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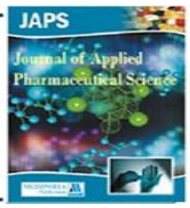

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Effectiveness of the medicinal plant R019 in the treatment of HIV infection: an observational study

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ABSTRACT

This study aims to evaluate the in vivo antiviral, immunologic, clinical effects and safety of a supposedly anti-HIV phytotherapy, code-named R019 used for the treatment of HIV/AIDS. This is an open observational study, which involved 32 HIV-1 infected patients, who were followed over a 3-month period. The efficacy evaluation was based on CD4 count, determination of viral load and clinical status. The safety evaluation was based on renal and liver function tests, fasting lipid and glycaemia levels as well as the frequency of other adverse events. The CD4 values increased significantly (mean±SD, 99.03±22.87 cells/µL; P<0.001), as well as Weight and Karnofsky score (2.94±0.67 kg, p<0.001; 4.9, p=0.005 respectively). The viral load decreased significantly (0.91±0.12 log viral load, P<0.0001). R019 did not impair renal or liver functions. Improvement of creatinine clearance was observed (p=0.02). Hemoglobin levels increased (0.38±0.16 gr/dL) whereas cholesterol and glucose levels decreased under R019 treatment (p=0.031, p=0.018 respectively). Main adverse effects were recorded: polyuria (40.5%), drowsiness (21.4%), orexis (19.1%). Immunological, anti-viral and clinical status improved under R019 treatment and a good safety profile was observed for this compound. Further studies would be required to optimize its efficacy and to define its appropriateness for the treatment of HIV disease.

Keywords: R019, HIV infection, Efficacy, Safety

INTRODUCTION

HIV/AIDS continues to be one of the public health concerns for policy makers and the entire community in the Republic of Benin. Available records show that the prevalence rate of HIV infection in 1990 was 0.3%, increasing to around 2% in 2002, with some regional variations. Current projections indicate that by 2010, the number of people living with HIV would be about 79,000 in Benin (Ministère de la Santé, 2004). Since 2006, a predicted quarterly growth of 250 patients has risen to the current figure of around 700. Based on this projection, 4,500 patients were meant to be supplied with antiretroviral (ARVs) in 2004, increasing by 1,000 patients per year until 2010. This has generated a shortfall that needs to be urgently addressed. Despite the efforts of governments and development partners, a considerable proportion of people living with HIV/AIDS, still have limited access to antiretroviral (ARV) medicines. This state of affairs led to the "3 by 5" initiative, which was launched in 2003 by the World Health Organization (WHO) and the Joint United Nations Program on HIV/AIDS's (UNAIDS, as a global TARGET to provide three million people living with HIV/AIDS in low- and middle-income countries with life-prolonging antiretroviral treatment by the end of 2005 (WHO, 2005).

Although, the initiative had not met its set target as of 2005, there was a significant increase in the number of people receiving ARV therapy, as well as renewed commitment to expanding prevention activities and strengthening health systems. Moreover, the initiative was instrumental in generating increased financial and technical support to deal with the pandemic.

Several other challenges exist to hamper the fight against the pandemic. These include lack of readily available reagents for the detection of new cases, limited access to ARVs (WHO Benin, 2007), lack of trust in health care professionals, shortage of qualified health workers, the limitation of the criteria for inclusion in highly active antiretroviral therapy (HAART) regimen, and fear of stigmatization due to ARVs side-effects (MS, Benin, 2004) (Ministère de la Santé, 2004). In addition, most ARV treatment sites (40 testing sites based in 12 departments) are located in towns and urban centers, further hampering access for HIV-positive patients living in rural areas. Interestingly, support for HIV patients is still entirely dependent on donor funds (Zannou, 2006), which are inadequate to meet the rising demand.

Due to sociocultural beliefs, 60-80% of the population including many HIV patients, resort to traditional medicine (WHO, (Organisation Mondiale de la Santé (OMS) 2002-2005);). The use of phytotherapy is therefore frequent in HIV patients, irrespective of scientific validation of such shown medicines.

