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Antioxidant Activity of Diet Formulated from Selected Leafy Vegetables Commonly Available and Consumed in Abakaliki, Nigeria.

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Citation

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Abstract

The antioxidant effects of diet formulated from five leafy vegetables: Vitex doniana (Uchakiri), Ceiba pentandra (Akpuota), Ficus ottoniifolia (Ogbu), Zanthoxylum zanthoxyloides (Uko) and Sesamum alatum (Oboroto), commonly available and extensively consumed in the rural areas of Abakaliki, Ebonyi State, Nigeria, were studied using albino rats. A total of 84 rats (mean weight = 133.22 ± 0.33k g) assigned into seven groups of 12 rats per group were investigated. While group I was normal, groups II – VII were made diabetic by intraperitoneal injection of alloxan (200 mg/Kg body weight). Groups III-VII were administered graded concentrations (10 %, 15 % and 20 % w/w) of feed/powdered leaves mixture of the respective vegetables, while groups I & II served as non-diabetic and diabetic controls respectively and received normal rat feed. Feeding of the animals was done for twenty one days (21), after which the serum levels of malonylaldehyde (MDA), vitamin C, and total antioxidant activities (TAA) were determined using standard laboratory methods. The result showed that MDA levels were significantly ($p < 0.05$) lower in all the groups treated with the experimental diets, while vitamin C and antioxidant activities were significantly higher when compared with the diabetic controls. Ceiba pentandra and S. alatum showed the highest and lowest antioxidant activities, respectively. The results suggest appreciable antioxidant potentials of the vegetables, which could be of importance in the management of oxidative stress in diabetic condition.

INTRODUCTION

Increasing evidence from both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymic glycation of protein and the subsequent oxidative degradation of glycated proteins [1]. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defence mechanisms may lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance [2]. Oxidative stress may promote the development of diabetic or/and the progression of its complications. It is well known that alloxan administration causes severe necrosis of pancreatic beta-cells [3]. It has been suggested that alloxan induces the production of hydrogen peroxide and some free radicals such as superoxide and hydroxyl ions that first damage and later bring about the death of the cells [4, 5].

The curative properties of some plants have been discovered and exploited early in human history [3]. Over the years, the knowledge of herbal medicine has grown with a wide range of plants sought for their healing effects. In many developing countries, traditional medicine is still the mainstay of health care, and most of the drugs used come from plants [6]. Nigeria has a very long history of traditional medicine. At present, there are many medicinal plants that have already been promoted to be used in primary healthcare and classified according to their pharmacological actions such as treatment of stomach pain, respiratory problem, antidiabetic, and anti-inflammatory [6].

Herbal preparations from Zanthoxylum zanthoxyloides (Rutaceae), Vitex doniana (Verbenaceae), Ceiba pentandra, (Bombacaceae), Sesamum alatum (Pedaliaceae) and Ficus ottoniifolia (Moraceae) have achieved wide spread acceptability as therapeutic agents. These include uses as, antihypertensive, hepato-protective, anti-inflammatory, antidiabetic and lipid-lowering agents [5, 7, 8]. It is also believed that formulations from these plant families contain numerous types of antioxidants that can boost the body's defences against free radicals and other causes of oxidative stress. Varieties of bioactive compounds and their derivatives such as phenolic compounds, terpenes, steroids, alkaloids, glycosides, fats and oils derived from herbs have been shown to inhibit oxidative stress in a number of experimental systems [9]. Vitex doniana, C. pentandra, S. alatum, F. ottoniifolia, and Z. zanthoxyloides, are species widely cultivated in the Southeastern region of Nigeria. The leaves of these plants have been used as sausage. They have also been used traditionally for treatment of coughs, fever, thirst and sore throat. Besides these pharmacological actions, these plants have been demonstrated to possess antihyperglycaemic and antioxidant effects in alloxan-induced diabetic rats, especially the leaves [6]. The major advantage of using Nigerian traditional plants is their availability in our own locality, allowing easier access at lower cost. However, most of the traditionally used medicinal plants have not been given sufficiently scientific and medical scrutiny.

