Review

Title

The West African *Sorghum Bicolor* Leaf Sheath Extract Jobelyn® and Its Diverse Therapeutic Potentials

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Abstract

Background:

The West-African variety of *Sorghum bicolor* leaf sheath (SBLS) Jobelyn® is a natural remedy, which has gained international recognition for its anti-anemic effect and energy boosting qualities in debilitating diseases. The widespread use of traditional medicine in

the region usually confirms its safety, but not its efficacy or deep assessment of their pharmacological properties. The other major issue for herbal-based treatments is the lack of definite and complete information about the composition of the extracts. Despite limitations, efforts have been made in isolation and characterisation of active compounds in this specie of *sorghum* showing various subclasses of flavonoids including apigeninidin, a stable 3-deoxyanthocyanidin and potential fungal growth inhibitor, which accounts for 84% of the total extract. Non-clinical *in vitro* and *in vivo* studies support previous indications that this variety of *Sorghum bicolor* possesses several biologically active compounds with potent antioxidant, anti-inflammatory, anti-aging and neuro-protective properties. Clinical studies show that SBLS has the ability to boost hemoglobin concentrations in anemic conditions and most remarkably to increase CD4 count in HIV-positive patients. The multiple effects and high safety profiles of this extract may encourage its development as a therapeutic agent for the treatment of anemia, chronic inflammatory conditions or in the symptomatic management of HIV infections. This review describes the potential therapeutic aspects of SBLS extract and its potential benefits.

Methods: Text.

INTRODUCTION

Sorghum bicolor is an ancient plant that has been cultivated in North-eastern Africa for over 5000 years (Mann et al, 1983). This is a cane like grass, up to 6 meters tall with large branched clusters of grains. The individual grains are around 3-4 mm in diameter and vary in colour from white, red, brown, yellow, purple, to black. The leaves resemble those of maize and grow rapidly. *Sorghum* grains of different cultivars are a rich source of phenolic compounds. These are natural bioactive compounds found in plants, which offer potential health benefits. Examples include secondary metabolites and antioxidants. As plants absorb the sunlight they produce high levels of oxygen and secondary metabolites by photosynthesis, which results in medicinal components being produced and stored in plant leaves. Flavanoids and phenolic acids are the most important group of secondary metabolites and bioactive compounds in plant (Kim et al, 2003). In *planta*, antioxidant

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polyphenols have a range of roles including protection against herbivores and microbial infection, as allelopathic agents and UV protectants (Dewick 2009). In humans, there is now increasing evidence on the role of phenolic compounds as protective dietary agents (Del Rio et al, 2013). They are also considered to be natural and antioxidant substances capable of scavenging free superoxide radicals, supporting anti-aging mechanisms and reducing the risk of cancer.

The *sorghum* grain is a rich and diverse source of phenolic compounds, particularly phenolic acids and flavonoids. Similar to other cereals like maize and wheat, most of the phenols in the *sorghum* grain are located in the bran, but differ in that *sorghum* grain contains higher levels of phenolic compounds compared to most cereals and even fruits and vegetables depending on their variety (Awika et al, 2004a). Anthocyanidins are the primary flavonoids found in *sorghum* grain and include apigeninidin, apigeninidin 5-glucoside, luteolinidin, luteolinidin 5-glucoside and 3-deoxyanthocyanidins, which is the most common (Awika et al, 2004b). The biosynthetic pathway that is responsible for the accumulation of 3-deoxyanthocyanins flavanoid compounds is controlled by the yellow seed1 (ys1) gene present in most varieties of *sorghum* grains (Awika, 2011). The uncommonly high levels of flavonoid accumulation in the *sorghum* grain differentiates *sorghum* from other grains and certainly makes it an interesting grain for healthy dietary applications or a source of bioactive compounds. More intriguing is the fact that the West African variety of *sorghum* synthesizes exceptionally high amounts of 3-deoxyanthocyanins pigments in their non-grain tissue (Kayode et al, 2011). Compared to the *sorghum* grain the leaf sheath and glumes contribute to a greater biomass and may provide an easier and more cost-effective approach to obtain large quantities of stable 3-deoxyanthocyanins pigments.

The intensively coloured leaf sheaths of this wild variety of *sorghum* found within the Nigeria flora has been formulated into a commercial pharmaceutical product under the name Jobelyn®. The immediate interest in this herbal preparation originated from its unique phytochemical profile compared to other variants of *Sorghum bicolor* as it has

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been reported to contain significantly high amounts of anthocyanins. Anthocyanins are flavanoids believed to contribute to plant's high antioxidant capacity and provide overall disease protection *in vivo* through anti-oxidative mechanisms. With regards to the beneficial phytochemicals in medicinal plants and the shift towards natural products in pharmaceuticals, research on medicinal plants particularly is as important as the research on conventional drugs.

In this review, we discuss the features of the West African *Sorghum bicolor* leaf sheath (SBLS) extract on the human health and its diverse therapeutic potentials.

1. Isolation and characterisation of phytochemical compounds in *the* SBLS
2. SBLS extract role in anemia
3. SBLS extract role in inflammation
4. SBLS extract as antioxidants
5. SBLS Nutritional Composition
6. The role of *SBLS* in neurocognitive deficits associated with HIV Infection
7. HIV and Immunity
8. Toxicology
9. Conclusion

Results: Text.

