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# *Vernonia amygdalina* Extract and CD4+ Cell Counts: An Immune Study

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**Abstract:** *Vernonia amygdalina* Del. (Family Compositae) is used in Nigerian folk medicine as a tonic and remedy against constipation, fever, high blood pressure and many infectious diseases. Many minor components of foods, such as secondary plant metabolites, have been shown to posse antioxidant activities, improving the effects of oxidative stress on diabetes and other disease conditions. This study was carried out to access the effect of V*ernonia amygdalina* plant extract on the CD4+ cells count of diabetes rats. Three different concentrations were orally administered to the rats using nasogastric tube once daily for a period of 21 days. There was a significant increase in CD4+ cells as compared to the control group ($p > 0.05$). The increase in CD4+ cells was concentration dependent. In conclusion, herbal-based and plant-derived products can be exploited with sustainable comparative and competitive advantage.

**Key words:** Evaluate · CD4+cell · Immune · *Vernonia amygdalina*

## INTRODUCTION

The use of plant-derived natural compounds as part of herbal preparations as alternative sources of medicaments continues to play major roles in the general wellness of people all over the world. Over 50 % of all modern clinical drugs are of natural product origin [1] and natural products play an important role in drug development programs of the pharmaceutical industry [2]. In developing countries, especially in rural contexts, people usually turn to traditional healers when in diseased conditions and plants of ethnobotanical origin are often presented for use. Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents [3]. Presently, many scientists and organizations are in search of traditional remedies as alternate medicine [4]. Thus many plants that are used in traditional practice are sold in a rounded urban settlement to meet the need of a public desire for panaceas which has resulted in the industrialization and large scale production of a great number of products of botanical origin widely consumed. In spite of the heterogeneous nature of the continent and a deluge of information on the composition and biological activity of many plant substances, there has been little effort devoted to the development of chemotherapeutic and prophylactic agents from these plants. *Vernonia 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 in making soup, while the water extract serves as a tonic drink for the prevention of certain illnesses. The leaves have found relevance in traditional folk medicine as antihelmint, a laxative herb and an antimalarial as they are known as quinine substitute. *V. amygdalina* has been reported for its use by wild chimpanzees for the treatment of parasite-related diseases in Tanzania [5]. Several stigmastane-type saponins such as vernoniosides A1, B1, A2, A3, B2, D3, A4 and C have been identified in the leaves [6]. Phillipson *et al.* [7] reported the antiplasmodial effects of some sesquiterpene and steroidal constituents of *V. amygdalina* and some were also effective against *Plasmodium falciparum in vitro*. The antioxidant activities of luteolin, luteolin 7-0,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 [8]. In Nigeria, recently, there has been an increase in research into medicinal plants, with the view to providing alternatives

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to or supplementing imported drugs thereby preserving our foreign exchange reserves. But, toxic actions on certain special cells, especially the T lymphocytes are a limitation to the potential usefulness of any agent and may even lead to loss of immunity and *Vernonia* species are the leading focus in medicines. It is for this reason, that this study is primarily designed to determine the effect of the extract on the CD4+ cells count on diabetes rats.

## MATERIALS AND METHODS

**Materials:** *Vernonia amygdalina* plant, Albino rats, check bead, alloxan monohydrate (Sigma St. Lious, USA), CD4+ reagents test kits, End runner mill, Filter paper, nasogastric tube. All other reagents are purchased from it local sources and used without modification.

### Methods

**Extraction of *Vernonia amygdalina* Leaf Constituents:** The fresh leaves of *Vernonian amygdalina* plant were collected from University of Nigeria campus within Nsukka zone of Enugu state, Nigeria and identified at Bioresources Development and Conservation Program (BDCP) Nsukka. The clean leaves were sorted, washed and remove all the dirty without squeezing and sun dried for seven days. The dried leaves were milled into coarse powder. A 500 g of the resulting milled coarse powder was soaked with two litres of distilled water and the mixture shaking for 10 mins and allowed to stand for 48 hrs before filtering. The filtrate was then placed in an oven to evaporate at a temperature of 40°C and the residue refers to as extract was stored in an air tight container until used.

**Phytochemical Screening of *Vanonia amygdalina* Extract:** The water leaves extract of *Vernonia amygdalina* were subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Brain and Turner [9].

