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Immune Boosting Herbs: Lipid Peroxidation in Liver Homogenate as Index of Activity

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Abstract: *In vitro* antioxidant activity of aqueous extracts of *Phyllantus amarus* (Schum and Thonn.) Euphorbiaceae, *Sida acuta* Burman f. (Malvaceae), *Sida cordifolia* Linn. (Malvaceae) and *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) was assessed by lipid peroxidation (LPO) and reduced glutathione content (GSH). Inhibition of peroxidation and GSH oxidation in all concentrations was dose-dependent. % inhibition at 1 mg mL^{-1} concentration activity for lipid peroxidation was mixture of all plant extracts with *S. cordifolia* (86.83±1.23) > mixture with *S. acuta* (72.92±0.96) >*P. amarus* (72.03±0.39) > *S. cordifolia* (56.82±0.54) > *S. acuta* (42.77±1.76) > *X. eathiopica* (39.48±2.07). Inhibition of GSH oxidation was 97.47±0.42, 83.56±0.39, 78.33±0.09, 61.69±0.87, 56.48±0.19 and 49.44±1.13, respectively in the same order.The extracts inhibited lipid peroxidation and GSH oxidation and thus could slow down aging process and improve immune responses.

Key words: Medicinal plants, lipid peroxidation, GSH oxidation, synergism, immunostimulants

INTRODUCTION

The importance of building a strong, healthy immune system has received much attention lately. Many illnesses so prevalent in the world today, such as cancer, Acquired Immune Deficiency Syndrome (AIDS), candidiasis and chronic intestinal infections are now believed to be immune-related disorders.

Imbalances or a severe disruption of the immune function can result in a vast array of diseases. T-cell defects are associated with recurrent viral infections, as well as fungal infections such as candidiasis. So when the defense system is weakened, not only do infections occur more frequently, but there is the danger of drastic health problems arising. In the condition of AIDS, it is the T- and B-cell systems that function inadequately.

Some of the factors leading to a weakened immune system include stress, exposure to environmental toxins, faulty diet, sedentary lifestyle, inadequate sleep, alcohol and tobacco abuse, malabsorption, antibiotics, chemotherapy, birth control pills, cortisone and other drug therapies.

The immune system is subject to free radical damage which will suppress their activity. Ability of antioxidants to destroy free radicals protects the structural integrity of cells and tissues. Traditional medical practitioners in Nigeria know that certain herbs make the body more resistant to diseases. According to medical researchers, it is quite possible that some cases of diabetes and infertility, chronic hepatitis, atopic dermatitis, some cases of asthma and many other inflammatory and several degenerative disorders with no other known causes may also be autoimmune problems.

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Oxygen Free Radicals (OFRs) have been implicated in the pathogenesis of an increasing number of diseases and inflammatory states. They may cause cell and tissue damage by their chemical modification of proteins, carbohydrates, nucleotides, lipids of cell membranes and DNA. As a result, they can easily initiate the peroxidation of the membrane lipids. The harmful activities of free radicals are associated with damage to membranes, enzymes and DNA. Under physiological conditions, OFRs are part of normal regulatory circuits and are neutralized by antioxidants. Infections are one cause of increased OFR production. The ability of antioxidants to destroy free radicals protects the structural integrity of cells and tissues. Recent clinical trials have found that antioxidant supplementation can significantly improve certain immune responses (Bendich, 1993). Antioxidant vitamins C, E, A or beta-carotene protect immune responses in individuals exposed to certain environmental sources of free radicals. Supplementation with glutathione or antioxidants may also improve immunologic and virologic indexes in HIV-infected persons and decrease morbidity and mortality associated with measles infections in children (Kagan *et al*., 1992; Muller *et al*., 2000).

Most herbs for the immune system are general immunostimulants. They increase the activity of the immune system by mobilizing effector cells which act against all foreign particles, rather than just one specific type. Echinacea is the best known and one of the most researched of immunostimulants. Astragalus extract may also restore immunocompetence; potentially for cancer as well as AIDS patients (Blair, 2004). Low levels of glutathione in the body are almost always a sign of illness, especially of poor immune function. A number of other herbs are powerful tonics which strengthen the immune system. They have been known to support T-cell function, activate macrophages and help rebuild bone marrow reserves (Bendich, 1993).

Oxidative stress can be assessed by measuring lipid peroxidation in the body (Halliwell and Chirico, 1993). The lipid peroxidation process is initiated by a free radical attack on a polyunsaturated fatty acid. A lipid radical is formed that reacts with oxygen, leading to formation of a peroxyl radical that may further react with other lipids and produce a new lipid radical. Thereby a propagation reaction starts and is maintained, until a termination reaction occurs including for example chain breaking antioxidant (Basu, 2003).