The present study was therefore designed to evaluate the effectiveness of medicinal plant coded *R019*, commonly administered to HIV patients who are not yet eligible for antiretroviral therapy at the TANGUIETA hospital (WHO, 2006) (WHO, 2006).

R019, known as "kinkeliba" in the Republic of Benin and other West African countries, is commonly used for the treatment of numerous ailments such as infection, diarrhea, fever, and bowel dysfunction (Nikiema *et al.*, 2004b; Ogan, 1972; Bassene, *et al.*, 1986). The plant is also claimed to have cardiovascular and diuretic properties. Over the past twenty years, *R019* has been evaluated for its antiviral (Ferrea *et al.*, 2004; Pengsuparp *et al.*, 1995; Cohen, *et al.*, 1996; Ferrea, *et al.*, 1993b; Ferrea, *et al.*, 1993a), antimalarial, antibacterial, anti-inflammatory (Olajide, *et al*., 2003) and diuretic properties.

There are no clinical studies on its anti-HIV effects, probably due to the complex methodological and practical challenges associated with the clinical evaluations of traditional medicines such as *R019*. The above reason, together with the long history of use, led to the choice of an observational study, which is a relatively simple and inexpensive approach to assess the effectiveness of *R019*, with the expected outcomes being its immunological and anti-viral actions, its ability to increase the weight of the patients and to enhance their quality of life, as well as any associated adverse events.

MATERIALS AND METHODS

The study, which was carried out between March 2009 and January 2010, involved regular self-administration of measured doses of *R019* to HIV patients, who were not eligible for HAART, in a single centre, prospective, non-interventional 3-month observational study. Written informed consent was obtained from all patients, and the study protocol was approved by a Review Board and an independent Ethics Committee at the study centre (Letter's number: 0121-2009).

Plant material

Leaves of *R019* were collected in the Tanguieta district, Republic of Benin throughout one year. The plant was identified by Gaudence Djeigo of the Faculty of Agricultural Sciences of the Université d'Abomey-Calavi. The voucher specimen X123UAC was deposited in the herbarium of the University. The plant material was dried at room temperature and reduced to powder using a Thomas Scientific mill with a delivery tube of 2 mm diameter. The powder was then stored in a freezer at -10°C. Five grams of the powder were used to prepare one liter of decoction. For quality control purposes, the organoleptic characteristics of *R019* powder were assessed, and this gave a green powder with a greasy smell. Six classes of chemical compounds were identified in the pure *R019* (*R019*)-based extract: tannins, anthocyanins, leucoanthocyanins, and reducing sugars as the major compounds, whereas flavonoids and essential oils were present in small amounts.

Patients

Inclusion criteria

To be included in the study, all patients had to undergo an initial clinical and laboratory assessment. They were also screened for HBV surface antigen and HCV antibody.

HIV-1 infected patients were identified by ELISA and confirmed by HIV-1 RNA PCR. Patients included in the cohort were aged between 18 and 55 years, with $CD4^+$ T cell count between 200 and 500 cells/ µL, and naive for ARV. The patients with CD4>200 cells/µL were not eligible for ARV, according to local treatment guidelines in Benin.

Exclusion criteria: Patients with a history of severe psychiatric illness or any history of a chronic illness, such as cardiopulmonary disorder, were excluded from the study.

Treatment and Assessments

Dosage and administration

R019 was prescribed to the patients on a regular basis. During 3-month follow up period every day, patients received one liter of decoction of *R019*, to drink between the 08.00 and 16.00 hours in an unsupervised fashion.

Follow up

Each patient was followed over a three month period. Study visits were scheduled at Day 0 (D0), D14, D30, D60 and D90.

Clinical assessment

Study evaluations included physical examination; assessment of vital signs, symptoms, and review of adverse events reported by the patients.