**Preparation of the Samples for CD4+ Cells Count:** Twenty Wistar rats of either sex weighing 120-160 g fasted for 18 h were made hyperglycemic by a single intraperitoneal injection of alloxan monohydrate dissolved in normal saline as a vehicle at a dose of 200 mg/kg body weight. After 48 h of alloxan injection, rats exhibiting plasma glucose level of greater than 220 mg/dl were included in the study and divided into four batches (A, B, C, D) of five rats each. Rats were kept in cages which have wood shave as bedding and cleaned once a week. The feeds and water were provided *ad libitum* in earthen troughs. Rats were kept in groups, each of 5 animals in each cage marked A, B, C and D under the same environmental and management conditions. After 2 weeks of stabilization, rats in groups B, C and D were given aqueous extract of *Vernonia amygdalina* leaf at 200, 400 and 800 mg/kg' body weight, respectively, orally for 21 days using nasogastric tube to introduced the extract directly into the stomach through the oesophagus. Group A rats were the control with no administration of the extract. After 24 h of the last dose, blood samples were collected through the retro-bulbar plexus of median canthus of the eye into an EDTA bottle and used for the study.

**CD4+ Cells Count Study:** A 0.2 ml volume of blood sample from above mentioned rats was placed in a Wamble mixer (model A4-20003, Partec Germany). An automated 20 µl pipette was used to dispense 20 µl 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 mixed by tapping on the bench. The mixture was incubated in the dark at room temperature, followed by addition of CD4 buffer. This was 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.

**Statistical Analysis:** The mean percentages and standard error of mean were calculated for changing in CD4+ cells study. ANOVA (Analysis of variance) was used to establish any significant difference in this parameter.

## RESULTS AND DISCUSSION

The result of the phytochemical analysis (Table 1) shows that the *Vernonia amygdalina* aqueous extract contains alkaloids, flavonoids, steroids, glycosides, tannins and saponins, but does not contain cynogenetic compound. The results from phytochemical analysis have been observed to be consistent with findings in several *Vernonia* spp as reported by the previous authors [10, 11]. Glycosides are found commonly in *Vernonia* and include the galactose-arabitol glycoside (umbilicin) and the galactose-mannitol (peltigeroside) both of which abound in different *Vernonia* genera [12].

Table 1: Result of Phytochemical Tests on *Vernonia amygdalina* plant

| Test | Inference |
|---|---|
| Alkaloids | + |
| Cyanogenetic Glycosides | + |
| Cardiac Glycosides | + |
| Anthracene Glycosides | - |
| Steroidal Glycosides | + |
| Saponins | + |
| Tannins | + |
| Flavonoids | + |
| Proteins | + |
| Carbohydrates | + |

Key: + indicates presence of phytochemical secondary metabolite
- indicates absence of phytochemical secondary metabolite

However, the robust recuperation of the $CD4^+$ cells count in the *Vernonia* treated animal groups occurred after 21 days of administration, a relatively short time, that one cannot easily know whether such rise in $CD4^+$ count could be sustained for a long period of time. The use of diabetes rats in this study was to liken the animal to immuno-compromised condition, although losses of electrolytes in the system as a result of diabetes

**Effect of the Extract on the CD+ Cell:** The results showed sustained increases in CD4+ cells, averaging about 25 extra cells at the end of the study in contrast to the control group as shown in Fig. 1. This difference was statistically significant ($p>0.005$). The increase in $CD4^+$ cell count after administration of *V. amygdalina* extract was concentration dependent. It was observed that the groups of rats that had the highest dose of the aqueous extract (800, 400 and 200 mg/kg body weight) had their $CD4^+$ cell 79.8 ±6.0, 63.6±4.6 and 55.2±8.1 cell/µl, respectively) significantly higher ($P<0.05$) than that of the control group (50.2±6.3 cells/µl). The higher the dosage the more the effect of the extract on the $CD4^+$ cells (Fig. 1). The results of the activities of the extract on the $CD4^+$ cells was observed to be higher in the batch D as compare to B, C and A, this simply indicated that higher concentration gave a better result, the control show the least figure for $CD4^+$ cell. The results were in the following order: D>C>B>A as shown in Fig. 1.

