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Immunological effect of aqueous extract of *Vernonia amygdalina* and a known immune booster called immunace[®] and their admixtures on HIV/AIDS clients: a comparative study

Momoh MA^{1*}, Muhamed U², Agboke AA³, Akpabio EI³, Uduma Eke Osonwa³¹Department of Pharmaceutics University of Nigeria Nsukka, Enugu State²Department of Chemistry, Ahmadu Bello University Zaria³Department of Pharmaceutics and Pharmaceutical Technology University of Uyo

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ABSTRACT

Objective: To investigate the immunological effect of *Vernonia amygdalina* (*V. amygdalina*) leaf extract and immunace[®] on HIV infected patients taking highly active antiretroviral therapy.

Methods: Fresh *V. amygdalina* leaves were collected within Nsukka area in Enugu State. The leaves were rinsed with distilled water. Two handful of cleaned fresh leaves were soaked in 200 mL water and squeezed gently by hand to a mixture. Clients were divided into four groups and each group was given different combination. They took the medication for four weeks. The immune effect was tested against marketed immune booster in some retroviral clients. **Results:** The mean absolute CD4 count was increased in the client who took the extract or supplement. And the clients who took both the extract and supplement had a greater increase in the CD4 count. The increased CD4 was significant as compared with the control group ($P < 0.05$). The skin rashes were also improved in the entire groups. **Conclusions:** It can be concluded that the aqueous extract of *V. amygdalina* and immunace or both have immunological effect on HIV infected patients. Therefore, we suggest that the *V. amygdalina* extract or immunace or both could be used as adjuvant in the management of HIV/AIDS clients.

1. Introduction

Two decades have now rolled by, since the first case of AIDS was diagnosed in Nigeria in 1986. In that period, many individuals, families, communities, businesses and Nigeria at large have experienced the devastation of the epidemic. Every community or any aspect of the nation has been affected. Nigeria's burden of care/epidemic now ranks third in the world[1]. The key to the pathogenicity of this virus is its genetic heterogeneity which is the result of several features, such as the error-prone enzyme reverse transcriptase which is estimated to introduce an average of one error per genome per replication cycle and the rapid turnover of HIV *in vivo*[2]. Recombination occurs at a rate of about 2% per kilobase per replication cycle with its selective immune pressures by the host[3]. The

CD4 lymphocyte count plays a major role in HIV/AIDS patients[4]. It is said to be decreased in HIV state as a result of destruction and inappropriate nutrient in the patient. Insufficient nutrient in client was directly affecting patients' performances on antiretroviral therapy (ART)[5]. Several prospective, randomized clinical trials now suggest that the clinical outcomes have been improved in HIV-infected patients who take micronutrient supplements. Researches have shown that daily doses of a micronutrient supplement taken by HIV-infected Tanzanian women, in multiple of the recommended dietary allowances including B-complex, vitamin C, and vitamin E, produced a significant increase in CD4 and CD8 cell counts when compared with those given placebo[6]. This CD4 cell decrease has been traced to the activity of oxidative cell, thereby affecting the immune system and patients' recovery rate on ART.

Vernonia amygdalina (*V. amygdalina*) (compositae) is a small shrub that grows predominantly in the tropical Africa. In Nigeria, the plant is locally called bitter leaf due to its bitter taste. The macerated leaves of the plant are used to make soup while the water extract serves as a tonic drink

*Corresponding author: Momoh MA, Department of Pharmaceutics University of Nigeria Nsukka, Enugu State.

Tel: 08037784357

E-mail: jointmomoh@yahoo.com

for the prevention of certain illnesses. The leaves have been found relevance in traditional folk medicine as anthelmintic, a laxative herb and an antimalarial as they are known as quinine substitute[7]. *V. amygdalina* has been reported for its use by wild chimpanzees for the treatment of parasite-related diseases in Tanzania[8]. Several stigmastane-type saponins such as vernoniosides A1, B1, A2, A3, B2, D3, A4 and C have been identified in the leaves[9]. The antioxidant activities of luteolin, luteolin 7-O, β -glucuronoside and luteolin 7-O- β -glucoside flavonoid compounds isolated from the leaves of *V. amygdalina* have been reported using coupled oxidation of β -carotene linoleic acid[10]. In this study we try to assess the antioxidant activity of *V. amygdalina* on the immune level in HIV sero-positive clients and compare it with immunace[®].

2. Materials and methods

2.1. Collection and preparation

Fresh *V. amygdalina* leaves were collected in Nsukka zone, Enugu State, Nigeria. The leaves were rinsed with distilled water. Two handful of cleaned fresh leaves were soaked in 200 mL of water and squeezed gently by hand to a mixture. The mixture was then filtered through clean white cloth to remove the particulate matter. The water extract was taken using glass tumbler twice a day. All the clients were administered with the extract for four months.

