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Protective Effect of Aqueous Root Extract of *Tamarindus indica, Psidium guajava and Balanite aegyptica* on Oxidatively Stressed Human Erythrocytes.

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Abstract
Oxidatively induced cell damage has been proposed to play an important role in the etiology of numerous pathological conditions. The present study evaluates the antioxidant potential of aqueous root extract of three selected medicinal plants *Tamarindus indica*, *Psidium guajava* and *Balanite aegyptica* against chemically induced oxidatively stressed human erythrocytes. Exposure of erythrocytes significantly increased lipid peroxidation and decreased the levels of glutathione and the antioxidant enzymes. When treated with the root extract (1.5mg/ml, 3mg/ml and 6mg/ml) it could effectively inhibit lipid peroxidation and enhance the activities of the antioxidant enzymes and glutathione content significantly. The observed results indicate that the cytoprotection may be due to its direct free radical scavenging activity and thereby modulating the antioxidant defence system and preserving the functional integrity of the erythrocytes.
Keywords: Antioxidant Potential, Aqueous Root Extract, Erythrocyte Human.

1. Introduction

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repairing the resulting damage. Disturbance in the normal redox state of tissue can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell including proteins, lipids, and DNA. Some reactive oxidative species can even act as a messenger through the phenomenon called Redox Signaling. In humans oxidative stress is involve in many diseases examples includes Sickle Cell Diseases, Amer *et al* (2006), Atherosclerosis, Parkinson diseases, Heart Failure, Mycocardial infarction, Alzheimer's Disease, Bi- polar disorder and fragile X syndrome Diego *et al* (2009).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects Farnswoth (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).

Tamarindus indica belongs to the Family: Caesalpiniacae. The roots are employed in the treatment of heart pains while the bark is used to cure sore throats and the leaves are used for stomach complications

.The fruit are widely used as a remedy against fever, intestinal disease and diarrhoea. The pulp is used against malaria and on wounds and haemorrhoids Mother Herbs and Agro Products (2011). *Psidium guajava* commonly known as guava belongs to the Family: Myrtaceae. It is known as gwabaa in Hausa, ugwoba in Igbo and guafa in Yoruba languages The roots are employed in the treatment of childhood, diarrhoea, dysentery and astringent in Ghana and India Peter (2001). Guava leaves are used for treatment of ailment like diabetes, fever, cough, ulcers, boils and wounds in South Africa. The fruit contains vitamin C, vitamin A, iron, calcium and phosphorus Hernandez (1980) and Burkill (1997). *Balanites aegyptica* is a deep root arid zone tree. The tree is valued for it fruits, seed, roots. It has been used over thousands of years Von (1986). It contains 64 – 72 % carbohydrates, crude protein steroidal saponins, vitamin C, ethanol and other minerals Al – Futuh (1983). It is steroidal saponins which yield

diasgenin a source of steroidal drugs such as corticosteroids, contraceptives and sex hormones Farid *et al* (2002) and Pettit *et al* (1991).

1.1 Materials and Methods

Preparation of Crude Extracts

Three selected medicinal plants roots (*Tamarindus indica, Psidium guajava and Balanite aegytiac*) 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 40^0C 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 4^0C 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 root extract has increased significantly in (1.5mg/ml, 3mg/ml and 6mg/ml), Table below treated erythrocytes as compared to untreated cells (control) thereby showing protective effect which could be due to the activity of all the antioxidant enzymes were significantly enhanced. Lang *et al* reported that invitro incubation of erythrocytes with silymarin markedly increased the expression of antioxidant enzymes.

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 protective effect of aqueous root extract of the selected medicinal plants. 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: Malonyldiahyde Formation in Human erythrocytes (nmol/h) at Concentration 1.5mg/ml, 3mg/ml and 6mg/ml

Plant Extract	Untreated Cell (Control)	Treated Cell (1.5mg/ml)	Treated Cell (3mg/ml)	Treated Cell (6mg/ml)
Tamarindus indica	9.65±0.10	13.84±0.10	16.88±0.14	24.19±0.06
Psidium guajava	18.81±0.07	22.27±0.07	19.69±0.09	21.73±0.14
Balanite aegyptica	7.77±0.10	14.55±0.13	26.61±0.20	38.27±0.10

Values are in mean ± SEM, n=3

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