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# Oxidative Stress Attenuation in Hiv/Aids Patients on Antiretroviral Drugs by Calyx Juice of *Hibiscus sabdariffa Linn* (Malvaceae)

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**Abstract:** The present study was aimed at evaluating the *in vivo* antioxidant potential of *Hibiscus sabdariffa Linnaeus* calyx juice (foléré) in HIV/AIDS patients on antiretroviral therapy. In the study, 5g of dried calyces were decocted with 250 mL of fresh tap water at 95°C for 15 minutes. The experimental design was a case-control randomized blinded trial in which, 58 patients on antiretroviral drugs were divided into 2 groups age-and-sex-matched. The experimental group was served a glass of 250 mL calyx juice per day alongside their antiretroviral treatment while the control group was exclusively on antiretroviral drugs with no available placebo. The trial was run for three (3) months after which blood samples were analyzed for the biochemical aspartate aminotransferase (ASAT) and alanine aminotransferase ALAT) and the hematological CD4, white blood cells (WBCs), red blood cells (RBCs), and hemoglobin (HGB)] parameters. The results show an increase in RBCs and WBCs in the experimental group which on the other hand experienced a decrease in CD4 and HGB. The control group experienced a decrease in RBCs, less decrease in HGB, and a higher decrease in CD4, but with increased WBCs. There was a parallel increase in ALAT and ASAT in both groups, more elevated in the control group.

**Keywords:** Oxidative stress, HIV/AIDS, Antiretroviral, Antioxidant, *Hibiscus sabdariffa Lin*

## 1. Introduction

More than three decades [1] after its outbreak, the Acquired Immune Deficiency Syndrome (AIDS) remains a great mystery because there is neither an existing vaccine against its causative agent (the human immune deficiency virus) nor a cure against it. Worst of all is the popular attention that is tuned to the AIDS virus, ignoring oxidative stress which is the major cause of mortality in HIV/AIDS as in many other chronic diseases. Antiretroviral drugs introduced in 1996 [2] have been shown to increase oxidative stress among other drug-related complications [3, 4]. With these backdrops, an antioxidant therapy is necessary to accompany antiretroviral treatment without which its beneficial effects are null.

Interestingly, research findings have reported the antioxidant properties of the calyces of *Hibiscus sabdariifa* of the sabdariffa Linn variety [5-12] (Roselle), highly consumed as a juice commonly called 'foléré' in the Fulfulde language of northern Cameroon. To the best of our knowledge, the verification of this antioxidant property *in vivo* in HIV/AIDS patients on antiretroviral drugs is still lacking. Thus the present study was aimed at evaluating the *in vivo* antioxidant potential of *Hibiscus sabdariffa Linnaeus* (foléré) calyx juice in HIV/AIDS patients on antiretroviral therapy.

## 2. Materials and Methods

### *2.1. Procurement of Dried Calyces*

Dried calyces of *Hibiscus sabdariffa* L. were purchased

from the "Pétit marché", a local market in Ngaoundéré, Adamawa Region of Cameroon and this, according to the retailer, was imported from Ndjamena-Chad. The calyces were approved by Dr. TCHOBSALA, Department of Biological Sciences in the Faculty of Science, University of Ngaoundéré.

### *2.2. Preparation of Calyx Juice*

in the study, one hundred and fifty grams (150g) dried calyces were weighed out using an electronic balance (Gilbertini serial number 125185, Italy) and decocted in the ratio w/v: 1/50 at 95 °C for 15 minutes after which the extract was filtered using a traditional sieve. To the extract destined for consumption, sugar was added in the ratio of 1:16 (w/v) to improve on the taste.

### *2.3. Phytochemical Analyses of Extract*

A sample of the crude extract was analyzed for total polyphenol content as described by Siddhuraju and Becker in 2007 [13], total flavonoids as described by Zhishen et al in 1999 [14] and by Ozgen et al in 2006 [15] and the antioxidant potential (% radical scavenging activity) as described by Philip Molineux in 2004 [16]. All analyses were carried out in triplicates.

