



WOUND HEALING AND ANTIOXIDANT PROPERTIES OF LEAF EXTRACTS OF ZAPOTECA PORTORICENSIS

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Abstract Various parts of *Zapoteca portoricensis* are used in the management/treatment of several disorders in Eastern Nigeria without any scientific basis. This study was set up to evaluate the wound healing and antioxidant potentials of aqueous and ethanol extracts of fresh leaves of *Zapoteca portoricensis*. Nine groups (A-I) of albino rats (five in each group) were used. Groups A, B, C, and D were treated orally with 100, 200, 400 and 600mg/kg body weight respectively of the aqueous extract, while groups E, F, G and H received the same order of doses of the ethanol extract for seven consecutive days. Group I was the control. Serum levels of total protein, proteinases and vitamin C were used to assess wound healing property, while malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) were measured to examine antioxidant activity of the extracts. The total protein concentrations of treated groups were not significantly different ($P > 0.05$) from those of the control. Proteinases activity and vitamin C concentration were significantly higher ($P < 0.05$) in the administered groups than in the untreated group. Activity of SOD, CAT and GR increased significantly ($P < 0.05$), while MDA levels decreased significantly ($P < 0.05$) in the treated groups relative to the control. These findings are indications that fresh leaves of *Zapoteca portoricensis* may be useful in the treatment of wounds due to their antioxidant potential.

Key words: Albino Rats, Serum, Oxidative Stress, Proteinases, Vitamin C.

INTRODUCTION

Plants continue to be a major source of medicines as they have been throughout human history. Drug discovery, ethnobotany, and traditional/indigenous medicines have long been basic to medicinal plant research. More than 150,000 plant species have been studied, and many contain therapeutic substances [1].

Zapoteca portoricensis, commonly called white stick pea, belongs to the family Mimoseceae [2]. It is a perennial plant and streamlined erect shrub, with small and oval green leaves. Different parts of the plant are used in the treatment/management of several disorders in Eastern Nigeria and other parts of the world. In Eastern Nigeria, the leaves (in various forms) are used in the treatment/management of convulsion, external wounds, constipation, prolonged labour, mental disorder (inducement of calmness) and skin infections

Wound healing is a natural restorative response to tissue injury. It refers to the interaction of a complex cascade of cellular events that generate resurfacing, reconstruction and restoration of the tensile strength of the injured skin [3]. The process of wound healing involves three major phases

inflammatory, proliferative and maturation phases. In maturation phase, collagen forms tight cross-linkage to other collagen and with other protein molecules increasing the tensile strength of the scar [4].

Proteinases are involved in proteolytic processing of nascent polypeptide chains that accompany protein synthesis and degradation [5]. They are present in all wound exudates, and play an essential role in the healing of acute and chronic wounds, via cellular invasiveness, apoptosis and remodeling [6]. Proteinases enable cells participating in wound healing (eg. macrophages, mast cells, fibroblasts, etc.) to digest the cellular barriers across their path to the site of injury [7]. Vitamin C (ascorbate), a water-soluble vitamin involved in anti-oxidative processes in cellular metabolism, plays an important role in synthesis of collagen and norepinephrine by maintaining the necessary enzymes in their active forms [8]. It is a co-factor for the hydroxylase involved in the hydroxylation of proline residues of collagen which is vital to normal wound healing [9].

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Reactive oxygen free radicals (ROS) have been implicated in many diseases and in aging process. These free radicals, which cause tissue damage via oxidative stress, are generated by aerobic respiration, inflammation and lipid peroxidation. Antioxidant systems minimize or prevent deleterious effects of the ROS [10].

Lipid peroxidation is an established mechanism of cellular injury, and is used as an indicator of oxidative stress. Polyunsaturated fatty acids peroxides generate malondialdehyde (MDA) and 4-hydroxyalkanal upon decomposition [11]. Superoxide dismutase (SOD) decomposes superoxide anion into hydrogen peroxide and oxygen at very high rates. Superoxide radical is involved in diverse physiological and patho-physiological processes [12]. Catalase (CAT) is an antioxidant enzyme ubiquitously present in aerobic cells. It catalyses the decomposition of hydrogen peroxide to water and oxygen. High concentration of hydrogen peroxide is deleterious to cells, and its accumulation causes oxidation of cellular targets such as DNA, proteins and lipids, leading to mutagenesis and cell death [13]. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells glutathione is maintained in its reduced form by glutathione reductase (GR), and in turn reduces other metabolites and enzymes, as well as react directly with oxidants [14].