Laboratory assessments

Laboratory analysis were performed at D0, D30 and D90 (serum chemistry and hematology tests, CD4 cell counts by flow cytometry and HIV-1 RNA level by quantitative reverse-transcriptase-polymerase-chain reaction (PCR) assay). A fasting metabolic assessment (i.e., analysis of total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, glucose), and orthostatic blood-pressure monitoring were performed at D0, D14, D30 and D90. The evaluation of efficacy was based on the change in viral load (measured as log 10-transformed copies/mL), change in CD4 cell counts and clinical status (weight, BMI and Karnofsky index as a scoring of physical performance at each visit). Safety was assessed using total cholesterol [TC], triglycerides, liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], Gamma-glutamyltranspeptidase [GGT]) and bilirubin levels at D0, D30 and D90 and the frequency of adverse events during treatment with *R019*. The renal function was evaluated by measuring serum creatinine and creatinine clearance (using Cockroft-Gault formula). Adverse events (AEs) were defined as all disorders of well being, subjective and objective disease symptoms reported by patient, significant laboratory changes, concomitant illnesses, and accidents (death or hospitalizations) occurring during the course of the study. AEs were evaluated by the investigators based on severity (mild, moderate or serious). A serious adverse event (SAE) was defined as an event which is fatal or life-threatening, results in persistent or significant disability/incapacity, constitutes a congenital anomaly/birth defect, and requires inpatient hospitalization or prolongation of existing hospitalization (FDA, 2010). Adverse events were documented during clinic visits. They were also recorded after visits, either by self-reporting by the subject or by detection of clinical signs and changes in laboratory parameters by the physician.

Statistics

All patients participated in the cohort were included in the analysis. Descriptive summary statistics were used for this cohort analysis, describing changes in $CD4^+$ cell count and viral load from baseline, the type and frequency of adverse events observed, and changes in safety-relevant laboratory parameters. For continuous variables, the following statistics were calculated: mean; standard deviation, median, minimum, maximum and 95% interval confidence of mean. For categorical variables, the number of values in each category and percentage of the values with regard to the number of patients in the study population were calculated. Explorative statistical methods were used with regard to the efficacy endpoints and changes in safety-relevant laboratory parameters. Changes from baseline were tested for significance using the Wilcoxon signed-rank test. The sample size was determined using the sampsi function in STATA version 9 (STATA Corp., College Station, TX, USA). A change in CD4 counts of 50 cell/microliter was considered clinically significant. This study needed 32 patients to detect a significant change of 50 cells $CD4^+$/microlitre, with a standard deviation of 100 cells $CD4^+$/microlitre with a power of 80%.

RESULTS

Thirty two patients were included in the study. There were no dropouts. Baseline characteristics including demographic data are summarized in Table I. Four (12.5%) patients were infected with hepatitis B at the start of the study and 1 (3.1%) patient had co-infection with hepatitis C. No patient had both hepatitis (B and C). A total of 20 (62.5%) of the patients were at stage 2 and the remaining, i.e. 37.5%, were at stage 3 according to WHO classification.

Table. 1: Patients' baseline characteristics.

Sex: n (%)	
Male	12(37.5)
Female	20(62.5)
Age (years)	
Mean	31.13
Range	18-55
Weight (kg)	
Mean	53.53
Range	34-75
Nationalities: n (%)	
Beninese	30(93.7)
Togolese	1(3.1)
Guineans	1(3.1)
CD4 count (cells/µL)	
Median	274
Range	208-500
Viral load (log_{10} copies/mL)	
Mean	4.93
Range	3.87-5.00
Stage (WHO)	
1 and 2	20(62.5)
3 and 4	12(37.5)
HCV *n (%)*	1(3.1)
HBV *n (%)*	4(12.5)

CD4 cell count

At initiation the mean CD4 cell count was 289 cells/mm^3 and the majority of the patients (78.125%) had an initial CD4 count below 350 cells/µL (Table 2). At D90, the mean CD4 cell count was 387.84, i.e an increase by 99 cells/mm^3 (p=0.010). The CD4 cell count decreased only in one patient and remained stable in eight patients.