P. amarus has a long history of use as a supportive herb, assisting with circulatory, digestive and skeletal system function. Infusion of the leaves is used for hemorrhoids, veneral diseases, tachycardia and female sterility (Burkill, 1994). In addition, *P. amarus* is considered one of the best herbs for treating liver disorders. It harnesses the ability to block DNA polymerase, the enzyme needed for the hepatitis B virus to reproduce. The main constituents in *P. amarus* include; lignans (phyllanthine and hypophyllanthine), alkaloids, bioflavonoids (quercetin) and repandusinic acid. Repandusinic acid has been shown to have anti-viral properties *in vitro*, inhibiting HIV and HTLV-I replication; this agent also has HIV reverse transcriptase activity (Thyagajaran *et al*., 1988; Venkateswaran *et al*., 1987; Rajeshkumar and Kuttan, 2000; Notka *et al*., 2003, 2004).

S. cordifolia grows as wasteland weed. The plant is tonic, astringent, emollient and useful in blood, throat, respiratory and urinary system related infections, piles and aphrodisiac. Both *S. cordifolia* and *S. acuta* (synonym *S. carpinifolia)* are referred to locally as OSONKOTU/ ISANKOTU among the Yoruba tribe in Nigeria. They are interchangeably used in traditional medicine for the same ailment. *S. cordifolia* is an erect, perennial undershrub, up to 1 m tall with ovate leaves and yellow flowers in axillary peduncles. *S. acuta* is a much branched undershrub 1-2 m tall with lanceolate leaves and pale yellow ciliated flowers. Decoction of the leaves is used for hookworm, diarrhea, parasitic skin diseases, catarrha, dysentery and nephritis (Burkill, 1997).

X. eathiopica is a tropical West African evergreen tree bearing pungent aromatic seeds used as a condiment. The fruits decoction is used in the treatment of bronchitis, asthma and rheumatism (Burkill, 1985). They are also used in many traditional herbal preparations to produce xylopic acid, a substance which has been found to have antimicrobial effects (Karioti *et al*., 2004).

The present study evaluated the *in vitro* antioxidant activity of aqueous extracts of *P. amarus*, *S. cordifolia*, *S. acuta* and *X. eathiopica* used in traditional medicine in Nigeria as immune stimulants to strengthen and harmonize degenerative body systems and assists the immune system in its fight against invading antigens (bacteria and viruses). Oxidative stress was accessed by quantifying the ability of different concentrations of plant extract to suppress iron (Fe^{2+}) induced lipid peroxidation in rat liver homogenates and results were compared with that of vitamin E a known natural antioxidant.

MATERIALS AND METHODS

Sample Collection

X. eathiopica was purchased from a local market in Lagos Nigeria. *P. amarus*, *S. cordifolia* and *S. acuta* were collected by Odukoya on Babcock University Road Ilishan Remo, Nigeria in January 2004 and all samples were in good condition. All herbs used were identified with the assistance of Mr. Wale Ekundayo by comparing with herbarium specimens at FRIN (Forestry Research Institute of Nigeria) herbarium. They were cut into pieces, dried in the oven at 50°C and powdered before use.

Preparation of Extracts

Fifty grams from each sample was extracted with 200 mL of water by boiling and extract obtained was filtered through doubled-folded cheesecloth placed on a funnel and using fingers to press the sample where necessary.

Preparation of Rat Liver Homogenates

Liver homogenates were prepared from male Wistar albino rats (180-250 g) fed on a standard laboratory diet and receiving water *ad libitum*. The animals were fasted overnight but allowed free access to drinking water, killed the next day, dissected and abdominal cavity was perfused with saline. The liver from each animal was collected; a weighed amount of liver was processed to obtain 20% homogenate in ice cold phosphate buffer, pH 7.4 and centrifuged for 15 min to remove the cell debris. The supernatant was used for the in vitro studies (Anuradha and Pavikumar, 1998).

Lipid Peroxidation in Liver Homogenate

The degree of lipid peroxidation was evaluated by estimating the thiobarbituric acid-reactive substances (TBARS) using modified standard methods (Ohkawa *et al*., 1979; Al-Mamary, 2002; Govindarajan *et al*., 2003). In brief, different concentrations of the extract (200-1000 μg mL^{-1}) were added to the liver homogenate. The intensity of the pink coloured complex was measured at 532 nm in a spectrophotometer (TBARS1). As the control, the homogenate was peroxidized with Fe_2SO_4 without the antioxidant extracts (TBARS2). The reactions without Fe_2SO_4 were carried out for each of the test substance as the blank (TBARS3 is the blank for test and TBARS4 is the blank for control). Vitamin E was used as a standard. All tests were done in triplicate and expressed as Mean±SD.

The antioxidant potential of the sample was calculated by using the following equation (Govindarajan *et al*., 2003):

$$\text{Antioxidant activity (\%)} = (1- (\text{TBARS1} - \text{TBARS3}) / (\text{TBARS2} - \text{TBARS4}) \text{ X } 100.$$

Reduced Glutathione (GSH)

This was determined by Ellman's method (Tripathi and Sharma, 1998). Liver homogenate of different extract concentrations (200-1000 μg mL^{-1}) was used and absorbance measured at 412 nm.