The medicinal uses of *Zapoteca portoricensis* have not being investigated. There is a strong correlation between antioxidants and wounding [15]. In this communication, this study assessed its wound healing and antioxidant properties.

MATERIALS AND METHODS

Collection of fresh leaves of *Zapoteca portoricensis*

The leaves were obtained in October, 2010 from a bush in Abakaliki, Ebonyi State of Nigeria. They were identified by Professor. SC Onyekwelu of Biology Department, Ebonyi State University in Abakaliki, washed with distilled water and used immediately for the research, which lasted fifteen days.

Preparation of leaf extracts

The methods of extraction used by Agbafor [16] were adopted, utilizing distilled water and ethanol (separately) as solvents. The extracts were concentrated using rotor evaporator to get gel-like dark brown extracts.

Animals and handling

Forty-five adult male albino rats, weighing 125-136g were bought from the animal house of Biochemistry Department, University of Nigeria, Nsukka. They were placed in nine groups (A-I) of five rats in each group and kept in animal house of Biochemistry Department, Ebonyi State University Abakaliki for seven days to acclimatize. All the rats were allowed free access to feed (rat chaw) and water before and throughout the experiment.

Animal groups and treatments

Solutions of the extracts were made with distilled water. Groups A, B, C and D were treated orally with 100, 200, 400 and 600mg/kg body weight respectively of distilled water extract, while E, F, G and H received the corresponding doses of ethanol extract for seven consecutive days. Group I, the control, was given distilled water.

Collection of samples from the animals

Blood samples were collected from the animals following an overnight fast through cardiac puncture under mild anaesthesia using diethylether.

Assessment of wound healing property

Total protein, proteinases and vitamin C levels were used to study the wound healing property of the extracts. The method of Lowry [17] as described by [18] was used to measure total protein. Proteinases activity was measured by the method of Im and Hoopes [19]. Vitamin C was determined by the method of Tietz [20].

Measurement of antioxidant property

The method of Ohkawa et al., [21] was used to measure the level of MDA. SOD, CAT and GR activities were determined by the methods of Kakkar et al., [22], Aebi [23] and Bentler [24] respectively.

Data Analysis

Statistical analysis was done using analysis of variance (ANOVA). Means were compared for significance using Duncan's multiple range test ($P < 0.05$) [25]

RESULTS AND DISCUSSION

The references cited in this section are in agreement with the major findings of the study. There was a dose-dependent decrease in average body weight, physical activity, and food and water intake in the treated groups compared with the control, which continued to thrive (data not shown). The actual reason for these observations is a subject of an ongoing research. However, an upset in general metabolism may be responsible for the decrease in

physical activity and food and water intake, while the observed decrease in food and water intake may explain the decrease in body weight. Agbafor and Akubugwo [26] made a similar observation on treating albino rats with ethanolic extract of *Cymbopogon citratus*.

The significant increase ($p < 0.05$) in proteinases activity and vitamin C concentration of the treated groups (table.1) indicates that the extracts possess wound healing potentials. Proteinases play a vital role in the healing of acute and chronic wounds, via cellular invasiveness, apoptosis and remodeling [6]. Proteinases enable cells participating in wound healing (eg. macrophages, mast cells, fibroblasts, etc.) to digest the cellular barriers across their path to the site of injury [7]. The exact chemical constituent(s) of the extracts responsible for this increase in proteinases activity and their mode of action are currently under investigation. Vitamin C plays a key role in the synthesis of collagen (a structural protein required for the maintenance of connective tissues) by maintaining the necessary enzymes in their active forms. It is a cofactor for hydroxylase involved in the hydroxylation of proline residues of collagen. This hydroxylation is responsible for stability and tensile strength of collagen [8].

The wound healing property of the extracts may not depend on total protein concentration in serum. This was reflected by the no significant difference ($p > 0.05$) in serum total protein of the treated groups and the control.

