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Fractionated Neem Leaf Extract is Safe and Increases CD4$^+$ Cell Levels in HIV/AIDS Patients

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The safety and effect of an acetone-water neem leaf extract (IRAB) on CD4$^+$ cells was investigated in 60 HIV/AIDS patients as part of an ongoing study to determine the influence of neem on immunity and viral load in HIV/AIDS. Patients were confirmed as HIV I or II positive, as having CD4$^+$ cell count, less than 300 cells/μL, and as antiretrovirally naïve. They were given oral IRAB (1.0 g daily for 12 weeks). Clinical and laboratory tests were carried out at baseline and at 4 weekly intervals. Thus, the patients served as their own controls. Sixty patients completed treatment. Fifty (83.33%) were completely compliant with respect to laboratory tests. Increase in mean CD4$^+$ cells, 266 cells/μL (159%), for the 50 patients was significant ($P < 0.001$) between baseline and week 12. Erythrocyte sedimentation rate (64 mm/hr at baseline) was 16 mm/hr at week 12, whereas total number of incidences of HIV/AIDS-related pathologies decreased from 120 at baseline to 5. Mean bodyweight, hemoglobin concentration, and lymphocyte differential count increased significantly by 12% ($P < 0.05$), 24% ($P < 0.0001$), and 20% ($P < 0.0001$), respectively. There were no adverse effects and no abnormalities in kidney and liver function parameters. The results support the safety of IRAB in HIV/AIDS, and its significant influence on CD4$^+$ cells may be useful in the formulation of multidrug combination therapies for HIV/AIDS. However, its antiretroviral activity is being evaluated in our laboratory.

Keywords: neem leaf extract, IRAB, HIV/AIDS patients, safety, CD4$^+$ cells

INTRODUCTION

The advent of protease inhibitors as part of a multidrug combination therapy with reverse-transcriptase inhibitors known as highly active antiretroviral therapy brought hope to millions of people infected with the human immunodeficiency virus (HIV).[1,2] With this combination therapy, significant and sustained viral suppression was achieved in most treated patients. This success was, however, soon followed by limitation to therapy brought about by a number of factors, which include toxicity,[3] drug resistance,[4,5] drug-drug and drug-food interactions,[6] as well as a need for perfect patient adherence[7] to complex treatment regimens.

Thus, in the face of highly active antiretroviral therapy, viral rebound occurred with grave challenges for future treatment options.[8] Enfuvirtide was recently introduced with a mode of action targeting the envelope glycoprotein 41 of the HIV-I critical for fusion of the virus with the cell membrane.[9] The introduction confirmed the general class of anti-invasion agents as viable targets for novel antiretroviral drug development.

Fractionated acetone-water neem leaves extract (also known as IRAB) is a complex molecule (202 Daltons)

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with functional groups that include sodium salt of carboxylic acid and nonaromatic dialcohol. IRAB was found to have both schizonticidal and gametocytocidal activities against *Plasmodium falciparum* malaria parasites in vitro,[10] inhibited the invasion of human lymphocytes by HIV-I in vitro, and caused significant improvement in $CD4^+$ cell count in a small number of HIV/AIDS patients in a phase I clinical trial.[11] In the present study, IRAB was investigated to further determine its efficacy and safety in a larger number of HIV/AIDS patients treated for a longer period of time in an open-label, multicenter clinical trial.

MATERIALS AND METHODS

Patients and study design

The study was a multicenter investigation consisting of a 2-week recruitment period followed by 12-week treatment and follow-up period. During a patient's initial-screening visit, a complete history was taken, and HIV positivity was confirmed. Laboratory investigations were carried out including $CD4^+$ cell counts, erythrocyte sedimentation rate (ESR), white blood cell count, and hemoglobin concentration. Patients qualified for inclusion in the study were ambulatory, with $CD4^+$ cell counts equal to or less than 300 cells/μL. They had not been treated with any antiretroviral drug and had no primary organ failure. Excluded were pregnant women and patients below 16 years of age as well as those with established major organ failure. Before the initiation of therapy, additional laboratory tests that included liver function tests and urinalysis were carried out on the qualified patients.