1. ISOLATION AND CHARACTERISATION OF PHYTOCHEMICAL COMPOUNDS IN *SBLS*

1.1 Extraction Methods

The dried powdered *SBLS* was extracted with 50% v/v aqueous ethanol for 24 hours following thorough agitation. The extract was subsequently filtered through cotton wool and concentrated *in vacuo* to give the crude extract designated (J). This was then

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dissolved in 80% v/v aqueous methanol and partitioned into equal volumes of the solvent 50% v/v ethyl acetate (EtOAc), 50% v/v n-butanol and 50% v/v distilled water successively. The EtOAc, n-butanol and aqueous fractions were concentrated *in vacuo* to give fractions labelled JE, JB and JA, respectively.

1.2 Phytochemical characterisation of bioavailable compounds in the ethanolic extract (JE)

To evaluate the qualitative and quantitative presence of various subclasses of flavonoids from the *SBLS*, ethanolic extract (JE) was dissolved in methanol (70%) at 10mg/mL, filtered in a vacuum and HPLC analyses was performed as previously described in Geera et al. (Geera et al, 2012). Flavanoids were identified by comparison of HPLC retention times, UV Spectra and co-elution with authentic samples analysed in the same condition. The concentration of the measured peaks were determined from the calibration lines by linear regression analysis and the sample accuracy was estimated as the percentage of each measured concentration from the nominal (added) concentrations. Various subclasses of flavanoids, namely apigeninidin (3-deoxyanthocyanidin), luteolinidin (anthocyyanidin), apigenin and luteolin (flavones), and naringenin (flavanone) were successfully identified and analysed from the ethanolic extract (JE) of the *SBLS*. Apigeninidin was the predominant compound in the ethanolic extract (JE), which accounted for 83.5% of the total amount of identified phenolic compounds. The proportions of luteolinidin, apigenin, luteolin and naringenin as a percentage of the total quantities of the 5 compounds were 1.0%, 13.7%, 1.5%, and 0.4%, respectively.

1.3 Isolation and characterisation of bioavailable compounds in the ethanolic extract (JE)

As previously described in Geera et al, JE of the *SBLS* was sub-fractionated adopting a Medium Pressure Liquid Chromatography (MPLC) technique (Geera et al, 2012). Fractions of 15 ml each were collected in test tubes and monitored by thin layer

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chromatography (TLC). The fractions, which had the same TLC characteristics were bulked as appropriate and concentrated *in vacuo* to give 4 major fractions labelled JE-5 to JE-8 (Figure 1). Fractions labelled J, JA, JB, JE and JE-5 to JE-8 were subjected to COX-1 and COX-2 inhibition bioassays and the fraction, JE-5, which had the least ratio of COX-2: COX-1 activity hence suggesting greater anti-inflammatory activity, was subjected to repeated column chromatography to produce two further fractions (monitored on a thin layer plate to be relatively pure components) which were concentrated *in vacuo* and labelled P8 and P9, respectively. The P8 and P9 were further analysed using an MDS Sciex API QStar Pulsar mass spectrometer with electrospray ionization (AB SCIEX, Foster City, California, USA). Identification of the compounds was based on matching UV-Vis spectra analysis, and MS data with authentic standards. The absorbance profiles of P8 and P9 revealed the presence of 2 peaks in P8 and a single peak in P9. One of the MS data of the compounds in P8 showed *m*/*z* 271 and was later identified as apigenin when matched against apigenin standard. The second compound in P8 and the single compound in P9 showed *m*/*z* 523 and 509 respectively and were later identified as dimeric flavonoid molecules differing from each other in one methyl group. Using both UV-Vis data and LC elution profiles as described in Geera at al. the two compounds were inferred to be 3-deoxyanthocyanidin dimers. The second compound in P8 was identified as 7-methoxyflavone-apigeninidin adduct while that in P9 was identified as flavone-apigeninidin adduct (Geera et al, 2012). The amount of total flavonoids in the extracts P8 and P9 were measured spectrophotometrically as previously reported in Geera et al. The quantity of apigenin (29.87±9.85 mg per g of dried leaf sheath) in P8 was approximately ten times that of 7-methoxyflavone-apigeninidin adduct which was 2.8 mg/g while that of flavone-apigeninidin adduct was 7.7 mg/g (Geera et al, 2012).

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2. *SBLS EXTRACT ROLE IN ANEMIA*

The anti-anemic potential of the S*orghum bicolor* leaf sheath has been extensively reported (Oladiji et al, 2007; Solawu et al, 2014; Majolagbe et al, 2013). In studies involving rats and rabbits made anemic by inoculation with trypanosoma brucei, findings demonstrated significant increases in the red cell count, hemoglobin and packed cell volume within 5 weeks of administration of the *SBLS* (Okochi et al, 2003; Erah et al, 2003).

The anti-anemic potential use of the extract was also investigated in a randomized, open label clinical trial in women with preoperative anemia being prepared for myomectomy. A group of the subjects were given *SBLS* plus hematinics for 4 weeks and the other group was placed on hematinics alone. There was an increase in the red blood cell count, hemoglobin and packed cell volume in the subjects that took *SBLS* such that there was a 15% increase in subjects that met the criteria for surgery (packed cell volume of 36%). In this trial, there was no evidence of hepatotoxicity or nephrotoxicity. White blood cells and platelet counts were reduced though not significantly and were within the normal ranges. (Tayo et al, 2016).

In separate studies *SBLS* was used on HIV patients to assess its potentials in preventing anemia originating from HIV infection. This was a pilot study conducted on 10 HIV subjects and surprisingly results showed that *SBLS not* only increased hemoglobin levels but also the CD4 count in antiretroviral naive patients (Table 1). These findings suggest that *SBLS* could assist as a dietary supplement in the management of anemic HIV-positive patients to improve anemia, primarily related to iron depletion due to chronic disease status. The early haematological changes observed in this pilot study could serve as basis for a clinical study that would describe the prevalence and characteristics of anemia in a larger cohort of HIV-infected patients.