### *2.4. Experimental Design*

The study design here was a randomized blinded case-control study in which fifty-eight (58) enrolled participants were divided into two equal groups (n = 29) that were age and sex-matched, ignoring their socio-economic backgrounds after having taken their baseline blood biochemical (ASAT and ALAT) and hematological parameters . The age distribution gave 35±9 and 35±7 years for groups 1 and 2 respectively. Group 1 constituted HIV/AIDS patients on highly active antiretroviral drugs (HAART) and receiving 250 mL of the *Hibiscus sabdariffa* L. beverage/day (experimental group). Group 2 on the other hand was composed of HIV/AIDS patients exclusively on antiretroviral drugs (control group), but with no available placebo. The trial was run for a period of three (3) months after which, various biochemical (ASAT and ALAT) and hematological (CD4, WBCs, RBCs, HGB) parameters were analyzed.

#### *2.4.1. Collection (Day 0)*

This was done on day 0, prior to initiation of trial. Blood samples from each of the retained participants were withdrawn for immediate biochemical and hematological analyses.

#### *2.4.2. Collection (Day 90)*

At the end of 3 months, samples were withdrawn from each of the 2 groups for the same analyses.

### *2.5. Biochemical and Hematological Assays*

The liver function enzymes ASAT and ALAT were assayed using BA-88A Semi-Auto Chemistry Analyzer from Shenzhen Mindray Bio-Medical Electronics Co., LTD, complete blood cells count was carried out with BC-3200 Auto Hematology Analyzer from Shenzhen Mindray Bio-Medical Electronics Co., LTD; meanwhile the BD FACSCount ™ System was used for the CD4 count.

### *2.6. Statistical Analyses*

The results were analyzed for statistical significance by the PAIRED T-TEST and completed by the more relevant SIGN-TEST and SIGNED RANK TEST (for within-group analyses). Between-group analyses were carried out using the non-parametric KOLMOGOROV-SMIRNOV TEST. The PEARSON CORRELATIONS TEST established links between studied parameters. The results are expressed as mean ± standard deviation with $P < 0.05$ meaning significantly different. The statistical package used was STATGRAPHICS® Plus, version 5.0.

## 3. Results

***Table 1.*** *Phytochemical composition and antioxidant potential of calyx extract.*

| Daily intake | Total polyphenols | Total flavonoids | Antioxidant potential |
|---|---|---|---|
| 5g/250mL | 1.38±0.058mgGAE/g DW | 1.02±0.060 mg/g DW | %RSA: 54.030% |

Values are mean ± standard deviation (S.D), n = 3; DW = dry weight; GAE = Gallic acid equivalent

Results in figure 1 show that the CD4 counts of both study groups on day 90 decreased, though not significantly P = 0.225893 for the control group while in the experimental group, P = 0.689123. Between-group non-parametric analysis using the KOLMOGOROV-SMIRNOV TEST shows no significant difference between the baseline CD4 count of the two groups (P = 0.122476) meanwhile; there was a significant difference between the groups in their day 90 CD4 count (P = 0.0308301). The rate of decrease was higher in the control group than in the experimental group as seen in their P values

There was a general increase in the white blood cells population in the 2 study groups in the face of a decreasing CD4 count. This increase was significant in the experimental group (P = 0.00831813), but not in the control group (P = 0.0528035).The KOLMOGOROV-SMIRNOV TEST shows no significant difference between the two groups in both the baseline and final WBCs population (P = 0.781826). The PEARSON correlations test gives a statistically significant non-zero correlation between the CD4 and WBCs at the 95% confidence interval (P = 0.0133; correlation coefficient = 0.3233).

The experimental group witnessed an increase in the red cells population which however decreased in the control group. The observed changes in both groups were not significant at the 95% confidence interval (P > 0.05). Non-

parametric analysis using the KOLMOGOROV-SMIRNOV test shows that there is no statistically significant difference between the two groups for the various pairs in both the baseline and final values (P = 0.997757for the baseline; and P = 0.122476 for the final values).