Study location

Recruitment and drug administration took place in three centers: University of Nigeria Teaching Hospital, Enugu, Nigeria; Annunciation Specialist Hospital, Emene, Enugu, Nigeria; and Mother of Christ Specialist Hospital, Enugu, Nigeria. Laboratory tests were performed at a central laboratory at the University of Nigeria Teaching Hospital, Enugu, Nigeria.

Study medication and monitoring

An oral formulation of IRAB encapsulated in clear, transparent gelatin capsules (250 mg IRAB per capsule) was administered to the patients. The dosage was 1000 mg daily in two divided doses of 500 mg each. Patients were given an initial supply of IRAB to last 2 weeks and resupplied biweekly thereafter at the outpatient clinics of the study centers and on presentation of the drug container of the preceding week's supply. Instructions on how to take the drug accompanied each supply. During each visit, complete clinical evaluation was performed and any opportunistic infections treated. Laboratory evaluations were performed every 4 weeks during the study and follow-up periods.

Study endpoint

The primary efficacy determinations were the change from baseline to week 12 in the $CD4^+$ cell count and ESR. $CD4^+$ cell counts were determined using TRAX CD4 Test kits. Other study efficacy parameters included changes in incidence of opportunistic infections, hemoglobin concentration, and bodyweight.

Safety assessment

Safety was assessed on all the patients who completed a minimum of 4 weeks of treatment. The following parameters were analyzed: adverse events such as gastrointestinal disturbances, dizziness, rash, and vital sign changes. Laboratory tests included total blood cell counts, blood chemistry, and urinalysis.

Statistical analysis

Efficacy data were analyzed on a treatment basis. Changes from baseline to week 4, 8, and 12 were evaluated using Friedman's nonparametric analysis of variance for repeated measurements.

Consent and approvals

The ethical committees of the participating centers reviewed and approved the study protocol before the study began. Informed consent was obtained from all participating patients.

RESULTS

Study population and adherence

Sixty patients were enrolled in the study. Of this number, 50 (83.33%) completed the 12-week treatment program and had complete clinical and laboratory test data. The remaining 10 patients had omissions of different laboratory data points but otherwise also completed the treatment program.

Baseline characteristics of patients

The baseline characteristics of the enrolled patients are shown in Table 1. All the patients had one or more HIV/AIDS-related symptoms at enrollment. The majority (64%) had $CD4^+$ cell counts below 200 cells/μL. A similarly high proportion (64%) had relatively elevated ESR values (51 to >100 mm/hr).

Table 1. Patient characteristics at entry (before treatment).

Parameter	Value
Total number of patients	60
Males, n (%)	34 (57)
Females, n (%)	26 (43)
Age (yr)	
Median	30
Range	23–50
Weight (kg)	
Median	58
Range	41–74
Prior retroviral therapy	0
Hemoglobin concentration (g/dL)	
Median	10
Range	6.6–14.3
White blood cell count	
Total (cells/mm^3)	
Median	4600
Range	2800–8900
Differential cells (%)	
Neutrophils	67
Lymphocytes	31

Influence on CD4⁺ cells

Fifty patients completed CD4⁺ cell counts at all designated time points and were the only ones included in the CD4⁺ cell count analysis. As shown in Table 2, CD4⁺ cell count increased consistently between baseline and all time points from week 4 through week 12. At week 12, the change from baseline in the mean CD4⁺ cell count was +266 cells/μL, and the mean percent change was 159%. The changes were statistically significant ($P < 0.0001$). The median (range) of 167 (50–300) cells/μL at baseline increased to 411 (262–610) cells/μL at week 12. In patients' responses by week 8, 50 (100%) had increases in CD4⁺ cell counts greater than 10 cells/μL; at responses greater than 50 cells, 40 (80%) and 50 (100%) responded by weeks 8 and 12, respectively. There were no declines in CD4⁺ cell count in any patient at any time point during the treatment and follow-up.

Erythrocyte sedimentation rate

Mean ESR declined significantly ($P < 0.0001$) by 48 mm/hr (75%) and consistently between baseline (64 mm/hr) and week 12 (16 mm/hr) (Table 2). Median (range) at baseline of 59 (14–120) mm/hr decreased by week 12 to 16 (12–22) mm/hr. In patient responses defined by a decrease of ESR from a baseline range of 20 or greater to 120 mm/hr to less than 20 mm/hr, 80% responded at week 12. None of the patients had an increase in ESR values throughout the study.