RESULTS AND DISCUSSION

The most prominent and currently used assay as an index for lipid peroxidation products is the thiobarbituric acid assay (TBA test). It is based on the reactivity of an end product of lipid peroxidation, malondialdehyde (MDA) with TBA to produce a red adduct. The assessment of the extent of lipid peroxidation relied on individual determinations of MDA contents in sample supernatants. MDA is an end product of peroxidative decomposition of polyenoic fatty acids in the lipid peroxidation process and its accumulation in tissues is indicative of the extent of lipid peroxidation (Draper and Hadley, 1990). Addition of different concentrations of the extracts to the (Fe^{2+}) containing homogenates, showed inhibition of peroxidation in all concentrations. This was found to be dose-dependent (Table 1). *P. amarus* was the most potent of the individual plant extracts. While *S. cordifolia* was more active compared to the other specie *S. acuta* and *X. eathiopica* had the least activity. However, a mixture of all the plant extracts showed greater activity than the individual plant extracts. The mixture with *S. cordifolia* was more active than the mixture with *S. acuta*. This may be as a result of additive effects, related to synergism and/or the contribution of other antioxidants. The inhibition value of the standard, vitamin E was higher than that of the extracts.

Determination of GSH levels was performed using the DTNB. Extracts inhibited the oxidation of reduced glutathione in a dose-dependent manner as in TBA test (Table 2). Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous anti-oxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage to macromolecules. Reduced glutathione, a free radical scavenger, protects cells and tissue structures including activation of T cells and macrophages and regulation of immune function (Fidelus and Tsan, 1986; Meister, 1983). Free radicals are the signalling entities in T cell activation. GSH, act as antioxidants by reacting with free radicals and thus interrupting the propagation of new free radical species.

% inhibition at 1 mg mL^{-1} concentration activity for lipid peroxidation was recorded as mixture of all plant extracts with *S. cordifolia* (86.83±1.23) > mixture with *S. acuta* (72.92±0.96) > *P. amarus* (72.03±0.39), > *S. cordifolia* (56.82±0.54) > *S. acuta* (42.77±1.76) > *X. eathiopica* (39.48±2.07) and inhibition of GSH oxidation was 97.47±0.42, 83.56±0.39, 78.33±0.09, 61.69±0.87, 56.48±0.19, 49.44±1.13 (mean±SD of 3 separate determinations), respectively in the same order.

Table 1: Effects of extracts on ferrous sulphate induced lipid peroxidation in rat liver homogenate

	Inhibition of lipid peroxidation LPO (%) at concentration of extract (μg mL^{-1})				
Plant materials	200	400	600	800	1000
P. amarus	30.62±0.06	42.09±0.13	58.92±1.09	69.11±1.26	72.03±0.39
S. cordifolia	24.44±0.11	30.51±0.07	37.47±0.22	48.56±2.01	56.82±0.54
S. acuta	20.75±0.20	25.91±0.72	31.98±0.16	34.74±0.34	42.77±1.76
X. eathiopica	14.39±0.08	22.67±0.14	28.96±0.09	31.92±0.07	39.48±2.07
Mixt. and *S.acuta*	36.92±1.04	47.49±0.01	58.42±0.14	67.86±1.92	72.92±0.96
Mixt. and *S.cordifolia*	41.73±0.57	58.81±1.21	59.01±0.37	69.35±0.22	86.83±1.23

Control = 0% inhibition; Vitamin E = 95.82±0.11%

Table 2: Effects of extracts on oxidation of GSH in rat liver homogenate

	Inhibition of GSH oxidation (%) at concentration of extract (μg mL^{-1})				
Plant materials	200	400	600	800	1000
P. amarus	33.01±3.27	48.55±0.63	67.27±0.08	70.83±0.12	78.33±0.09
S. cordifolia	28.97±0.61	30.86±1.01	39.03±0.36	52.79±0.14	61.69±0.87
S. acuta	28.44±0.17	29.00±0.29	36.39±0.01	40.18±1.06	56.48±0.19
X. eathiopica	16.59±0.12	33.49±2.07	34.47±1.71	38.02±0.79	49.44±1.13
Mixt. and *S.acuta*	40.05±0.04	46.91±1.61	60.88±2.06	79.24±0.58	83.56±0.39
Mixt. and *S.cordifolia*	48.76±0.18	68.93±0.48	72.26±0.07	88.07±1.91	97.47±0.42

Control = 0% inhibition; Vitamin E = 98.41±0.07%

The reduction in oxidative stress, by the antioxidant effect of these plant extracts and inhibition of GSH oxidation could slow down the cellular aging process and boost the immune system thus explaining the underlying mechanism for using these herbs as immunostimulants in immune mediated disease conditions in Nigeria.

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