Viral load (VL)

Median log (VL) at baseline was 4.91; after 3 months of treatment a decrease of 0.91 was seen (P=0.000). However, no patient had an undetectable viral load (< 50 copies/ml) at D90 (Table 2).

Health status

The mean values of BMI of patients at different follow-up times were normal. However, a significant increase was noted in these values (p <0.040) (Table 2). The Karnofsky index of the patients increased at D30 and D90 and showed significant improvement at D90 compared to D0 (Table 2).

Table. 2: One and three month changes in CD4 count, viral load, BMI and Karnofsky score in 32 HIV patients on *R019* in Benin.

Parameters	Day 0		Day 30		Day 90		
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	P-value (D90 vs D30)
CD4 count (cells/ µL)	288 (66)	208-500	340 (94)	215-623	388 (140)	201-776	
p-value (vs D0)			0.002		0.000		0.008
Viral load (log_{10}/ml)	4.93 (0.65)	3.87-5.7	4.44 (0.65)	3.38-5.7	4.10 (0.55)	3.09-5.7	
p-value (vs D0)			<0.001		<0.001		<0.001
BMI (kg/m^2)	19.9 (2.82)	13.6-26.6	20.9 (3.95)	15.4-30.6	21.4 (3.33)	16.8-33.5	
p-value (vs D0)			0.013		0.000		0.028
Karnofsky index	93.2 (8.3)	80-100	96.3 (5.5)	80-100	98.1 (4.7)	80-100	
p-value (vs D0)			NS		0.005		0.014

Table 3: One and three month changes in some biological safety parameters in 32 HIV patients on *R019* in Benin.

Parameters	Day 0		Day 30		Day 90		
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	P-value (D90 vs D30)
Hemoglobin (g/dl)	11.6 (1.76)	8.0-16.2	11.8 (1.76)	8.6-15.3	12.0 (1.71)	8.7-15.5	
p-value (vs D0)			0.023		0.027		NS
ASAT (U/L)	44.6 (23.2)	18-134	38.2 (14.5)	16-68	38.8 (17.0)	14-94	
p-value (vs D0)			0.018		0.06		NS
ALAT (U/L)	30.3 (12.7)	11-75	27.3 (11.7)	12-65	29.1 (14.4)	12-81	
p-value (vs D0)			0.032		NS		NS
Serum creatinine (mg/L)	10.1 (1.62)	7.2-14.9	9.8 (1.60)	7.4-14.7	9.6 (1.48)	7.4-14.6	
p-value (vs D0)			0.019		0.008		NS
Creatinine clearance (ml/min)	74.8 (18.9)	35.7-113.9	76.9 (19.5)	35.5-116.3	78.1 (17.5)	41.8-113.6	
p-value (vs D0)			0.018		0.008		0.05
Total Cholesterol (mg/dL)	145.6 (21.7)	103-215	133.8 (18.2)	113-198	134.6 (18.1)	111-200	
p-value (vs D0)			0.02		0.001		NS
Glycemia (g/L)	0.84 (0.13)	0.69-1.20	0.81 (0.12)	0.64-1.10	0.79 (0.11)	0.55-1.03	
p-value (vs D0)			0.02		0.000		0.000

Laboratory assessments

Hemoglobin

Treatment with R019 did not adversely affect hemoglobin levels. No decrease hemoglobin rate among patients was observed (Table 3). In contrast, the hemoglobin level at D90 increased significantly compared to that of D0 ($p = 0.02$). Red blood cell count was not affected by the treatment. The values obtained at different times of follow-up were not significantly different from each other ($p > 0.31$). Leukocyte values also remained unchanged.

Liver enzymes

AST or ALT were not adversely affected. Instead, there was a significant decrease in the level of the enzymes between at D30, remained stable at D90. A significant improvement over time was observed (Table 3). No statistically significant difference was observed for transaminase ALT during the follow-up period ($p > 0.164$).

Renal function

The renal function (serum creatinine and clearance of creatinine) was not affected, but seems improved (serum creatinine decreased and clearance of creatinine increased). No significant difference was observed between D0 and D30 ($p = 0.093$) and between D30 and D90 ($p=0.132$). However serum creatinine and clearance of creatinine at D90 were significantly improved compare to D0 values ($p=0.010$).

