)	758.35 ± 180.28	733.35 ± 261.20	552.33 ± 133.13	568.00 ± 206.08	665.60 ± 212.67	675.50 ± 206.24
CD8$^+$ T cell (17.10%-44.60%)	29.59 ± 5.03	28.26 ± 7.24	29.33 ± 6.09	30.07 ± 5.30	26.91 ± 11.38	26.32 ± 11.00
CD8$^+$ T cell (360.00-1,250.00 cells/mm^3)	658.94 ± 144.31	630.06 ± 193.74	591.67 ± 49.90	579.00 ± 57.71	545.30 ± 215.87	527.90 ± 220.67
CD4$^+$CD8$^+$ Ratio (0.65-2.49)	1.19 ± 0.30	1.20 ± 0.30	0.94 ± 0.26	1.00 ± 0.43	1.43 ± 0.72	1.54 ± 0.83

Data are presented as mean ± SD. Asterisk '*' indicates a significant difference ($p < 0.05$) compared to the baseline value.

Table 5

Comparative effect of HCFP on the pharmacokinetic parameters of single oral dose of efavirenz (EFV) and tenofovir disoproxil fumarate (TDF).

Pharmacokinetic parameters (Unit)	EFV Group		TDF Group	
	alone	combination with HCFP	alone	combination with HCFP
AUC_{0-t} (h/µg/mL)	22.78 ± 1.55	23.49 ± 1.12	49.04 ± 1.18	50.04 ± 1.26
C_{max} (µg/mL)	1.72 ± 0.22	1.93 ± 0.10	3.30 ± 0.70	3.72 ± 0.67
T_{max} (h)	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
$T_{1/2}$ (h)	48.24 ± 3.47	45.19 ± 5.23	19.56 ± 7.34	23.30 ± 2.65
CL (mL/kg/h)	1.12 ± 0.04	1.06 ± 0.07	0.44 ± 0.107	0.43 ± 0.096
Vd (mL/kg)	78.13 ± 5.20	69.44 ± 5.31	12.50 ± 1.81	14.67 ± 2.15

Data are expressed as mean ± SD. AUC: area under the plasma concentration-time curve; C_{max}: maximum drug concentration/peak concentration; T_{max}: time to maximum concentration; $T_{1/2}$: half-life; CL: total body clearance; Vd: volume of distribution.

Figure captions

Figure 1. Flow diagram showing trial design and subject recruitment and progression through the study.

Figure 2. The effect of HCFP on WBC (A, B) and percentage of neutrophils (C, D) between male (B, D) and female (A, C). Healthy volunteers of both genders were given HCFP for 8 weeks. The results represented before and after the intervention. Box plot expressed as mean with SD. Asterisk '*' indicates a significant difference ($p < 0.05$) compared to the baseline value.

Figure 3. Plasma concentration-time curve of EFV (25 mg/kg) (A) and TDF (22 mg/kg) (B) with or without co-administration of HCFP (3.08 ml/kg) in Sprague Dawley rats. Data are expressed as mean ± SEM (n = 6).