Thus, to ensure the safety of these plants in clinical use, their scientific evaluations are necessary. Therefore, the aim of this study was to evaluate the antioxidant properties of the leaves of these selected vegetables in a diabetic model.

MATERIALS AND METHODS

PREPARATION OF EXPERIMENTAL FEED

Fresh leaves of Monk’s pepper (Vitex doniana), Silk cotton (Ceiba pentandra), Gazelle’s Sesame (Sesamum alatum), Ficus ottoniifolia and Zanthoxylum zanthoxyloides were collected from Ugwulangwu village in Ohaozara local government area of Ebonyi State, Nigeria. They were duly identified and confirmed by a plant taxonomist from Botany Department of the University of Nigeria Nsukka, Nigeria. The fresh leaves were washed clean with sterilzed distilled water and sun-dried to a constant weight and ground into powder with a mortar. The resulting powder was sieved and stored in polyethylene bags at room temperature. The experimental feed was prepared by mixing 10g/90g, 15g/85g and 20g/80 w/w of the powder and feed, representing 10 %, 15 % and 20 % respectively. A little quantity of water was added to get the feed in pellet form. Thereafter, the pellets were sun-dried and ready for use

EXPERIMENTAL ANIMALS

Eighty four (84) adult Wister albino rats weighing 100-165g of both sexes, obtained from the Laboratory Animal Facility of the Faculty of Biological Sciences, University of Nigeria, Nsukka (UNN) were used for the study. The animals were acclimatized for about 7 days under standard environmental conditions and maintained on a regular livestock feed (Pfizer Plc, Lagos, Nigeria) and water ad libitum.

EXPERIMENTAL DESIGN

The rats were divided into seven groups (I – VII) of 12 rats each. Diabetes was induced in groups II – VII animals by a single intraperitoneal injection of 200mg/Kg body weight of alloxan monohydrate dissolved in distilled sterilized water while animals in group I were normal. Fasting blood glucose level was checked by a glucometer (ACCUTREND GC (Boerhinger, Mannheim, Germany), using blood from the tail tips and diabetes mellitus was confirmed by elevated blood glucose >7.8 mmol/l.

The animals in groups III, IV, V, VI & VII were sub-grouped into 3 of four (4) rats per sub-group and administered graded concentrations (10 %, 15 % & 20 % of the experimental feed) prepared from Zanthoxylum zanthoxyloides, Ceiba pentandra, Ficus ottoniifolia, Vitex doniana and Sesamum alatum respectively. However, groups I & II animals were fed on normal feed and served as non-diabetic and diabetic control respectively. The experimental feeds were administered to the rats once daily for 21 days after which the animals were sacrificed and blood samples collected for biochemical analyses. The blood was collected exactly eighteen hours after the last feed by ocular puncture into EDTA (1mg/ml) bottle. Plasma was obtained by centrifuging the whole blood in a whisperfuge (Model 684) centrifuged at 2500 x g for 5mins.

DETERMINATION OF ANTIOXIDANT INDICES

Lipid peroxidation assay: The quantitative measurement of lipid peroxidation in plasma was performed according to the method of Albro et al. [10] as modified by Das et al. [11].

Measurement of antioxidant activity: The antioxidant activity in the plasma was measured in accordance with the method described by Diordjevic and Koracevic [12].

Determination Of Vitamin C (Ascorbic Acid): Vitamin C was determined as described by Omaye et al. [13].