2.2. Study design

This was a prospective, randomized, double-blinded, placebo-controlled clinical trial designed to determine the effect of aqueous extract of *V. amygdalina* leaf and immunace capsule on HIV-infected patients who took the first line antiretroviral drugs in an approved ART centre.

2.3. Study subjects

Forty HIV-infected patients on a ART regimen for at least a year and whose base CD4 line was between 120–212 with little symptoms of rashes in the beginning were selected from the treatment site. All the clients involved in the study were requested to give their consent willingly. Information obtained was treated confidentially. The clients' name and address were completely avoided. The patients were divided into four groups i.e. A, B, C and D with ten clients in each group. Group A took immunace[®] drugs alone. Group B took *V. amygdalina* aqueous extract alone. Group C took immunace[®] plus *V. amygdalina* aqueous extract and group D served as the control group. The aqueous *V. amygdalina* extract and immunace[®] capsule were consumed twice daily with food. Antiretroviral medication was taken without any restriction in all the groups and clients were also allowed to take both together at the same time. All the clients were taught how to prepare the aqueous *V. amygdalina* extract

and the quantity to consume while immunace[®] capsules were taken according to the manufacturer specifications. All the medications were taken twice daily for a period of four months.

2.4. Clinical and laboratory evaluations

HIV/AIDS clients come to the clinic every month for drug refills and see their physician and pharmacist for any new complaint. Laboratory testing was performed at baseline every month for four months. It included a cluster designation CD4 lymphocyte count using Leftron machine, and the haematological indices such as plasma cell volume (PCV) were investigated using Abacus junior machine.

2.5. CD4⁺ cells count

A volume of 0.2 mL of blood sample collected was placed in a wamble mixer (model A4–20003, Partec Germany). An automated 20 μ L pipette was used to dispense 20 μ L of the CD4⁺ monoclonal antibody in a test tube. An equal volume of the sample blood was also transferred into the test tube and the content was mixed by tapping on the bench. The mixture was incubated in the dark at room temperature, followed by addition of CD4 buffer. This was further standardized using green check bead. The sample was plugged in the sample pot of the Cyflow machine (model: D–481611, Partec GMBH, Germany) and the cell count was recorded. This was done for all the batches.

2.6. PCV analysis

A volume of 2 mL of the blood samples collected in ethylene diamine tetraacetic acid (EDTA) were used. The samples were mixed in a Roller mixer at the rate of 30/min for 5 min. The following blood parameters including white blood cell (WBC), red blood cells (RBC) were determined using an automated haematological analyzer machine (Abacus Junior, Model S/N 111244).

2.7. Effects on the physiology of the body system

The effects of the medication on the sleeping pattern, food intake, stress and normal daily activities were also assessed.

2.8. Statistical analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS) and multiple comparison ANOVA was used.

3. Results

The phytochemical screening of the ethanolic extracts of the leaves revealed the presence of protein, saponins, flavonoids, cardiac glycosides, and tannins as shown in Table 1. These may be the active principles that account for the changes observed in this study.

Table 1
Result of phytochemical tests on *V. amygdalina* plant.

Tests	Inference
Alkaloids	+
Cyanogenetic glycosides	+
Cardiac glycosides	+
Steroidal glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Proteins	+
Carbohydrates	+

+: indicating presence; -: indicating absence.

Table 2
The results of characteristics study.

Groups	CD4 cell count		Skin rashes		PCV	
	Initial value	Final value	Initial value	Final value	Initial value	Final value
A	234.00±1.00	238.00±0.00	++++	+	24.00±0.00	26.00±0.41
B	196.00±0.30	205.00±0.00	+++	+	36.00±0.00	38.00±0.50
C	134.00±0.50	141.00±0.00	++	-	28.00±0.00	33.00±0.21
D	219.00±0.00	219.50±0.00	+++	-	31.00±0.10	32.00±0.23

A= immunace[®] alone, B = *V. amygdalina* extract alone, C = *V. amygdalina* and immunace[®], D = control group (only on ART).

4. Discussion

The phytochemical screening of the leaves revealed the presence of chemical compositions. The presence of these alkaloids may be the bases for the pharmacological effect as seen in this research.