Figures

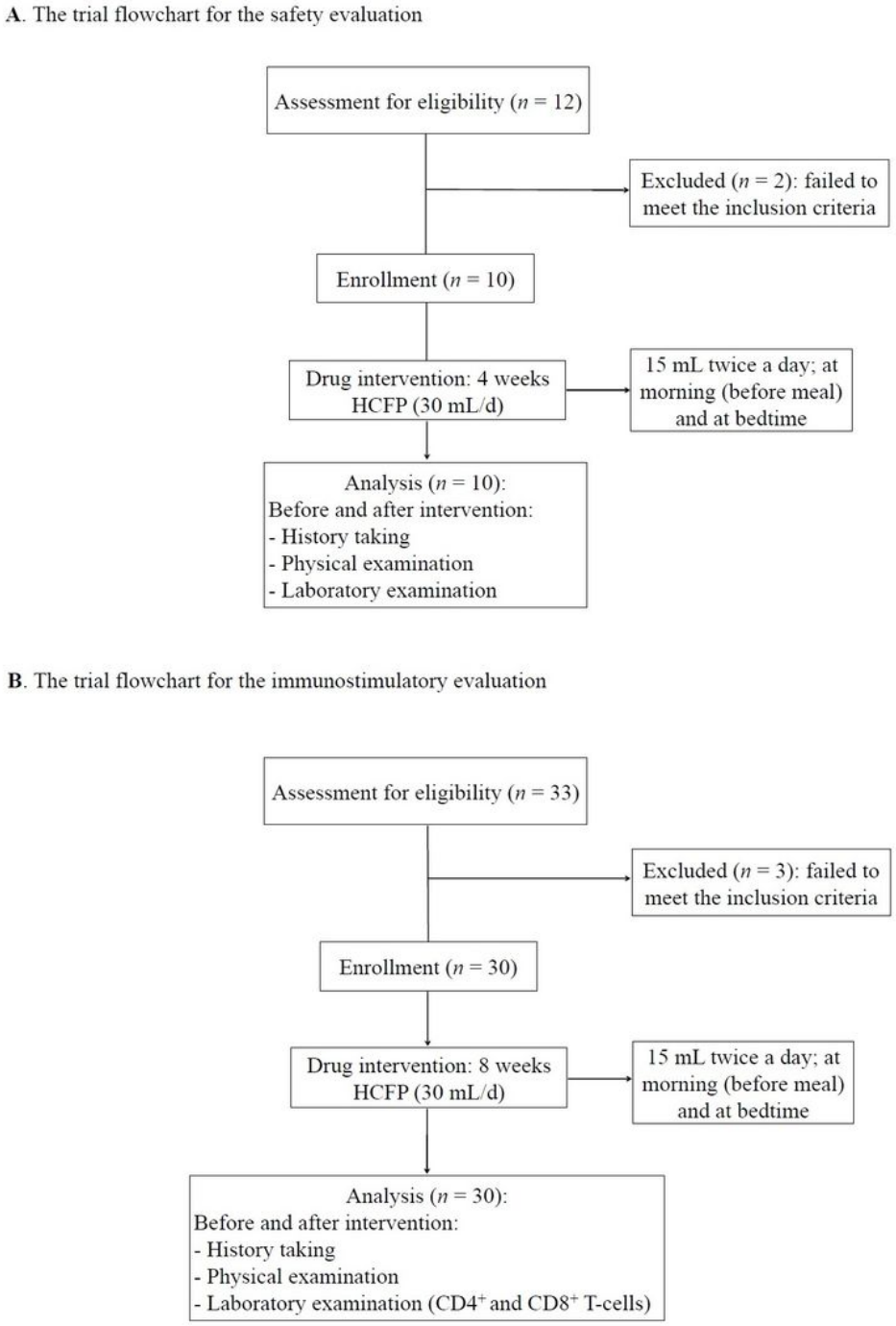

Figure 1

Flow diagram showing trial design and subject recruitment and progression through the study.