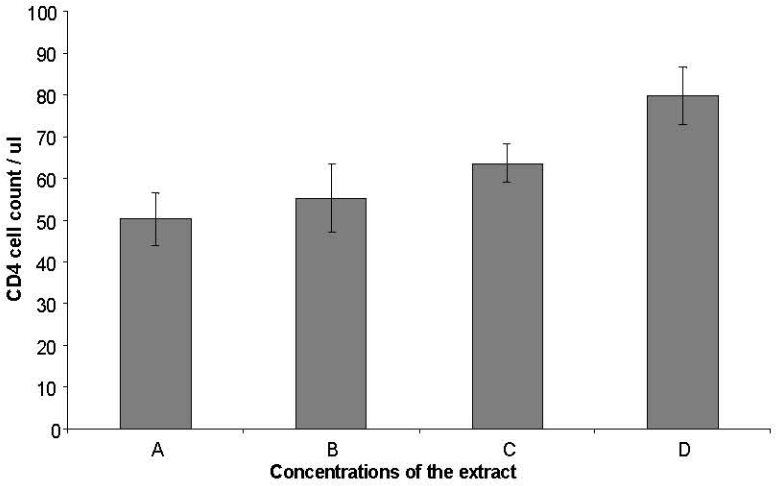


Fig. 1: Shows the effect of various concentration of the extract on $CD4^+$ cell
Note: Values are given as mean ± SD for 5 rats in each group; experimental groups are compared with control and various doses. Values are statistically significant at = $P<0.05$.

**Composition of Immunace According to Manufacturer Directive:**

**Descriptives**

CD4+ Cell count

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| A | 5 | 50.2000 | 6.30079 | 2.81780 | 42.3765 | 58.0235 | 43.00 | 57.00 |
| B | 5 | 55.2000 | 8.13634 | 3.63868 | 45.0974 | 65.3026 | 45.00 | 63.00 |
| C | 5 | 63.6000 | 4.61519 | 2.06398 | 57.8695 | 69.3305 | 58.00 | 69.00 |
| D | 5 | 79.8000 | 6.83374 | 3.05614 | 71.3148 | 88.2852 | 69.00 | 87.00 |
| Total | 20 | 62.2000 | 13.01659 | 2.91060 | 56.1080 | 68.2920 | 43.00 | 87.00 |

ANOVA

| CD4+ Cell count | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2523.600 | 3 | 841.200 | 19.349 | .000 |
| Within Groups | 695.600 | 16 | 43.475 | | |
| Total | 3219.200 | 19 | | | |

condition is said to impair immune development [13]. The mechanism of precisely how this plant extract may increase the $CD4^+$ cells count is presently unknown. Although it is not possible to determine from this study which of the components of the plant extract or their combination is responsible for the observed increased $CD4^+$ count, flavonoids, tannins and saponin have previously been shown to have antioxidant activity which invariably enhance cellular integrity [13]. Moreover, the phytochemical study of this plant revealed a lot of constituents that have antioxidants properties (saponnins, flavonoid and tannins). Each of these constituents has individually been shown to produce antioxidant effect [14]. Another proposed mechanism of action, could be as a result of enhanced early maturation and release of leucocytes, since transient interactions between leucocytes have long been known to be critical for the normal function of the immune system [15]. In addition, antioxidant supplementation has previously been shown to produce a significant decrease in $CD4^+$ cells apoptosis, especially in immuno compromised situations [16]. Studies have shown that, flavonoids were also found in several medicinal plants and herbal remedies containing flavonoids such as quercetin (e.g. *Gingko biloba, Grindelia camporum, Marrubium vulgare*) have been used in folk medicine for the treatment of several diseases, including those affecting the respiratory tract [17]. Among the many different flavonoids present in plants, quercetin was the most abundant [18]. Quercetin shows a wide range of biological activities, including inhibition of $Na^+$ / $K^+$ ATPase, histamine release, protein kinase C, tyrosine kinase, angiogenesis, angiotensin-converting enzyme II and intestinal peristalsis. Moreover, antioxidant, anticarcinogenic, antihypertensive, antiinflammatory, pro-apoptotic, hepatoprotective effects, a superoxide dismutase-like activity and modulation of cell cycle and improved immune status have also been reported for quercetin [19].

It can be therefore concluded that dosages between 200-800 mg/kg body weight of the aqueous leaf extract of *Vernonia amygdalina* for as long as 2l days will have a positive effects on the $CD4^+$ cells of the Wistar rats used for the study and so it is advised that aqueous leaf extracts of *Vernonia amygdalina* can be used as immune booster in immune compromised health conditions.

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