

Comparative Assessment of Cytoprotective Effect of Aqueous Extract of Stem bark and Root of *Azadirachta indica* and *Cassia italica* on Oxidatively Stressed Human Erythrocytes

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Abstract

The present investigation shows comparative assessment of aqueous stem bark extract and aqueous root extract of *Azadirachta indica* and *Cassia italica*, possesses an antioxidant activity that exhibits its protective action on lipid peroxidation and contributes to enhance its effect on cellular antioxidant defense. This activity thus proves to be helpful to offer adequate protection against oxidative damage in human erythrocytes. Exposure of erythrocytes to oxidative stress significantly increased lipid peroxidation and decreased the levels of glutathione and the antioxidant enzymes. When erythrocytes treated with aqueous stem bark extract (1.5mg/ml, 3mg/ml and 6mg/ml) Table 1: it could effectively and highly inhibit lipid peroxidation and enhance the activities of the antioxidant enzymes and glutathione content significantly. It could also be observed that when erythrocytes treated with aqueous root extract (1.5mg/ml, 3mg/ml and 6mg/ml) Table 2: *Azadirachta indica* and *Cassia italica* indicate less cytoprotective effect when compared with aqueous stem bark extract at the same concentration.

Keywords: Oxidative stress, Cytoprotection, Aqueous Stem Bark Extract, Aqueous Root Extract, Erythrocyte.

1. Introduction

Cellular damage or oxidative injury arises as a result of an increase in the free radicals or reactive oxygen species (ROS) load and decrease in the efficiency of the antioxidant systems has been implicated to be the fundamental mechanism underlying a number of many human diseases such as neurodegenerative disorder, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorder Saltman (1989). Oxidative/ nitrosative stress generally describes a condition in which cellular antioxidant defences are unable to completely inactivate the ROS and reactive nitrogen species (RNS) generated because of excessive production of ROS/RNS, loss of antioxidant defences, or both. Stocker and Keaney (2004).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects Farnsworth (1989) and Eisner (1990). Over the past two decades, an expanding body of evidence from epidemiology and laboratory studies have demonstrated that some edible plants as a whole or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis Surh and Fergusson (2003) Park and Pezzuto (2002). Medicinal plants are recognized as a source of natural antioxidant that can protect the erythrocyte from oxidative stress and thus play an important role in reactive oxygen species (ROS) Liessuy (2002).

Azadirachta indica popularly known as Neem tree is known as the wonder tree for centuries. It becomes an important global context because it offers answers to the major concern facing mankind. Neem fruits, seed, oil, leaves, bark and roots have such uses as general antiseptics, antimicrobials, treatment of urinary disorders, diarrhoea, fever and bronchitis, skin diseases, septic sores, infected burns, hypertension and inflammatory diseases (Bases et al, 2006). *Cassia italica* is a small medium sized tree with compound leaves and large shining dark green leaflets. The leaves contain anthraquinone derivatives and little tannin. The root bark contains tannins, phlobaphenes and oxy-anthraquinone derivatives and a small amount of volatile oil. The roots of cassia tree is a tonic and useful in reducing fever, it is useful in common cold, in case of running nose, smoke from the burning roots can be inhaled (Home remedy guide, 2011).

1.1 Materials and Methods

Preparation of Crude Extracts: The stem bark and root of *Azadirachta indica* and *Cassia italica* was collected from around Sangere village near Modibbo Adama University of Technology, Yola Adamawa State of Nigeria. They were washed, chopped into small pieces shade dried and pulverized with laboratory mortar and pestle, followed by sieving with Endicott's test sieve to obtain a fine powder. 50g of the fine powder was extracted in a soxhlet with 200ml MeOH for 24 hours. It was filtered; the filtrate was evaporated in a vacuum below 40°C on a rotary evaporator. The final dry weight of the solid extract was used to estimate the yield (g/kg) of each plant, the main values was presented and also stock solution was prepared from these plant extracts by dissolving the dried solid extracts in dimethyl sulfoxide (DMSO) final concentration not exceeding 0.2% prior to being diluted with phosphate buffered saline (PBS), all solutions were stored in a refrigerator at 4°C until use.

Cell Separation: Blood samples from healthy human volunteers were collected in sterile heparinised glass tubes. Erythrocytes were separated by centrifugation (3000 rpm for 10 min) at 4°C. The red cells were then washed three times with 5 volumes of phosphate buffered saline (PBS). Isolated erythrocytes were divided into appropriate aliquots for various treatment schedules. The blood was stressed by applying oxidative reagent.

Study design: The erythrocyte fraction was divided into four groups; in each group three samples were processed. Group I - Control (untreated erythrocytes); Group II - Erythrocytes treated with 1.5mg/ml of root extract for 20 min. at 37°C; Group III - Erythrocytes treated with 3mg/ml of root extract for 20 min. at 37°C and Group IV - Erythrocytes treated with 6mg/ml of root extract for 20 min. at 37°C Trotta (1982). The various parameters were examined in the haemolysate.