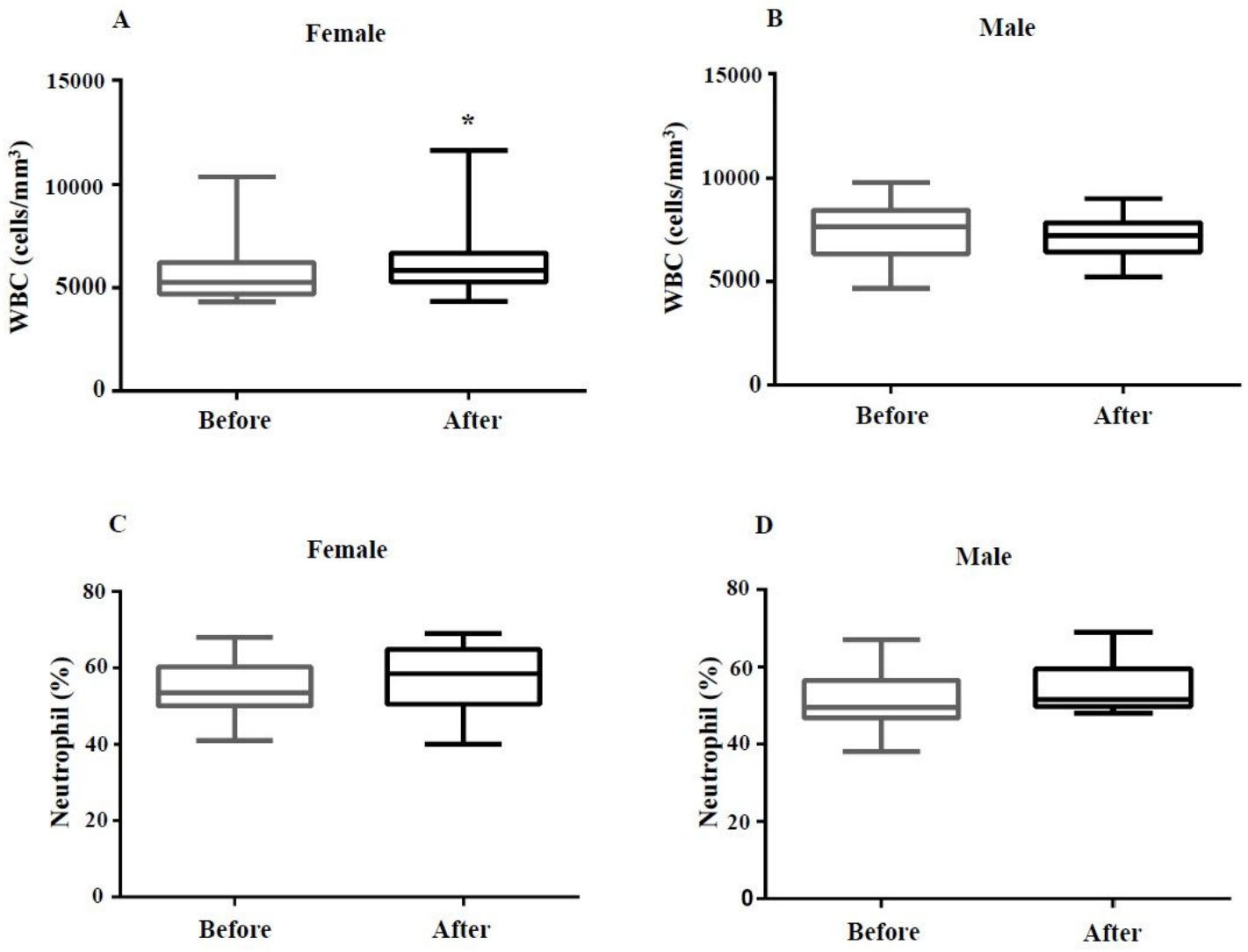

Figure 2

The effect of HCFP on WBC (A, B) and percentage of neutrophils (C, D) between male (B, D) and female (A, C). Healthy volunteers of both genders were given HCFP for 8 weeks. The results represented before and after the intervention. Box plot expressed as mean with SD. Asterisk '*' indicates a significant difference ($p < 0.05$) compared to the baseline value.

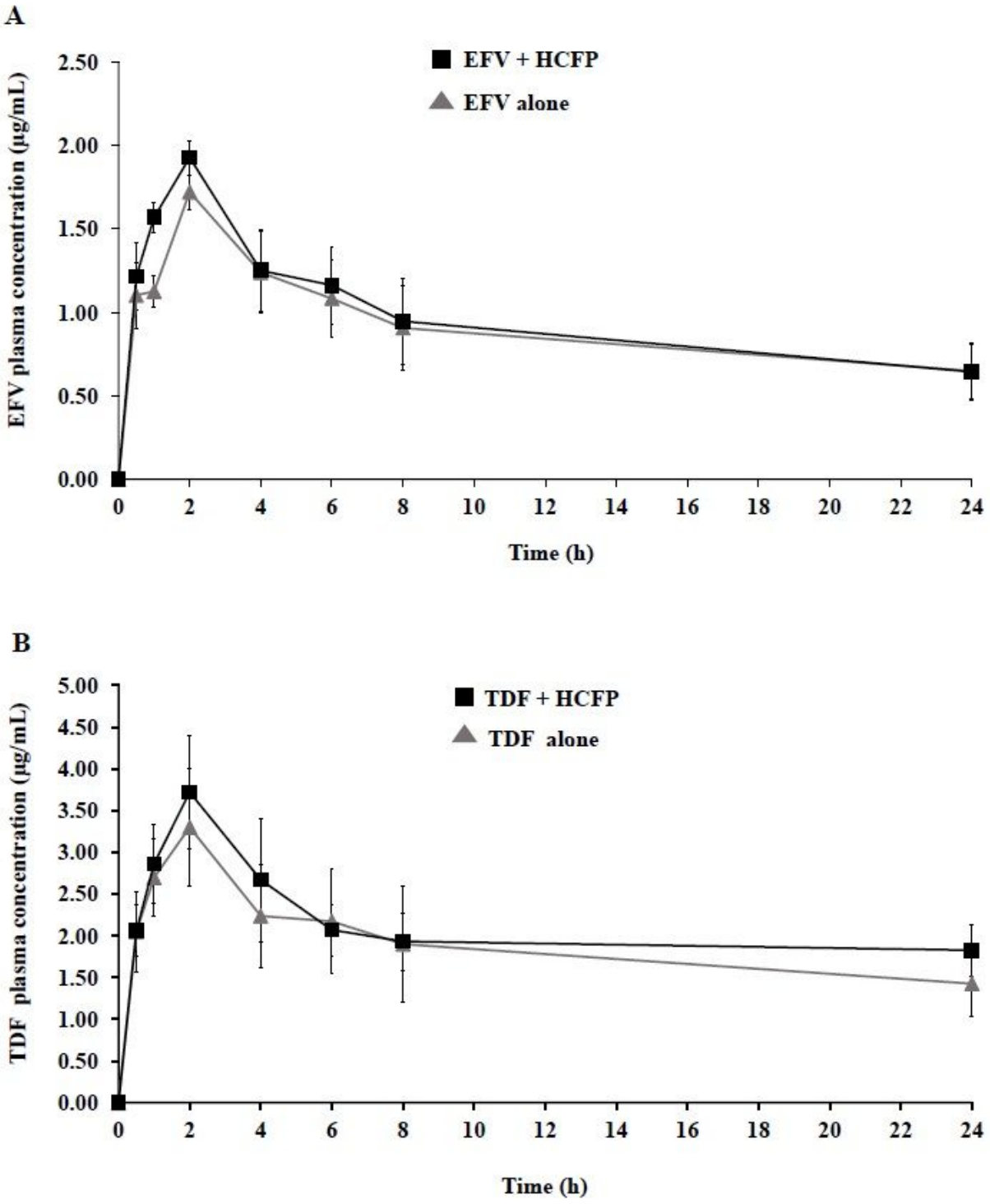

Figure 3

Plasma concentration-time curve of EFV (25 mg/kg) (A) and TDF (22 mg/kg) (B) with or without co-administration of HCFP (3.08 ml/kg) in Sprague Dawley rats. Data are expressed as mean ± SEM (n = 6).

Supplementary Files

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- SupplementaryFig.S1.pdf