Other efficacy endpoints

At week 12, mean lymphocyte differential, mean hemoglobin concentration, and mean body weight were significantly increased by 20% ($P < 0.0001$), 24% ($P < 0.0001$), and 12% ($P < 0.05$), respectively (Table 3). The mean neutrophil differential in cells was significantly decreased by 21% ($P < 0.0001$) from a baseline value of 68%. The total incidence of opportunistic infections and other HIV/AIDS-related conditions was reduced from a baseline number of 129 to 5 at week 12, and the categories of conditions were reduced to 4 from a baseline of 13 (Table 4). Sixty (100%) patients had one or more conditions at baseline. By week 12, all conditions had been resolved in 57 (95%) patients. Of the remaining three (5%), each had unresolved lymphadenopathy, and in addition, one still had cough, and another was persistently underweight.

Table 2. CD4⁺ cell count and erythrocyte sedimentation rate (ESR) response during treatment of HIV/AIDS patients with IRAB.

	Weeks of treatment			
	0	4	8	12
CD4⁺cell count (cell/μL)				
Median	167	220	305	411
Range	30–331	107–434	109–589	262–610
Change in mean from baseline				
CD4⁺cell count	—	+60	+138	+266
Percentage	—	36	83	159
P value	—	<0.005	<0.0001	<0.0001
ESR (mm/hr)				
Median	59	43	22	16
Range	4–120	12–98	12–47	12–22
Change in mean from baseline (%)	—	−20 (31)	−40 (63)	−48 (75)

Table 3. Change in laboratory parameters in HIV/AIDS patients between baseline and week 12 of treatment with IRAB.

Parameter	Mean change	*P* value
WBC differential cells		
Neutrophil (%)	−21	
Lymphocyte (%)	+20	<0.0001 (ANOVA)
Hemoglobin concentration (g/dL)	+24	<0.0001 (ANOVA)
Bodyweight (kg)	+12.3	<0.05 (ANOVA)

WBC, white blood cell count; ANOVA, analysis of variance.

Adverse events

The adverse events profile in the treated patients was consistent with underlying HIV/AIDS condition. Monitoring by laboratory tests including hematology, kidney and liver function tests, and urinalysis did not reveal any treatment-related side effect. As shown in Table 5, kidney function as determined by serum electrolyte balance was not significantly altered by treatment. The median concentrations in millimoles per liter for sodium (Na^+), potassium (K^+), bicarbonate (HCO_3^-), chloride (Cl^-), urea (U), and creatinine (Cr) were, respectively, 140, 4.0, 24.5, 104, 5.9, and 89 before and 137.5, 3.7, 24.5, 101.5, 4.6, and 96 after treatment. Liver function monitored by concentration of total bilirubin, direct bilirubin, alkaline phosphatase, alanine phosphatase, and aspartate transferase was not adversely affected by treatment. The median values in millimoles per liter for total bilirubin (11.0) and direct bilirubin (4.4) before treatment were, respectively, 8.8 and 4.4 after treatment. Values in International Units per liter for alkaline phosphatase (51.0), alanine phosphatase (9.0), and aspartate transferase (12.0) before treatment became, respectively, 37.5, 6.0, and 11.0 after treatment. There was one report of transient flatulence in one patient and urticaria in another patient. These conditions were not determined to be treatment related.

Table 4. Incidence of AIDS-related conditions (ARC) in HIV/AIDS patients at baseline and week 12 of treatment with IRAB.

Condition	Number, baseline	week 12
Fever	27	0
Weight loss	17	1
Generalized lymphadenopathy	15	3
Diarrhea	13	0
Cough (nonspecific)	13	1
Skin rash (all types)	10	0
Oral thrush	10	0
Pallor	7	0
Loss of appetite	3	0
Headache	11	0
Dizziness	1	0
Chest pain	1	0
Zoster	1	0
Total incidence	129	5

DISCUSSION

This study was an open-label, nonrandomized investigation to further determine the efficacy and safety of IRAB in HIV/AIDS patients. The extract was administered for 12 weeks as an oral formulation in clear gelatin capsules at dose of 1.0 g daily and in the absence of any other antiretroviral medications. The patients included in the study had not been treated with any antiretroviral drugs previously. They had a variety of HIV/AIDS-related conditions at admission, including decreased $CD4^+$ cell counts, hemoglobin concentration, and body weight and elevated ESR and neutrophil differentials. Treatment with the extract caused marked improvements in all parameters measured.