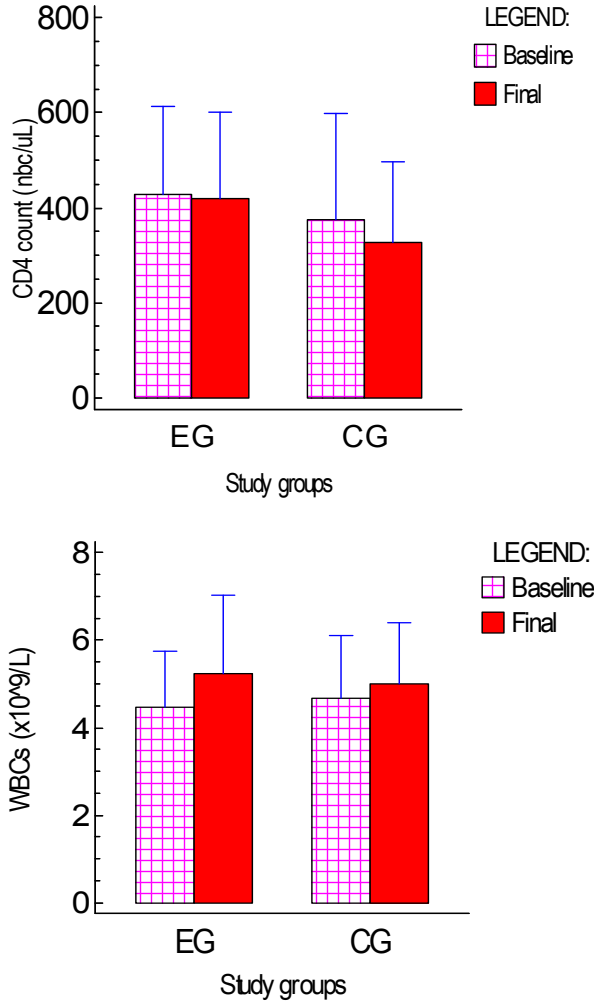
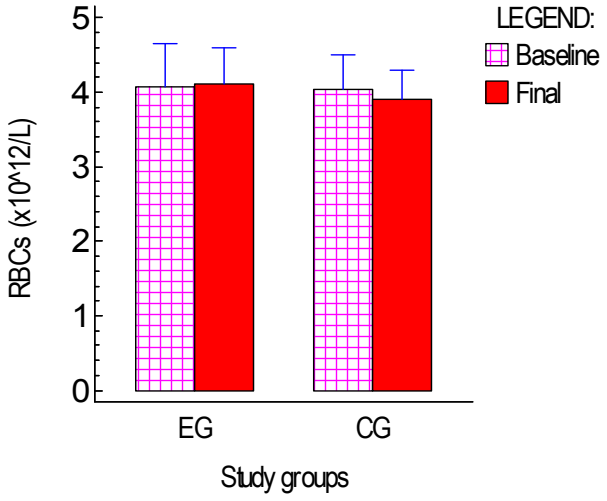