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Table 1: Pilot data on 10 HIV patients showing CD4+ T cell counts and hemoglobin levels after treatment with SBLS. *Data analysis utilised 2-tailed paired t-test to compare changes for Week 4 and Week 8 to baseline values. (Unpublished data).*

ID	Gender	ARBT	JOBELYN	Week 0		Week 4		Week 8	
1	M	No	Yes	CD4 HB	399 11.6	CD4 HB	617 12.6	CD4 HB	708 13.3
2	M	No	Yes	CD4 HB	656 12.3	CD4 HB	704 12.8	CD4 HB	824 13.0
3	M	No	Yes	CD4 HB	452 12.1	CD4 HB	662 12.8	CD4 HB	724 13.0
4	M	No	Yes	CD4 HB	518 12.0	CD4 HB	530 13.0	CD4 HB	560 14.0
5	F	No	Yes	CD4 HB	352 10.3	CD4 HB	390 12.0	CD4 HB	564 12.5
6	F	No	Yes	CD4 HB	460 10.6	CD4 HB	617 11.0	CD4 HB	669 11.0
7	F	No	Yes	CD4 HB	499 10.3	CD4 HB	550 11.2	CD4 HB	550 11.4
8	F	No	Yes	CD4 HB	830 9.8	CD4 HB	1082 10.3	CD4 HB	1203 11.4
9	F	No	Yes	CD4 HB	350 8.9	CD4 HB	461 9.6	CD4 HB	475 10.6
10	F	No	Yes	CD4 HB	385 10.2	CD4 HB	358 11.3	CD4 HB	622 12.0
Average				CD4 HB	490 10.8	CD4 HB	607 11.7	CD4 HB	690 12.0
SEM				CD4 HB	47.64 0.36	CD4 HB	61.29 0.37	CD4 HB	65.51 0.36
2-tailed paired t-test				CD4 HB	------- -------	CD4 HB	P<0.01 P<0.001	CD4 HB	P<0.001 P<0.001

3. *SBLS* EXTRACT ROLE IN INFLAMMATION

In response to fungal infection *Sorghum bicolor* has been shown to produce a complex mixture of flavanoid secondary metabolites, which are structurally related compounds to 3-deoxyanthocyanidins, apigeninidin, luteolinidin, luteolinidin 5-methylether and

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apigeninidin 7-methylether. This family of compounds may function as plant pigments (Harborne et al, 1993) or serve as phytoalexins (Hipskind et al, 1990). Phytoalexins are low-molecular weight antimicrobial compounds produced by plants in response to infection or stress (Smith, 1996). In the *sorghum* leaf, these phytoalexins first appear in the cells that are being invaded, where they accumulate inclusions in the cytoplasm (Snyder and Nilcholson, 1990; Snyder et al, 1991). The inclusions migrate to the site of attempted penetration, becoming pigmented and losing their spherical shape. Ultimately they release their contents into the cytoplasm, killing the cell and restricting further development of the pathogen. The accumulation of 3-deoxyanthocyanidin phytoalexins is a site-specific response localized around the site of attempted fungal penetration (Snyder and Nicholson, 1990) and prevents fungal proliferation through the tissue (Wharton and Julian, 1996). Similarly, inflammation in humans is a consequence of release of agents like leukotrienes; prostaglandins; thromboxane and other products of phospholipid metabolism; reactive oxygen species; cytokines like tumour necrotic factors, interleukins-1, -2, -4, -6 and -8; and chemokines like CCL5, CXCL4 and CCL3 by immunocompetent cells. The essence is to increase the blood flow and mobilise immunocompetent cells to sites of tissue damage as a result of invasive organisms, chemical, metabolic, radiation toxicity or physical damage. It is therefore a nonspecific protective event. However, uncontrolled stimulation of inflammation perturbs body hemodynamics, hemostasis and thermoregulation causing tissue damage and pains. The various combinations of these events will define most diseases.

3.1 The Effect of Crude and Purified Extracts of SBLS on Prostaglandins

Protaglandins E2 (PGE-2) is a principal mediator of inflammation. Selective cyclooxygenase-2 (COX-2) inhibitors reduce PGE-2 production to diminish inflammation. *SBLS* crude and purified extracts were tested for anti-inflammatory effects based on PGE-2 production from peripheral blood mononuclear cells (PBMC) in the presence of lipopolisacchraride (LPS). Ibuprofen, a non-selective COX-2 inhibitor and CAY10404, an inhibitor with greater COX-2 inhibitory activity compared to COX-1, were

used as controls for the study. The crude extract JE5 derived from the ethanol extraction (JE) of *SBLS* had the greatest inhibitory activity with the least COX2IC50: COX1IC50 ratio and reduced production of PGE2. Of the purified compounds, P8 (purified from JE5) had greater inhibitory activity with the least COX2IC50: COX1IC50 ratio and reduced PGE-2 production from LPS-stimulated PBMC, which was dose dependent. COX-2, the enzyme that catalises the synthesis of PGE-2 also shows enhanced expression in a number of cell types including circulating monocytes, tissue macrophages, lymphocytes and neuronal cells during chronic inflammation (Longo et al, 1993 and Liu et al, 2001). COX-2-derived PGE-2 production is also dependent on oxidative stress (Tian et al, 2012) as reports suggests that ROS is an up-regulator of COX-2 expression and activates COX-2 to release PGE-2 (Wong et al, 2010). Therefore, the role of *SBLS* in a) reducing PGE-2 production levels through inhibition of COX-2 expression and b) possibly reducing COX-2 expression through antioxidant capacity of the extract could be used as targets for therapeutic development in the management of chronic inflammation.