The absolute CD4 count was increased by an average of 12.0% in the immunace plus *V. amygdalina* extract in group C, while 5.6% in immunace[®] (group A) and 4.0% in *V. amygdalina* extract (group B), and 1.0% change in the control group D (ART alone). Skin rashes were improved in all the groups. The difference is statistically significant ($P < 0.05$). PCV, a component which is indirect or direct linked to CD4 cell was slightly increased and the extent of the increase depends on the substance administered. The increase in CD4⁺ cell count after administration of *V. amygdalina* extract and immunace[®] was the highest among all the groups. It may be attributed to the synergistic effect produced by *V. amygdalina* extract and immunace[®] which have similar effect on certain cells. The result in our study was in agreement with the previous research^[11]. However, the change in the CD4⁺ cells count in the treated groups occurred after 4 months of administration. Although it is a short term study, it is believed that with the increase in the cells count, one can easily say that the clients in the test groups are doing well and this increase can not be associated with extracellular CD4 which has been mopped up during immune reconstitution stage as reported in our previous study^[12]. The inconsistency observed in the CD4 cell count depends on the white blood cell count, the percentage of lymphocytes and the CD4 receptor bearing lymphocyte. In HIV state the CD4 cells are destroyed and subsequently reduced to a very lower level so that the ability to prevent infections is decreased, but the body may regain its ability

The results accounted for the relevant changes that took place especially in the area of the skin rashes, the improvement in the cluster designation CD4 cells and the changes in PCV (Table 2). PCV is an important factor because this determines the drugs effect on the RBC, in the principle of immunity and plays a major role in the management of the infection. Comparison of the immune increase in non-treated, extracts-treated and extract combined with drug treated subjects showed a little progression in all the groups. Formulation C change from (28.00±0.00 to 33.00±0.21), was the best as compared with A (24.00±0.00 to 26.00±0.41), B (36.00±0.00 to 38.00±0.50) and the control D (31.00±0.10 to 32.00±0.23).

over a certain period of time due to a process called immune reconstitution, which entails mopping the extracellular cells. The CD4⁺ T cells rush into the germinal centres to help β-cells fight the invaders and subsequently may become infected as they encountered HIV and trapped on follicular dendritic cells (FDCs). Once infected these cells may leave the germinal centre and infect other CD4⁺ cells that aggregate in the region. In and around the germinal centres, numerous CD4⁺ T cells are probably activated by the increased production of cytokinase such as tumour necrosis factor alpha (TNF-α) possibly secreted by β-cells^[13]. The importance of CD4 cells determination should be emphasized as it assists in predicting the stages of infections, the bases of therapeutics management and or a guide to both antiviral therapy and prophylaxis for opportunistic infection. The CD4 cell count is also a relatively consistent indicator of the response to ART^[14]. The precisely mechanism of how this plant extract and immunace[®] increase the CD4⁺ cells count is presently unknown. Moreover, the phytochemical study of this extract reveals a lot of constituents that have antioxidants properties and have been linked to immune stabilization^[15]. Other possible mechanisms include early maturation of leucocyte cell^[16], release of leucocyte cells^[17] and prevention of cell apoptosis^[18]. The theory of apoptosis has been supported by a study which claimed that antioxidant supplementation was implicated in decreasing the frequency of CD4⁺ cells apoptosis especially in immunocompromised situations^[19]. On the physiological effects none of the subjects shows any abnormalities in the sleeping patterns who were on pure ART without supplement. At the initial stage less than 0.5% complained of inability to have a sound sleep. This effect disappeared at the second stage of the study. Similar effect was observed in most of the clients in the ART centres who were placed on the first regimen of the ART, especially

combination that contained nevirapine, stavudine and zidovudine[20]. This effect was neither observed in the subjects taken *V. amygdalina* extract, immunace nor both in combination with ART. The food intake showed a better improvement in clients who took the combination of the supplement and the drug than in clients who took the drugs alone. This effect could be attributed to the nutritional value of the plant extract, as previous study has shown that the plant is often used as vegetable in the preparation of soup[21]. None of the clients complained of any problems in the daily activities such as going to work. All show a remarkable improvement. These information regarding the physiological effects was obtained orally from our clients, because they were made to document whatever effect they noticed while on the medications. Immunace[®] is mainly composed of vital essential vitamins and some antioxidants. The effect of immunace[®] could also be attributed to the various antioxidant composition as written in the manufacturer instruction.

Conclusively, the present study revealed immune stimulating activity of *V. amygdalina* leaves extracts and its combination with immunace[®] regarding the improvement of CD4 and enhanced PCV responses. Although the exact mechanism of this effect is not clear, it may be mediated by interactions between active components of extracts and cell surface molecules or growth factors involved in cells activation. Another possible action of extracts may be interference with cell signaling. Although further investigations are warranted, to our knowledge the results suggest that *V. amygdalina* leaves extract may have immunological actions through transient and early maturation of cells and hindrance of cell destruction. Therefore, *V. amygdalina* could serve as a nutritional supplement in a HIV-infected or immunocompromised condition such as cancer or diabetes patients to a less extent.

Conflict of interest statement

We declare that we have no conflict of interest.

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