***Figure 1.*** *Effects of calyx juice on the CD4 count and WBCs.*



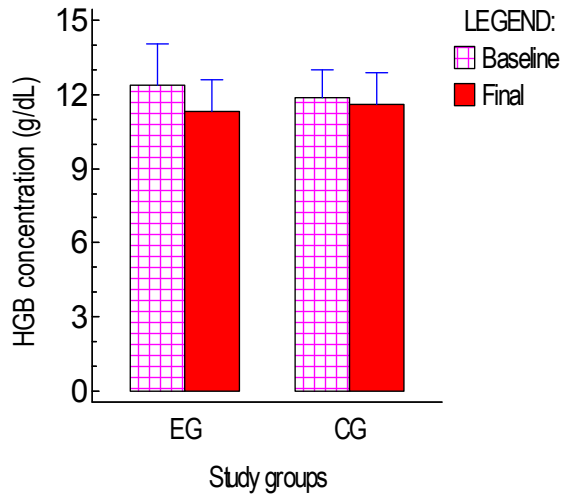




***Figure 2.*** *Effects of calyx juice on the RBCs count and HGB level.*

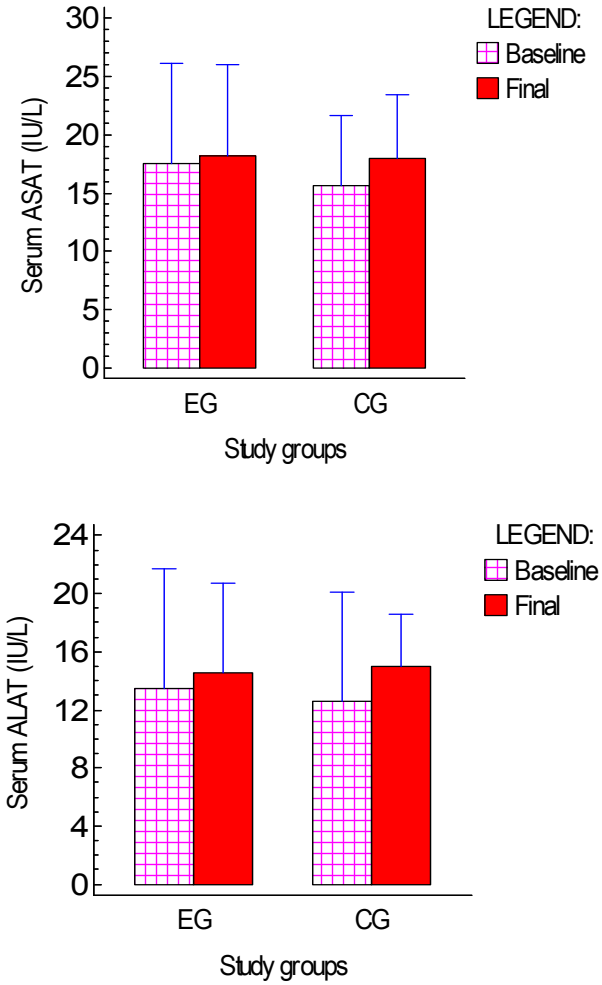



***Figure 3.*** *Effect of calyx juice on the enzymes activity (ASAT&ALAT).*

Hemoglobin is a component of the RBC which formation usually follows the later stages of erythropoiesis (RBCs formation). We observed a general decrease in both study groups as indicated in figure 2 and this decrease was statistically significant in the experimental group (P = 0.00001935) but not significant in the control group (P =

0.111687). The KOLMOGOROV-SMIRNOV test shows no significant difference between the two groups in both the baseline and final HGB content (P = 0.0636019for the baseline value, and P = 0.56405 for the final value). The PEARSON correlations test gives a statistically significant non-zero correlation between RBCs and HGB at the 95% confidence interval (P = 0.0046; correlation coefficient = 0.3669).

There was a parallel increase in ASAT and ALAT in both study groups, more marked in the control group. For both groups, the observed increases were not significant (P > 0.05) with the PAIRED TEST, SIGN-TEST and the SIGNED-RANK TEST except the ALAT of the control group which was statistically significant with the SIGN-TEST and the SIGNED-RANK TEST (P = 0.0223251with the SIGNED-RANK TEST). The KOLMOGOROV-SMIRNOV test show no significant difference between the two groups in the baseline and final values of the two enzymes (ASAT and ALAT) activities at the 95% confidence interval (P > 0.05). The PEARSON correlations test established a statistically significant non-zero correlation between ASAT and ALAT at the 95% confidence interval (P = 0.0000; correlation coefficient = 0.6925).