3.2 *Anti-inflammatory effects of the SBLS extracts on cytokines and chemokines*

A study on the effects of the *SBLS* on LPS-induced cytokine and PGE-2 release in human monocytes was performed and results revealed inhibition of LPS-induced release of cytokines (IL-1β; IL-6; TNFα; IL-8) and PGE-2 (Benson et al, 2013). The ability of the *SBLS* to inhibit these cytokines involved in inflammatory recruitment as well as inhibiting PGE-2 production possibly through inhibition of COX-2 enzyme activity could be explained by the extract's antioxidant properties. We speculate that the antioxidant capacity of *the SBLS* has the ability to reduce oxidative stress environment thus reducing COX-2 expression in macrophages and other cells, which will in turn reduce the production of PGE-2 as well as limit the release of pro-inflammatory cytokines that assist with immunocompetent cell recruitment during the onset of infection. Further studies are indeed required to validate such theory, however, there seems to be a direct correlation between *the SBLS* extract's antioxidant capacity: oxidative stress: COX-2 expression: PGE-2 and pro-inflammatory cytokines production during LPS-induced inflammation.

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In the same study, interferon –alpha (IFN-), an antiviral cytokine showed increased expression by 12-fold following treatment with *SBLS* extract (Benson et al, 2013). There seems to be a correlation between high IFN- production capacities and low HIV viral loads as well as high CD4 cell counts and lack of opportunistic infections (Soumelis et al, 2001; Finke, 2004). This effect can be related to the direct anti-viral role of IFN- on infected HIV cells. LPS-treated mononuclear cells after exposure with the *SBLS* extract showed a 12-fold increase in the production of IFN- suggesting that the extract may contribute not only in suppressing chronic inflammation originating from HIV infection but possibly in reducing the viral load and increasing CD4 count through IFN- stimulation.

4. SBLS EXTRACT AS A POWERFUL ANTIOXIDANT

4.1 Antioxidant Activities of the SBLS by ORAC

Studies carried out at the Brunswick Laboratories (Southborough, MA, United States) have shown that *SBLS* ranks among the highest Oxygen Radical Absorbance Capacity (ORAC) of all food plants with total $ORAC_{FN}$ of 37,622 µmole TE/g of the dry powder (Figure 1). This variety of *sorghum* was found to contain significant levels of antioxidant activities against peroxyl radicals (3,549 µmole TE/g), peroxylnitrite (269 µmole TE/g), hydroxyl radicals (18,387 µmole TE/g), superoxide ions 11,417 µmole TE/g) and singlet oxygen (4,000 µmole TE/g). In fact, the antioxidant capabilities of *SBLS* extract is greater than that of the anthocyanin-rich acai berry indicating its exceptionally high antioxidant potential (Figure 2). According to these findings it seems that specific varieties of *sorghum* like the one described here could positively assist in reducing the severity of several health conditions, as the antioxidant activity is able to contribute to cellular adjustments to oxidative stress.

4.2 *SBLS Cellular Antioxidant Protection*

Apart from ORAC measurements, *SBLS* was also submitted to relative antioxidant protection capacity analysis, which was carried out by Natural Immune Systems

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Laboratories (NIS), Oregon, USA. The extract was digested after exposure to gastric acid with pepsin, bile and pancreatic enzymes. Some of the products were further fermented by a blend of common probiotic bacteria for 24 hours. The products were centrifuged and filtrated in a spin column to remove the digestive enzymes. The cellular antioxidant protection (CAP-e) bioassay was performed and results showed that *in vitro* digestion though reduced the total antioxidant capacity of *SBLS*, it increased the cellular antioxidant uptake and protection from free radical damage in erythrocytes. In separate animal experiments carried out by (Umukoro et al, 2013), *SBLS* was shown to tolerate oxidative stress as oral administration of the extract (50-200 mg/kg) in rats decreased the levels of malondihydehide (MDA) in the serum suggesting antioxidant property. This variety of *sorghum* also elevated the concentrations of reduced glutathione (GSH) in inflammation exudates indicating free radical scavenging activity. Furthermore, it significantly inhibited red blood cells lysis caused by hypotonic medium, suggesting membrane-stabilising property (Umukoro et al, 2013).

4.3 The Impact of SBLS extract on Aging Protection

Analysis at the Brunswick Laboratories showed that the *SBLS* causes significant inhibition of collagenase, elastase and protein glycation. Such properties in *sorghum* should promote healthy skin and retard aging. Reported collagenase inhibition had 15-fold potency to that of vitamin C and 30-fold potency of ferrulic acid while elastin inhibition showed a 22-fold potency to that of vitamin C and 8-fold potency of ferrulic acid (Table 2). The on-going attacks of free radicals during HIV infection damages the elastin and collagen fibers (Harman, 1996). The skin protects itself against these impairments with the aid of radical scavengers. Effective elastase and collagenase inhibition reported with *SBLS* could be related to its exceptional antioxidant ability although further experiments are required in order to confirm these events.