Metabolic assessments

Lipid changes

The total cholesterol level was significantly reduced significantly at D30 and D90 compared to the pretreatment values. Triglycerides level was not affected during follow- up.

Glucose level

Glucose levels at different follow-up times were normal. However a trend towards lower values over follow-up was noticed. No significant difference was found between the values at different times (D0, D30 and D90) ($p>0.145$).

Blood pressure measurements

Blood pressure was also routinely measured at different times of follow-up. The values observed at different times were not significantly different. Patients under *R019* treatment did not develop hypertension or hypotension.

Adverse events (AEs)

Adverse events patterns identified with their respective frequencies are shown in the table IV. The most frequent adverse events were polyuria (53.12%), drowsiness (28.12%) and increased appetite (25%). No serious AEs were reported.

Table. 4: Nature and frequency of adverse events.

Adverse Events	*N(%)*
Headache	1 (3.12)
Drowsiness	9 (28.12)
Polyuria	17 (53.12)
Increased appetite	8 (25)
Asthenia	3 (9.37)
Diarrhea	4 (12.5)

DISCUSSION

Clinical, immunological and virological efficacy

A potent antiviral activity of *R019* was shown here by a significant reduction in viral load observed during the three months follow up of the AIDS patients. This result is consistent with ethno-pharmacological (Nikiema *et al.,* 2004a; Esimone *et al.,* 2005) and *in vitro* findings (Pengsuparp *et al.,* 1995; Neamati *et*

al., 1997; Cohen *et al.,* 1996; Esimone *et al.,* 2005; Phillips *et al.,* 1991) related to *R019*. However, none of the patients had an undetectable viral load at the end of the study period. The results obtained here were comparable with those obtained from Maroviroc in short term monotherapy (Fatkenheuer *et al.,* 2005). Compare to historical data, *R019* had superior antiretroviral activity compared with placebo (-0.82 *R019* vs -0.17 placebo) (Markowitz *et al.,* 2006). On the other hand, it is really important to determine the optimal dosage of *R019* using different doses and comparing it to placebo. Immunomodulatory effect of *R019* was clearly demonstrated with the increase of the CD4 cell count by a mean of 99 cells. Calabrese *et al.,* in a study of andrographolide in HIV infected patients found an increase of 96 CD4 cell counts after 6 weeks of administration (Calabrese *et al.,* 2000). Our results are in line with those seen in many clinical trials on lamuvidine, zidovudine, didanosine or ritonavir as well as in monotherapies or HAART (Markowitz *et al.,* 2006; Bartlett *et al.,* 1996; Gulick *et al.,* 1997) . Recently, Meynard *et al* in Kalesolo trial comparing lopinavir boosted by ritonavir(LPV/r) monotherapy versus current treatment continuation for maintenance therapy for HIV-1 infection found a gain of 98 CD4 cell counts in the LPV/r monotherapy arm (Meynard *et al.,* 2010) which is in the same range than the value obtained with patients under *R019* treatment . The respective increase of CD4 and decrease of viral load were more noticeable between D0 and D30 compared to D30 and D90. The decreased potency during the last period of treatment could be explained by the well known process of enzyme saturation or by a decrease in compliance. Although, the administration of the treatment is not supervised, it is probable that the low level of education and literacy of the majority of the patients could cause poor compliance and thereby impact negatively on *R019*'s efficacy. Moreover, the orexigenic effect of *R019* may have induced some habits such as administration of a lower dose than that recommended without notifying the health care professionals. In general, less literate patients with low incomes are more likely to engage in such practices. Although less probable, we can't ignore the development of resistance to *R019* treatment by the virus as explanation. Resistance tests have not been done in this study.