# 4. Discussions and Conclusions

Results from our phytochemical analysis confirm available literature claim that the flavonoids are the most abundant of all the classes of polyphenol. In our study, flavonoids constitute approximately 75% of total polyphenols present in the calyx extract. The calyx extract showed a total polyphenol content of 1.38±0.058mg GAE/g, slightly lower than 1.85 ± 0.11 mg GAE/g [12] obtained by some authors. This difference is accounted for by the differential heat treatment which destroys some of the bioactive substances [6]. In the authors' method, less heat treatment was applied; the calyces were lyophilized with a freeze dryer, stored in an air-tight container at -20 ˚C and their extraction was carried out at room temperature using an orbital shaker. On the other hand, a hot water extract by another group of workers yielded a total polyphenol content of 1.10 ± 0.03 mg GAE/g [10], slightly lower than our result. The said authors applied excessive heat treatment to calyces during post-harvest processing: oven drying of calyces and the prolonged extraction duration of 4 hours at 90˚C. However, even with similar post-harvest treatment procedures, differences usually arise in the phytochemical or nutritional composition and these are usually accounted for by a variety of factors among which are varieties, genetics, environment, ecology and harvesting conditions [20].The presence of polyphenols and flavonoids in the extract is a confirmation of previous findings which reveal that the calyces have an antioxidant potential and thus could attenuate oxidative stress in HIV/AIDS patients subjected to antiretroviral treatment. The percent radical scavenging activity (% RSA) of the extract evaluated at 54.03% is a measure of the total antioxidant (reducing) power of the extract. This parameter is more informative than the total polyphenols and flavonoids in that the total antioxidant power here includes all other reducing agents found in the juice such as vitamin C, other classes of polyphenols and the carotenoids.

Given the physiology in HIV/AIDS patients who generally experience oxidative stress complicated by the toxic effects of antiretroviral drugs, there is free radical-induced apoptosis of CD4 cells population, leading to the immunological suppression observed in the CD4 cells count of both groups (< 600 nbc/μL). Also worth-noting is the fact that most of our participants live in an economically low setting as our socio-demographic findings reveal. According to the information we gathered from our questionnaires, their daily meals were largely constituted of carbohydrates, with negligible intake of proteins, legumes and fruits. Legumes and fruits especially, were only consumed seasonally as many of the participants claimed and cognizant of the nutritional input of these two plant products in terms of antioxidants, an account of the generally observed immune suppression cannot undermine the influence of these two factors. Comparing the immunological status of the two groups, we see that there is a net amelioration of the state of the experimental group and this, despite the poor nutritional status, was thanks to the influence of the antioxidants present in calyx juice, especially the polyphenols.

The increase in the WBCs population observed in both groups is in conformity with recent findings which reveal that late–stage HIV/AIDS is characterized by profound immune deficiency while earlier stages are marked by ongoing immune activation and dysregulation, leading to the proliferation of the white blood cells population [3, 4, 17-21].The elevated white blood cells observed in both groups is an indication of the extent of immune stimulation by oxidative stress and this is usually mediated through inflammatory reactions by free radicals [22-24]. The higher WBCs population observed in the experimental group is an indication of the higher immune competence displayed by this group and this, through the antioxidant properties of the calyx juice.

Increased red cells population in the experimental group compared to the control group could be due to the beneficial effects of the calyx juice and this, thanks to the antioxidants present. Nevertheless, the increase could be significant if the levels of an anti nutrient like saponin in the extract are checked. Much research works on Roselle calyces have documented the presence of saponin among other anti nutrients like phytates and oxalates [10]. Anti nutrients are compounds which reduce nutrient utilization and/or food intake of plant products used as human or animal feeds [25]. Saponins (foaming glycosides) are glycosidic compounds whose structure is composed of two sub-units: a hydrophobic fat-soluble nucleus termed aglycone [85,86] which can either be of a steroid, alkaloid, or triterpenoid origin [26] linked to one or more water-soluble sugar side chains (glycone) through ether or ester bonds [27], giving them their particular surfactant property [26]. These substances have been widely documented for their pharmacological, medicinal and

hemolytic properties. Their hemolytic activity has been demonstrated *in vitro* and this is attributed to the aglycone moiety which has affinity for the phospholipids of the cell membrane with which they form insoluble complexes [27-29]. These complexes create pores on the membranes allowing free movement of substances in and out of the cell with their consequent lysis [27]. The pore-forming property has also been shown to increase the permeability of the brush-border region of the gastro-intestinal tract to large molecules that are normally un-absorbable [28].