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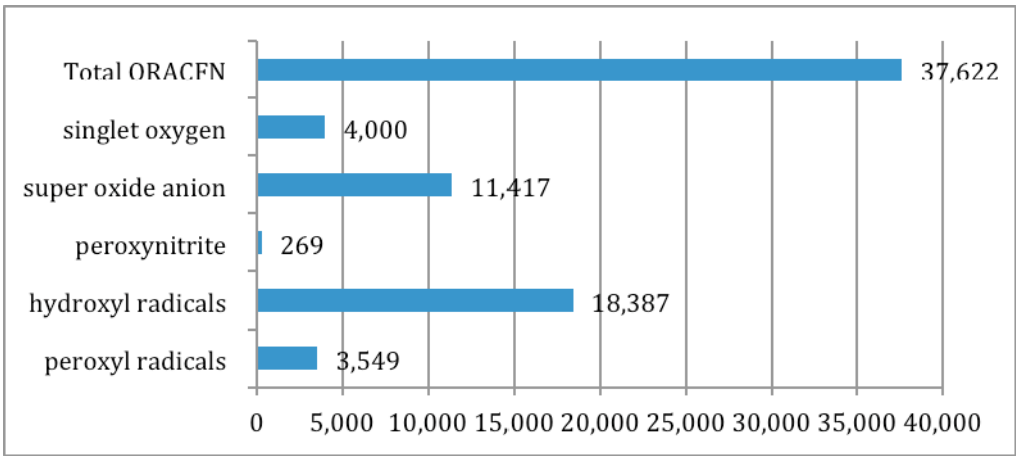

Figure 1: Antioxidant activities of SBLS ® assessed by ORAC. (Brunswick Laboratories).

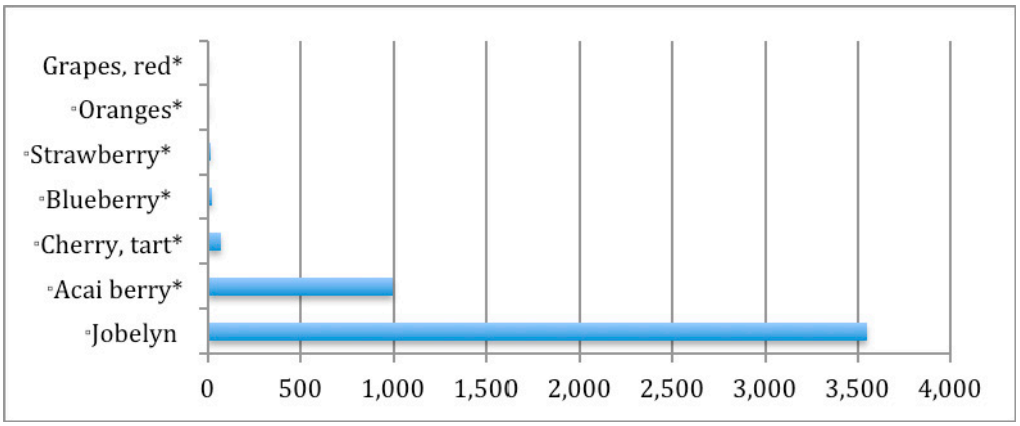

Figure 2: Comparisons in inhibition of peroxyl free radicals per gram of product between SBLS and other antioxidant-rich foods (USDA, 2010).

Table 2: Data showing the anti-aging properties of SBLS (Brunswick Laboratories).

Assays Performed	Results IC_{50}
Collagenase Inhibition[1]	60 µg/mL
Elastase inhibition[1]	17 µg/mL
Anti-glycation[2]	4 µg/mL

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5. SBLS Nutritional Composition

Over the course of a lifetime, significant chronic metabolism disruption may occur when consumption of micronutrient is below the current recommended dietary allowance but above the level that causes acute metabolic symptoms. When, a component of the metabolic network is inadequate, there may be a variety of setbacks in metabolism (Ames, 2006). As a result, dietary supplements, most commonly multivitamins and minerals are given to fill the nutritional gaps. In HIV infected persons, low serum concentrations of vitamins and nutrients has been associated with an increased risk of HIV disease progression and mortality (Kupka et al, 2004). In 1996, highly active antiretroviral therapy (HAART) became the new standard for HIV treatment. HAART restores the immunologic function (Autran et al, 1997), but does not eliminate weight loss, micronutrient deficiency and wasting syndrome (Tang et al, 2005), which are strong independent predictors of mortality (Tang et al, 2002).

5.1 *Mineral Contents in SBLS*

SBLS's nutritional contents were assessed by GMP Laboratories of America, Inc., CA, USA. SBLS was proven to be a good source of minerals like calcium (35.2% RDA), magnesium (59.03% RDA), sodium (95.83% RDA), selenium (28.0% RDA), Zinc (13.62% RDA) and copper (127.77% RDA) (Table 3). More interestingly, it was observed that 100g of this extract provides around 285% of the daily recommended levels of iron (Table 3). HIV infection increases the release of pro-oxidants, cytokines and ROS leading to increased utilisation and excretion of proteins and microminerals such as zinc, iron, selenium manganese and copper. This can result in an imbalance between pro-oxidants and antioxidants which may lead to increased oxidative stress and cause further damage to human cells, proteins and enzymes, thus accelerating HIV replication and mortality of the patient (Tang et al, 2002; Friis and Michaelsen, 1998). Therefore, vitamins and minerals are crucial in reducing HIV disease progression especially as the cost of effective strategy for reducing HIV disease progression, improving nutritional status and possibly reducing

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vertical transmission of HIV in low-income countries is high. Anemia, in particular is a common clinical finding in HIV-infected patients and iron deficiency or maldistribution may contribute to the development of low haemoglobin levels. Due to the effects of inflammation in HIV patients, iron is diverted from circulation into the reticulo-endothelial system and other storage sites. Iron maldistribution reduces iron availability in the circulation, increase susceptibility to opportunistic infections and accelerate disease progression (Wisaksana et al, 2013). Treating severe anemia in HIV-infected patients is critical because recovery from anemia is associated with increased length of survival, however, excess of iron in the storage sites is associated with an increased viral replication (Wisaksana et al, 2013). The SBLS contains large amounts of iron and as such it is used in the treatment of anemia. However, most of the excess iron from SBLS is chelated by the polyphenols in the *sorghum* extract and as a result the excess iron is not presented to the body. Such observation suggests that the use of SBLS can help improve hemoglobin concentrations and quality of life in anemic HIV-positive patients as well as reduce several micronutrients deficiencies, which are common in advanced HIV disease. Observational studies have shown low serum concentration of several micronutrients including selenium and zinc to be associated with low CD4 cell counts, advanced HIV-related disease, faster disease progression, or HIV-related mortality (Abrams et al, 1993; Campa et al, 1999). Similarly, anemia due to deficiency in iron is more common and more severe with advanced HIV disease progression (Belperio and Rhew, 2004).