Regarding the clinical efficacy of the remedy, a significant increase of weight was observed over time. Similar results were obtained also for the Karnofsky index. Significant weight increase was obtained between D0 and D90 whereas no change was found between D0 and D30. This is similar to result obtained by Idigbe *et al.,* in the Nigerian antiretroviral program, in 2005 (Idigbe *et al.,* 2005).

Tolerability and Safety

R019 was found to have better tolerance than ARVs, which tend to exhibit a pattern of side effects often requiring the discontinuation or modification of the treatment (Calza *et al.,* 2004; Arribas *et al.,* 2010). In addition, liver function was not affected under *R019* treatment and there was a noticeable decrease in transaminases. This confirms the hepatoprotective property previously described for the *R019* (Perrey *et al.,* 2004). Also, rather than the expected increases in baseline fasting total cholesterol, LDL cholesterol and triglyceride levels observed with ART, *R019* shows a good metabolic profile. The total cholesterol was reduced significantly and no increase of triglycerides was detected. The presence of inositol, tannin and unsaturated fatty acids in the *R019* would certainly contribute to this effect (Bassene *et al.,* 1981; Ogan, 1972). This is a major benefit of *R019* considering the high frequency of metabolic complications associated with some combinaison of antiretrovilal regimens (Calza *et al.,* 2004). In addition to these pharmacological actions, a probable antianaemic property of *R019* would also require further investigation since a slight increase in the level of hemoglobin was observed. This increase in hemoglobin is very interesting because several studies (Moore *et al.,* 1998; Sullivan *et al.,* 1998; Mocroft *et al.,* 1999; Mekonnen *et al.,* 2003; Srasuebkul *et al.,* 2009), showed that anaemia in HIV-infected patients is associated with higher rates of disease progression and death, independently of the CD4 cell count and other prognostic factors. Besides the immunomodulatory and antiviral activities of *R019*, it appears to possess other pharmacological properties such as being antioxidant, diuretic, hepatoprotective, orexigenic and antihyperglycaemiant (Chika and Bello, 2010), which could be also the subject of future investigations.

It is worthy of note that despite the promise that *R019* holds as a potential anti-HIV remedy, the limitations of the study cannot be ignored. For example, three months of follow-up is too short a period to assess the efficacy of a medicinal plant *in vivo* against HIV/AIDS.

In spite of these limitations, the huge therapeutic potential of the remedy cannot be ignored given the significant improvement in all the relevant indicators achieved after three months of treatment, which compares favorably with similar results obtained with HAART or monotherapy antiretroviral after treatment over the same period. The results of this preliminary study are significant, not only because of its public health relevance for Benin and other countries facing similar health challenges, but also because of the increasing need for alternative therapies. Since this herbal treatment, as well as other such therapies, are already being used as viable, local alternatives, it behooves the scientific community to take up the challenge to evaluate their safety and efficacy because of their potential impact on global public health. Importantly, this study adds to the developing field of "Reverse Pharmacology" (Kaya, 2009), in which indigenous knowledge combined with a history of safe use and ethno-pharmacological efficacy, is exploited as an effective and faster approach to discover new medicines. Following this approach, a novel class of integrase inhibitors, the dicaffeoylquinic acids (DCQAs), was isolated from medicinal plants in Bolivia (Zhu *et al.,* 1999; Robinson et al., 1996).

CONCLUSION

This preliminary study shows that *R019* has an anti-HIV activity characterized by an increase in CD4 T cell count and a

significant decrease in viral load in HIV1 infected patients after 3 months of follow-up. Improvement of health status based on weight, BMI and Karnofsky Index, was also observed among patients. Furthermore, this study has shown that *R019* is well tolerated in patients, with no evidence of safety concerns when compared with known ADRs of ARVs. However, more work will be needed to standardize the product and its method of preparation in order to optimize the level of its active compounds. Thus, more data related to its clinical pharmacology are needed to ensure potential place among the growing list of effective anti HIV therapies. In order to achieve this, randomized, controlled clinical trials, and more long-term prospective studies will be required. In addition, a more effective strategy will be needed to improve compliance and hence the efficacy of *R019*.

DECLARATION OF CONFLICT OF INTERESTS

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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