The significant decrease in HGB in the experimental group contradicts our hypothesis that extract increases HGB. The hypothesis is based on research findings which reveal that calyces contain iron (Fe) among other micronutrients [8] as well as vitamin C [6], a powerful promoter of non heme iron absorption [30] at the gastro intestinal tract. Iron is an essential element for erythropoiesis since it directly contributes to HGB production at the later stages of erythroid differentiation [31]. Indirectly, for an approximate number of 200 billion RBCs produced daily, 20-25 mg of Fe is required [31]. The results at hand show that unlike oxidative stress that is responsible for the reduction of RBCs and HGB in the control group, there should be some intrinsic factors in the calyces that are responsible for the decrease HGB content of the experimental group.

Phytate (myo-inositol(1,2,3,4,5,6) hexakisphosphate) is a common constituent of plant-based foods and behaves in a broad $P^H$ region as a highly negatively charged ion with a high affinity for food components with positive charges such as minerals, trace elements and proteins [32]. Its six (6) phosphate groups are very reactive, making the molecule to fulfill the requirements of a chelating agent [32]. The formation of insoluble metal cation-phytate complexes at physiological $P^H$ values accounts for the poor mineral availability since the complexes are un-absorbable from the gastro intestinal tract [32]. Oxalate (ethanedioate) acts in a similar manner as phytate to reduce absorption of iron [30, 32].

Serum ASAT and ALAT are an indication of hepatic intracellular enzymes that have leaked into the circulation [33]. ALAT being more specific to the liver, an increased serum concentration is indicative of liver damage. ASAT on its part exists in two (2) isoforms indistinguishable on standard ASAT assays: the mitochondrial form produced in the hepatocytes and the cytosolic form produced in the skeletal muscles, heart muscles and kidney tissues [34]. Parallel measurement of the two enzymes is usually aimed at separating liver damage from heart or skeletal muscle. The generally observed normal mean values for the two groups viz à viz the standard reference of ASAT < 31 UI/L and ALAT < 34 UI/L for women; ASAT < 35 UI/L and ALAT < 45 UI/L for men (IUPAC) indicates normal liver functioning and this may be accounted for, by two main findings from our study such as drug toxicity and duration of antiretroviral treatment. From our findings on the therapeutic regimens administered to participants, just one (01) of the study participants was administered the combination HAART-2 which is comprised of zidovudine + lamivudine + effavirenz (AZT+3TC+EFV). Studies reveal that this therapeutic combination is the most toxic of the regularly prescribed combinations [3, 4] reasons why it is recommended as a second line therapy following failure of other prescriptions. As well, our findings reveal that the duration of treatment of most of the participants in this study ranges from 2 months to 8 years with at least 95% of the total population having therapeutic durations below 8 years. The observed parallel increase in the two enzymes is an indication of future attainment to liver disorders and this, especially in the control group which recorded a significant increase in ALAT. The lower increase in the experimental group signifies improved treatment which can be due to the cytoprotective influence of the juice. Cytoprotection, attributed to the polyphenols, is associated with the induction of phase II and antioxidant enzymes [35]. These enzymes are principally involved in the biotransformation of harmful substances into less toxic forms which can be easily eliminated from the human system.

Calyx juice of *Hibiscus sabdariffa Linn* Possesses some beneficial health effects when associated with antiretroviral treatment; however, presence of anti nutrients should never be neglected when consuming this natural product.

## Authorship Contribution

All authors contributed to the design, preparation, editing, and final review of the manuscript.

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