5.2 *Vitamin Contents in SBLS*

The West African *sorghum* also contains substantial amounts of vital vitamin B_{12} (Table 3). Vitamin B_{12}, is a water soluble vitamin that acts as a cofactor in the conversion of homocysteine to methionine and methyl tetrahydrofolate to tetrahydrofolate. Polyglutamate is further added tetrahydrofolate to prevent the diffusion of folates out of the cell. Folates are essential for the building blocks of DNA in rapidly regenerating organs like the production of blood while excess homocysteine is known to cause endothelial

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damage and therefore cardiovascular disease. It is well documented that the body cannot produce B_{12} and that it can only be sourced from animal foods or in the form of B_{12} vitamin supplement. Deficiency usually results in anemia, impaired brain function, and symptoms of mental disorder (Tangney et al, 2011). The only good food sources of B_{12} are primarily animal foods like meat, fish and eggs producing 2.8 µg/100 1.95 µg/100; 4.15 µg/100 of B12, respectively. Interestingly, SBLS is a good source of B_{12} producing 0.83 µg/100 g. This is the first time a plant product has been reported to contain significant amounts (34%) of vitamin B_{12}. Low levels of B_{12} have been associated with a reduction in $CD4^+$ T cell count in HIV-infected patients (Baum et al, 1995). There have also been claims that nutrient supplementation could restore immune function and boost $CD4^+$ T cell counts in people with early stages of HIV infection (Baum et al, 1995). Vitamin B_{12} could potentially be a useful antioxidant as it directs reaction with reactive oxygen species and through glutathione sparing effect, can modify signaling molecules to decrease oxidative stress and increase total antioxidant capacity (Misra et al, 2016). However, such effects are yet to be evaluated in HIV-infected persons.

5.3 Fatty Acids Contents in SBLS

SBLS provides an impressive 1:3 ratio of Omega-3 to that of Omega-6 (Table 3) and as population studies indicate, our diet should contain no more than three parts of Omega-6 to one part of Omega-3, which is ideal for the heart's health (Gebauer et al, 2006). Omega-6 fats have pro-inflammatory effects while omega-3 fats have anti-inflammatory effects. The ratio of 3:1 will reduce the chronic inflammation that most people recognise as the root cause of many chronic diseases, including diabetes. Omega-3 fatty acids play an important role in every cell in the body. Omega-3 makes up cell membrane and helps in maintaining membrane fluidity, keeps the nervous system functioning by upregulating brain derived neurotropic factor (BDNF) and modulating neurotransmitters re-uptake, degradation, synthesis and anti-apoptotic effects once it gets converted into fatty acids eicosapentaenoic acid (EPA) and later docosahexaenoic acid (DHA). However, Omega-6 inhibits the conversion of Omega-3 into DHA and EPA, therefore, the adequate

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ratio of 1:2 or 1:3 is usually required to allow conversion of adequate amounts of Omega-3. The Omega balance is critical as Omega-3 and Omega-6 compete for absorption into the cells, and an excess of dietary Omega-6 will result in too few of Omega-6 being incorporated into cell membranes, from where they exert their essential effects (Mischoulon and Freeman, 2013). Earlier in the HIV epidemic the observation of increased oxidative stress and elevated cytokines led to a dietary intervention study on whether supplementation with Omega-3 fatty acids would decrease cytokines or markers of inflammation. Omega 3 showed beneficial effects on systemic inflammation mediated my multiple mechanisms including a decrease in the activation of NFkB by inflammatory stimuli. In a pro-oxidative stress environment, ROS has the ability as start a cascade as second messengers for the activation of NFkB, which increase the replication of HIV, because this factor controls the transcription for the HIV viral replication (Amador-Licona et al, 2016). Thus, the right supplementation of Omega 3 and 6 fatty acids would prove beneficial in a pro-oxidative stress environment as experienced in HIV infection.

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Table 3: Comparisons between daily recommended dietary intakes for vitamins, minerals and elements and amount found in SBLS (GMP Laboratories).

Principle	Nutrient value	Percentage of RDA
Energy	324 cal	
Carbohydrates	75.3g	
Protein	4.87g	
Dietary fiber	50.30g	
Vitamin		
Vitamin B12	0.83 µg	34.5%
Riboflavin	0.18 mg	16.36%
Niacin	3.55 mg	25.35%
Minerals		
Calcium	352 mg	35.2%
Magnesium	183 mg	59.03%
Iron	51.20 mg	640.0%
Zinc	1.09 mg	13.62%
Copper	900 µg	127.77%
Phosphorus	700 mg	20.14%
Selenium	15.40 µg	28.0%
Electrolytes		
Potassium	0.5 g	10.63%
Sodium	1.15 g	95.83%
Total Omega-3 fatty acids	36 mg	
Total Omega-6 fatty acids	110 mg	