STATISTICAL ANALYSIS

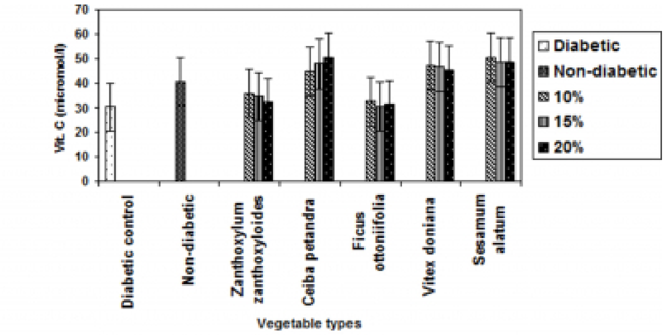
The data were analysed for mean and standard deviation and comparison of parameters among groups were done by one-way Analysis of Variance (ANOVA). Statistical significance was achieved at $p < 0.05$. Statistical Analysis System 6.21

package (14) was used for all analyses

RESULTS

Figure 1

Fig 1: Effect of Diet Formulated from Selected Leafy Vegetables Commonly Available and Consumed in Abakaliki on Plasma Vitamin C Concentration (μmol/l) of Alloxan-Induced Diabetic Rats

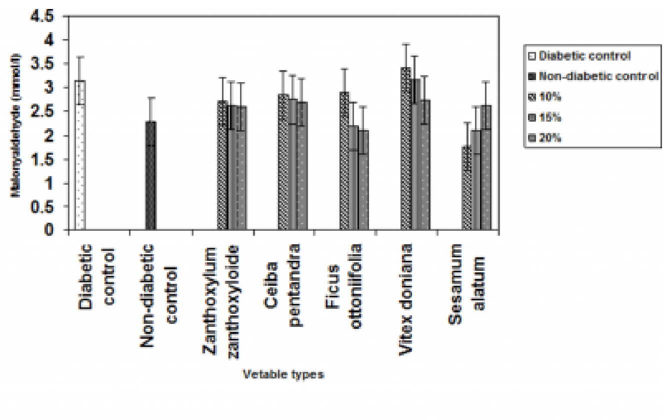


Values are mean ± S.D of three separate determinations.

While Vitamin C concentration was significantly (P<0.05) higher in Zanthoxylum zanthoxyloide, Ceiba pentandra, Vitex doniana and Sesannum alatum groups as compared to diabetic control, it was comparable (P>0.05) in the F. ottoniifolia and the contol groups.

Figure 2

Fig 2: Effect of Diet Formulated from Selected Leafy Vegetables Commonly Available and Consumed in Abakaliki on Plasma Malonylaldehyde (MDA) Concentration (mmol/l) of Diabetic Rats

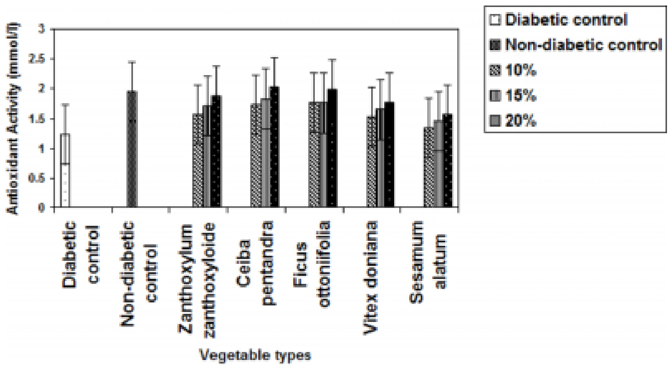


Values are mean ± S.D of three separate readings from rats.

Except for Ceiba pentandra and Vitex doniana groups which had comparable MDA concentration with the diabetic control, all other groups had significantly (p < 0.05) lower MDA concentrations, with Ficus ottoniifolia and Sesamum alatum having highest effect at 20 % and 10 % respectively.

Figure 3

Figure 3: Effect of Diet Formulated from Selected Leafy Vegetables Commonly Available and Consumed in Abakaliki on Plasma Total Antioxidant Activity (mmol/l) of Diabetic Rats



Values are expressed as mean ± S. D. of three separate readings.

All the treated groups showed significantly higher antioxidant activity, especially at higher dose (20 %) with Ceiba pentandra and Ficus ottoniifolia showing the highest total antioxidant effect.