Increase in $CD4^+$ cell count was rapid and sustained. The increase at week 12 in mean $CD4^+$ cell count (266 cells/μL) was remarkable and substantially higher than those reported in earlier similar studies, most of which were of a longer treatment duration.[12,13] The improvement in $CD4^+$ cell counts was paralleled by equally impressive rapid clearance of most of the HIV/AIDS-related infections and pathologies (Table 4). Resolution of these conditions probably played a role in the relatively precipitous drop in mean ESR values between baseline and week 12. Overall, it appeared that clinically significant immune restoration was achieved in the treated patients, perhaps also with suppression of retroviral activity. Such suppression is likely considering that increase in $CD4^+$ cell counts has been shown to be related to corresponding reductions in plasma viral load.[9,14,15] Furthermore, IRAB was shown in previous studies[11] to have direct antiretroviral activity in vitro, and we are presently investigating its effect on retroviral activity. Significant improvements in other clinically relevant parameters including hemoglobin concentration, bodyweight, and lymphocyte differentials contribute to substantiate the efficacy of IRAB.

Table 5. Values of kidney and liver function parameters in HIV/AIDS patients before and after 3 months treatment with IRAB.

Organ	Parameter	Median (range)	
		Before	After
Kidney	Sodium (Na^+) (mmol/L)	140.0 (135.0–145.0)	137.5 (133.0–144.0)
	Potassium (K^+) (mmol/L)	4.0 (3.5–5.0)	3.7 (3.5–4.2)
	Bicarbonate (HCO_3^-) (mmol/L)	24.5 (24.0–28.0)	24.5 (20.0–27.0)
	Chloride (Cl^-) (mmol/L)	104.0 (97.0–108.0)	101.5 (98.0–105.0)
	Urea (U) (mmol/L)	5.9 (2.5–6.2)	5.1 (3.8–6.6)
	Creatinine (Cr) (mmol/L)	89.0 (44.0–194.0)	96.0 (61.0–97.0)
Liver	Total bilirubin (mmol/L)	11.0 (1.0–17.0)	8.8 (8.8–13.2)
	Direct bilirubin (mmol/L)	4.4 (4.4–4.4)	4.4 (4.4–4.4)
	Alkaline phosphatase (IU/L)	51 (25.0–92.0)	37.5 (28.0–52.0)
	Alanine phosphatase (IU/L)	9.0 (3.0–15.0)	6.0 (5.0–8.0)
	Aspartate transferase (IU/L)	12.0 (5.0–18.0)	11.0 (6.0–13.0)

There were no reactions whatsoever associated with oral administration of IRAB. Likewise, there were no treatment-related adverse events throughout the 12 weeks of treatment and 4 weeks of follow-up. A rigorous and diligent monitoring of regular blood chemistry and urinalysis results as well as regular vital sign checks revealed no drug-related abnormalities. Although this report is for the initial 12 weeks of treatment, safety analysis of patients who continued and have taken the medication for 90 days has equally revealed no adverse side effects. The total absence of treatment-related adverse events, including the absence of those commonly associated with antiretroviral medications, constitutes a significant advantage for IRAB.

The mechanism of action of IRAB has not been conclusively determined. It is, however, known to have a broad spectrum anticyto-adhesive activity, and such action may be revealed in our ongoing study.

In conclusion, additional evidence has been presented to further demonstrate the efficacy and safety of the fractionated, acetone-water neem leaf extract (IRAB) in HIV/AIDS. The 12 weeks of treatment caused significant increases in all indices of clinical improvement measured, including a convincing increase in $CD4^+$ cells and resolution of infections. It was without any adverse side effects. In consideration of the frightening rate of development of drug resistance and limitations to therapy imposed by the toxicity of current drugs, IRAB has the potential to contribute significantly to the fight against HIV/AIDS.

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