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6. THE ROLE OF *SBLS* EXTRACT IN NEUROCOGNITIVE DEFICITS

SBLS may exert a beneficial role in the treatment of neuropsychiatric symptoms associated with depression, memory deteriorations and psychotic manifestations. Preclinical studies have shown that the extract ameliorated the characteristic feature of immobility in mice subjected to 'forced swimming test' (FST), a well recognized animal model of depression. Mice subjected to FST experienced a period of immobility, which indicates a depressive-like behavior and antidepressant drugs are known to decrease the period of immobility. These findings have provided the impetus for the proposed clinical trials of SBLS for treatment of psychotic ailment in Yaba psychiatric hospital Lagos, Nigeria. Results from the clinical study showed that *SBLS* suppresses amphetamine-induced stereotypy and antagonises hyperlocommotion due to amphetamine injection, which indicate antipsychotic potentials. More importantly, antipsychotic-like activity of the extract was devoid of the adverse effect of cataleptic behavior (Omogbia et al, 2013). In another neurological study conducted by Umukoro et al., *SBLS* was shown to reverse memory impairment induced by scopolamine in mice. Interestingly, the extract was found to attenuate memory deficits in mice subjected to unpredictable chronic mild stress (UCMS), which is an animal model that mimics the pathological changes seen in humans exposed to stress on daily basis (Umukoro et al, 2015).

Inhibition of inflammatory cytokines as previously reported by Benson et al. (Benson et al, 2013) may serve as an important target for development of drugs with potential efficacy against neurological disorders. A recent *in vitro* study showed that *SBLS* extract inhibited infiltrations of WBC, release of inflammatory mediators, and formation of free radicals (Heo et al, 2004), which are the major culprits involved in neurodegenerative diseases. Furthermore, Oyinbo et al., shows that the *SBLS* extract demonstrated anti-neuroinflammation and reduced the death of astrocytes (Oyinbo et al, 2015). Specific

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phytochemicals such as luteolin, naringenin, and apigenin found in this extract have previously demonstrated anti-inflammation activity in cultured cells (Bourin et al, 1986). Luteolin, in particular, was shown to inhibit nuclear factor-κB (NF-κB) signaling in immune cells, which supports its therapeutic efficacy in conditions associated with chronic inflammation (Devi et al, 2009).

7. HIV AND IMMUNITY

The sub-Saharan Africa is home to more than two-thirds (69%) of people living with HIV (World Health Organization (WHO)). Interestingly, it is also a region suitable for the cultivation of a unique variety of Sorghum bicolor. The SBLS extract has gained interest because of its antioxidant capability. Antioxidants are specific compounds that protect human, animal and plants against the damaging effects of free radicals or reactive oxygen species. Imbalance between antioxidants and free radicals results in oxidative stress, which may lead to cellular damage (Kukic et al, 2006). It has been observed that perturbations in antioxidant defense systems, and consequently redox imbalance, are present in many tissues of HIV-infected patients. Moreover, the level of production of free radical species in HIV infected individuals receiving highly active antiretroviral drugs (HAART) was reported to be higher than those who harbor HIV infection without receiving any treatment or normal and healthy subjects (Sharma, 2014). In HIV infection, the deficiency of total antioxidant status might markedly increase oxidative stress. The increase in reactive oxygen species may enhance viral replication by activating nuclear transcription factors, which ultimately could lead to viral gene expression (Schreck et al, 1991). Enhanced oxidative stress also occurs after initiating HAART due to persistent tumor necrosis factor-α (TNF-α) activation in HIV-infected patients (Muthu et al, 2008). Therefore, the antioxidant activity of SBLS may positively act on individual's immunological status and decrease HIV replication. Further, this variety of *sorghum* could reduce cellular damage due to oxidative stress originating from HAART treatment.

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HIV infection causes chronic inflammation, which is beneficial to HIV replication but detrimental for human host leading to senescence of the immune system (Dube and Sattler, 2010). The inflammation molecule PGE-2 levels are markedly enhanced during HIV infection as a result of chronic inflammation (Dumas et al, 1998). COX-2, the enzyme that catalises the synthesis of PGE-2 also shows enhanced expression in a number of cell types including circulating monocytes, tissue macrophages, lymphocytes and neuronal cells during HIV infection (Longo et al, 1993 and Liu et al, 2001).

HIV infection increases the release of pro-oxidants, cytokines and ROS leading to increased utilisation and excretion of proteins and microminerals such as zinc, iron, selenium manganese and copper. This can result in an imbalance between pro-oxidants and antioxidants which may lead to increased oxidative stress and cause further damage to human cells, proteins and enzymes, thus accelerating HIV replication and mortality of the patient (Tang et al, 2002; Friis and Michaelsen, 1998). Therefore, vitamins and minerals are crucial in reducing HIV disease progression especially as the cost of effective strategy for reducing HIV disease progression, improving nutritional status and possibly reducing vertical transmission of HIV in low-income countries is high. Anemia, in particular is a common clinical finding in HIV-infected patients and iron deficiency or maldistribution may contribute to the development of low haemoglobin levels. Owing to the effects of inflammation in HIV patients, iron is diverted from circulation into the reticulo-endothelial system and other storage sites. Iron maldistribution reduces iron availability in the circulation, increase susceptibility to opportunistic infections and accelerate disease progression (Wisaksana et al, 2013). Such observation suggests that the use of SBLS can help improve hemoglobin concentrations and quality of life in anemic HIV-positive patients as well as reduce several micronutrients deficiencies, which are common in advanced HIV disease.