DISCUSSION

The results showed that animals treated with the leaves of these vegetable had significantly (p < 0.05) lower MDA levels and significantly (p < 0.05) higher vitamin C levels and antioxidant activities when compared with the untreated animals with Ceiba pentandra and S. alatum showing the highest and lowest antioxidant activities, respectively. The antioxidant activities of medicinal plants have been documented by several studies [15-19]. In the present study, the significantly lower MDA in the animals treated with the leaves of these vegetables suggests lower rate of lipid peroxidation in the treated animal when compared the controls. MDA is one of the end products in the lipid peroxidation process, which elevation has been associated with increased lipid peroxidation leading to tissue damage [20]. Alloxan, a chemical diabetogen, has been found to induce diabetes mellitus via the induction of oxidative stress, which eventually destroy beta cells in islets of langerhans [21]. Studies have found increased lipid peroxides or reactive oxygen species (ROS) and oxidative stress (or both) in different animal models of diabetes [22, 23]. Moreover, it is suggested that transition metals such as iron, zinc and copper may be involved in alloxan toxicity [24]. The role of transition metals in alloxan-induced diabetes was also documented in the study of El-Hage et a.l [25] who found that ICRF-187 (dexrazoxane, Cardioxan), a metal chelator with no free radical scavenging activity prevented alloxan-induced hyperglycaemia in mice.

The present study demonstrates that diet formulate from Zanthoxylum zanthoxyloides, Ceiba pentandra, Ficus ottoniifolia, Vitex doniana and Sesamum alatum prevented the rise in oxidative stress induced by alloxan in alloxan-induced diabetic rats. However, the results in clinical practice are not unequivocal and the usefulness of antioxidant therapy in diabetic patients is not convincing [26].

The lack of significant difference in the body weight of the treated and non-treated animals observed in the present study reflects equal growth rate in the animal groups, which indicated that the vegetables do not impose any acute fluid loss, proteolysis and lipolysis on the treated groups. It has been reported [27] that in diabetic mellitus, acute fluid loss, proteolysis and lipolysis are responsible for weight loss.

Decreased activities of non-enzymic antioxidant vitamin (vitamin C) noticed in diabetic rats groups indicate the extent of free radical-induced damage due to hyperglycaemia. It is well known that, when there is imbalance between free radical production and antioxidant defences, “oxidative stress” occurs resulting in deregulation of cellular functions [2]. The significant increase in the concentration of the ascorbic acid in V. doniana, S. alatum, C. pentandra, Z. zanthoxyloides and F. ottoniifolia diet groups showed the antioxidant properties of those vegetables.

The significant increases in the total antioxidant activity (TAA) observed in the treated animals in comparison to their non-treated counterparts reaffirms the antioxidant capacity of these vegetables. This antioxidant property was also found to be dose-dependent as most of the vegetable showed highest activity at 20% concentration. Although we did not come across any study on these vegetables and phytochemical analysis was not part of the present study, the antioxidant properties of some vegetables and fruits have been partly attributed to the presence of low molecular weight phenolic compounds, which are known be potent as antioxidant [28]. Studies have shown that the antioxidant activity of these phenolic compounds were due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers as well as chelators of metal ions [29]. Total phenolic content had been positively correlated with antioxidant capacity. The mechanism employed by flavonoids, which are abundantly present as polyphenols in vegetables in the defence against the harmful effect of reactive species include breaking of the chain reactions of lipid peroxidation via the removal of hydroxyl radical [16], chelation of transition metal, including copper, iron and zinc and inhibition of enzymatic steps responsible for the generation of free radicals [30, 31]. The free radical scavenging ability of plant phenolic compounds has been attributed to their hydroxyl group [32] and related to the hydroxylation patterns [17].

In conclusion, this study has demonstrated the antioxidant properties of V. doniana, C. pentandra, Z. zanthoxyloides, S. alatum and F. ottoniifolia and reassures the safety of their consumption as vegetables, especially in the rural communities. Therefore, they seem promising in alleviating diabetes-associated oxidative stress. The active substances of these vegetables when identified, isolated and administered in purer forms may have better effects.

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