Table.2 presents the effect of the extracts on serum levels of MDA, SOD, CAT and GR. There was a significant decrease ($p < 0.05$) in the concentration of MDA of the treated groups relative to the control. The activity of SOD, CAT and GR in the treated groups was significantly higher ($p < 0.05$) in the untreated (control) group. These results are indicative of the antioxidant potential of the extracts. The antioxidant enzymes are the first-line defense against damage by free radicals, and thus provide protection against the deteriorating outcome [27]. Oxidative injury and lipid peroxidation can be monitored by measuring MDA. Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage caused by free radicals [28][29].

This antioxidant property is a contributing factor in the wound healing potential of *Zapoteca portoricensis*. Antioxidants have been reported to play significant roles in wound healing. It is consented that ROS are deleterious to wound healing due to harmful effects on cells and tissues [30]. Free radical scavenging enzymes are cytoprotective enzymatic

group that has an essential role in the reduction, deactivation and removal of ROS as well as regulating wound healing process. Wound results in loss of different free radical scavengers, both enzymatic and non-enzymatic, that either partially or completely recover following healing. Increase in free radical production and diminished antioxidant activities may worsen the situation and account for delay in wound healing [15].

Table I: Serum Proteinase Activity, Vitamin C and Total Protein of the Rats after Extracts Administration

Animal group	Proteinases activity ($\mu\text{ mol/hr/ml}$)	Vitamin C (mg/dl)	Total protein (mg/ml)
A	3.89 ± 0.11^b	1.93 ± 0.13^b	0.61 ± 0.05^a
B	4.75 ± 0.08^b	2.25 ± 0.09^b	0.60 ± 0.04^a
C	5.77 ± 0.12^b	2.66 ± 0.11^b	0.58 ± 0.03^a
D	4.54 ± 0.14^b	2.84 ± 0.10^b	0.55 ± 0.03^a
E	1.88 ± 0.08^b	1.14 ± 0.10^b	0.64 ± 0.06^a
F	2.21 ± 0.11^b	1.84 ± 0.07^b	0.60 ± 0.03^a
G	2.56 ± 0.14^b	2.33 ± 0.05^b	0.48 ± 0.03^a
H	2.62 ± 0.07^b	2.59 ± 0.12^b	0.57 ± 0.06^a
I	0.44 ± 0.06^a	0.73 ± 0.10^a	0.76 ± 0.04^a

Values are mean \pm SD. $n = 5$. Values in the same column having different superscripts differ significantly ($p < 0.05$).

Table II: Serum levels of MDA, SOD, CAT and GR of the animals after treatment.

Group	MDA (nmols/ml)	SOD (U/mg protein)	CAT (U/mg protein)	GR (U/mg protein)
A	2.44 ± 0.24^b	7.15 ± 0.43^a	114.40 ± 3.43^a	8.67 ± 0.44^a
B	2.06 ± 0.30^b	11.66 ± 1.14^b	130.45 ± 2.87^a	10.35 ± 1.16^a
C	1.13 ± 0.08^b	16.45 ± 1.06^b	169.70 ± 3.66^b	17.60 ± 1.24^b
D	1.65 ± 0.36^b	16.12 ± 1.40	161.33 ± 4.20^b	14.40 ± 2.11^b
E	2.97 ± 0.78^b	5.38 ± 1.24^a	120.16 ± 1.84^a	8.15 ± 0.53^a
F	2.54 ± 0.60^b	8.70 ± 0.67^b	124.30 ± 3.14^a	9.45 ± 1.20^a
G	2.01 ± 0.05^b	13.73 ± 1.67^b	148.77 ± 2.80^b	14.63 ± 1.55^b
H	2.35 ± 0.04^b	12.81 ± 1.22^b	145.41 ± 3.35^b	12.34 ± 0.77^b
I	4.72 ± 0.36^a	4.88 ± 0.25^a	96.38 ± 2.14^a	6.90 ± 0.33^a

Values are mean \pm SD. $n = 6$. Values in the same column having different superscripts differ significantly ($p < 0.05$).

A = 100mg/kg aqueous extract B = 200 mg/kg aqueous extract
 C = 400mg/kg aqueous extract D = 600mg/kg aqueous extract
 E = 100mg/kg ethanol extract F = 200mg/kg ethanol extract
 G = 400mg/kg ethanol extract H = 600mg/kg ethanol extract
 I = Control

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