Multiple studies have shown increased levels of oxidative stress after HIV infection in the Central Nervous System (CNS) (Turchan et al., 2003) and have even correlated increased levels of oxidative stress with the severity of the disease. Neurons are exposed to extensive amounts of oxidative species with the reduced concentration of endogenous

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antioxidant defenses such as glutathione during HIV neuropathogenesis (Louboutin and Strayer, 2014). Despite the advent of combination of HAART, the CNS complications associated with HIV infection still present a great challenge in the management of neuroAIDS. As survival with chronic HIV-1 infection improves, due to the use of HAART, the number of people harboring the virus in their CNS increases because the brain is largely impervious to HAART (Louboutin and Strayer, 2014). Moreover, it is becoming clear that the brain is an important reservoir for the virus, and that neurodegenerative and neuroinflammatory changes may continue despite HAART and thus, HAND remains a significant independent risk factor for AIDS mortality (Louboutin and Strayer, 2014).

Several studies carried out to assess the potentials of SBLS in treating indicators of HIV infection such as chronic inflammation, oxidative stress, HAND, anemia and other micronutrient deficiencies has led to speculate that SBLS extract may contribute to many aspects in the management of HIV infection. As previously described in SBLS role in anemia, a pilot study on 10 HIV subjects was conducted and results showed that SBLS not only increased hemoglobin levels but the CD4 count in antiretroviral naive patients (Table 1). This encouraged a randomised controlled clinical trial in HIV patients on antiretroviral therapy (HAART). Findings from the trial (Figure 3) show significant increase in CD4 count for HIV patients with initial CD4 count >350/µl and placed on SBLS alone and also significant increase in the CD4 count for patients with initial CD4 count <350/µl placed on SBLS + antiretroviral drugs (AVRs) compared to the CD4 count for patients with initial CD4 count <350/µl placed on antiretroviral drugs alone (Figure 6) (Ayuba et al, 2014). Such achievements within 3 months of treatment suggests that the SBLS extract should be evaluated in a long-term clinical disease progression to establish if the supplementary or alternative treatment with SBLS for HIV/Aids patients can serve as an immune system booster and a probable “virus-cidal” factor.

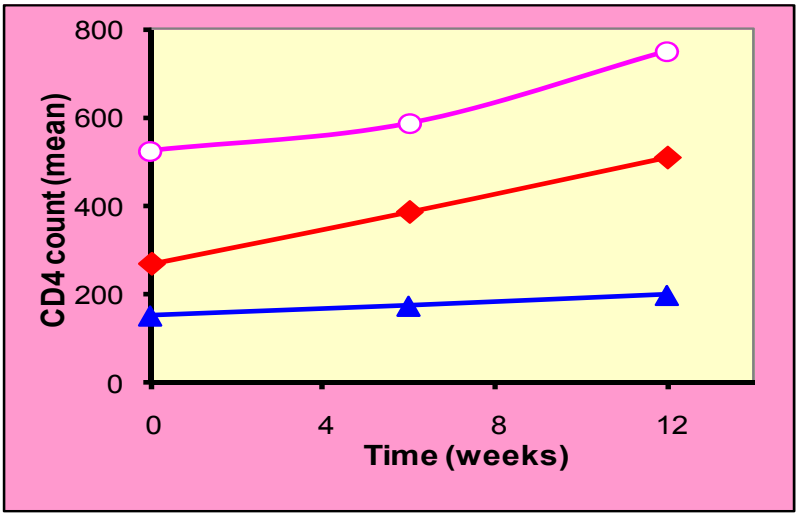

Figure 3: Data from the randomised controlled clinical trial in HIV patients on antiretroviral therapy (HAART) and SBLS therapy (Ayuba et al, 2014).

8. TOXICOLOGY

A toxicological evaluation of SBLS on both the acute and short-term chronic administration in mice was performed. Acute toxicity studies revealed that the LD50 values for oral and intraperitoneal routes were 215.06mg/kg and 193.37mg/k, respectively. SBLS would produce a lethal effect via oral route in about 10 times the maximum recommended dose per day, which is 6 capsules. Behavioral changes including reduced motility and sedation were observed at high doses. Histopathological examination of the liver, lung, spleen and kidney tissues did not indicate any organ damage. At toxic levels, however, there was some degree of congestion in the lungs, liver, spleen and kidney tissues. Short-term chronic studies using sub-lethal administered over 14 days showed no serious behavioral abnormalities or histopathological changes in the lungs, liver, spleen and the kidneys (Eniojukan et al, 2009).

Conclusions: Text.

9. CONCLUSION

In summary, it is widely reported that increased levels of oxidative stress and depletion of endogenous antioxidant defense systems are associated with HIV pathology. It is also believed that increased oxidative stress triggers inflammation changes and immunosuppression that contribute to the severity and symptomatologies of the disease. Therefore, antioxidant supplementation might be a better option in reducing the severity of the devastating effects of HIV on the immune system and body organs including the brain.

The standardised dried powder from the West African SBLS described in this review has been shown to possess a unique combination of phytochemicals known to exhibit a wide range of biological activities. Phytochemical antioxidants found in SBLS may assist in decreasing oxidative stress-mediated cellular damage and improve immune functions. The nutritional contribution of SBLS will increase hemoglobin concentration and micronutrients deficiencies. While the anti-inflammatory effects of the extract will improve neurocognitive activities in psychiatric patients as well as address the uncontrolled inflammation that causes tissue damage and pains in HIV-infected individuals.

More importantly, SBLS will promote increase in CD4 count as it targets chronic inflammation, oxidative stress and the destruction of the adaptive immune response (CD4 T cells), which are the most significant factors in the pathogenesis of HIV-AIDS.

There are no reported cases of adverse events or allergic reactions during the use of this extract over the years and acute and chronic studies revealed that SBLS was well tolerated by laboratory animals.

The high safety profile of this extract coupled with its potent antioxidant properties makes this unique *sorghum*-based natural product a potential therapeutic target in the management of HIV-AIDS, chronic inflammatory conditions and anemia.

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