Biochemical Assay: Lipid peroxides in terms of malondialdehyde (MDA) were determined by thiobarbituric acid reaction as described by Ohkawa *et al* (1979). The reduced glutathione (GSH) Moron *et al* (1979), superoxide dismutase (SOD) Misra and Fridovich (1972), catalase (CAT) Bergmeyer *et al* (1974) and glutathione peroxidase (GPx) Rotuck *et al* (1973). Calculated data were statistically analysed and compared with that of the student's t-test, taking $P < 0.05$ as significant.

1.1.1 Result

Study show that the levels of lipid peroxides (malondialdehyde formation) and antioxidant potential of the aqueous stem bark extract has increased significantly in (1.5mg/ml, 3mg/ml and 6mg/ml), Table 1 below shows that treated erythrocytes as compared to untreated cells (control) show protective effect which could be due to the activity of all the antioxidant enzymes were significantly enhanced. Table 2. Shows when treated erythrocytes as compared to untreated cell (control), (1.5mg/ml, 3mg/ml and 6mg/ml) also provide cytoprotective effect of the erythrocyte against oxidative damage. Lang *et al* reported that invitro incubation of erythrocytes with silymarin markedly increased the expression of antioxidant enzymes. The comparative assessment of antioxidant effects, anti pro-oxidant, cytoprotection and potential of the extract to provide protection against cellular damage and oxidative stress. Table 1 (malonyldialhyde formation in human erythrocytes of aqueous stem bark extract) offers high protection of the treated cell at various concentrations of the extract when compared to untreated cell (control) and Table 2 (malonyldialhyde formation in human erythrocytes of aqueous root extract) of the treated cell which could be due to its evidence which demonstrates that aqueous stem bark extract have higher chemoprevention capacity of ethnobotanicals and components of vegetable diets with free radical scavenging potentials on ulcer, diabetes, lipid peroxidation, cognitive function, cardiovascular, renal disorder and other human disease.

1.1.2 Discussion

Erythrocytes are very sensitive to toxic influences and serve as an interesting model for assessing the potential of a drug. They are commonly employed in the evaluation of oxidative stress, since they are prone to oxidative reactions because of relatively high oxygen tension and the presence of polyunsaturated lipid – rich membranes. Tapped (1973), Stern (1985) and Mallozi *et al* (1995). Experimental evidences have pointed that lipid peroxidation and oxidative membrane alterations or change in haemoglobin as factors responsible for haemolysis. Hence human erythrocyte exposed to oxidative stress was use to evaluate the comparative assessment and cytoprotective effect of aqueous stem bark and aqueous root extract of *Azadirachta indica* and *Cassia italica*. In the present study erythrocyte exposed to oxidative stress showed increased in lipid peroxidation. The polyunsaturated fatty acid side chain's of the membrane lipids are susceptible to attack by oxidizing radicals with the formation of lipid hydroperoxides, which causes alteration in the physiological properties of the cell and oxidative changes in the membrane, causing protein polymerization leading to an increase in membrane rigidity and permeability Rice-Evan *et al* (1973), Kaplan *et al* (1995) and Nowak *et al* (2002). Such elevation in lipid peroxides due to oxidative stress in RBC has been reported by many workers Nowak (2002) and Bukowsk (2004). Oxidative stress leads to a decline in the activity of the defensive properties of the cell Laskowska and Chelchowska (2001). Erythrocytes exposed to oxidative stress showed a marked decrease in their antioxidant defences. This impairment in the cellular defence system renders the cell more vulnerable to oxidative stress Clark (1988). Increased lipid peroxidation leads to the depletion of intracellular GSH, indicating cellular detoration Shivarajashankara *et al* (2001) When erythrocytes are exposed to oxidative stress, GSH is oxidised to GSSG leading to GSH depletion Srivastava *et al* (1970). In physiological processes, glutathione acts as a protective agent against reactive oxygen species Demir *et al* (1996).

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Table 1: Malonyldialdehyde Formation in Human erythrocytes (nmol/h) at Concentration 1.5mg/ml, 3mg/ml and 6mg/ml of Aqueous Stem Bark Extract

Plant Extract	Untreated Cell (Control)	Treated Cell (1.5mg/ml)	Treated Cell (3mg/ml)	Treated Cell (6mg/ml)
<i>Azadirachta indica</i>	10.26±0.25	13.51±0.37	21.64±0.34	33.61±0.23
<i>Cassia italica</i>	14.12±0.07	23.95±0.05	40.50±0.34	51.07±0.49

Values are in mean ± SEM, n=3

Table 2: Malonyldialdehyde Formation in Human erythrocytes (nmol/h) at Concentration 1.5mg/ml, 3mg/ml and 6mg/ml of Aqueous Root Extract

Plant Extract	Untreated Cell (Control)	Treated Cell (1.5mg/ml)	Treated Cell (3mg/ml)	Treated Cell (6mg/ml)
<i>Azadirachta indica</i>	13.54±0.13	20.73±0.14	25.53±0.10	27.92±0.13
<i>Cassia italica</i>	18.81±0.07	22.27±0.07	19.69±0.19	21.73±0.14

Values are in mean ± SEM, n=3