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# The *In Vivo* Antioxidant Potentials of the Crude Ethanol Root Extract and Fractions of *Sphenocentrum jollyanum* on Oxidative Stress Indices in Streptozotocin-Induced Diabetic Wistar Albino Rats

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**ABSTRACT**

The *in vivo* antioxidant potentials of the crude ethanol root-extract and fractions of *Sphenocentrum jollyanum* (SJ) on oxidative stress indices in streptozotocin (STZ ) induced diabetic Wistar albino rats were carried out with a total of 48 albino rats. The *in vivo* antioxidant properties of the ethanol root extract of SJ was analyzed using standard laboratory methods. Forty eight male albino rats were randomly assigned into eight groups, each containing six animals. Diabetes was induced by intraperitoneal injection of a single dose of 70mg/kg body weight of STZ. The treatment started after confirmation of diabetes and lasted for 21 days. Groups 1, 2 and 3 served as positive control (diabetic rats treated with 0.5 ml of normal saline), standard control (diabetic rats treated with 0.5mg/kg body weight of glibenclamide) and negative control (non diabetic rats treated with 0.5ml of normal saline) respectively. Groups 4, 5 and 6 rats were induced with diabetes and were treated with 250, 500 and 1000 mg/kg body weights of the crude ethanol extract of SJ respectively while rats in groups 7 and 8 were induced with diabetes and treated respectively with 250 mg/kg body weight of methanol and ethylacetate fractions of SJ. The treatment of STZ-induced diabetic albino rats with crude ethanol root-extract of *Sphenocentrum jollyanum* at doses of 250, 500 and 1000 mg/kg body weights and 250 mg/kg body weight of ethylacetate and methanol root fractions of *Sphenocentrum jollyanum* significantly ($p<0.05$) increased the activities of catalase, superoxide dismutase, glutathione peroxidase, the levels of serum vitamins C and E significantly ($P<0.05$) lowered the level of malondialdehyde in the treated groups. The result also showed significant ($p<0.05$) differences in the effect of crude ethanol extract relative to fractions except at the dose of 1000 mg/kg body weight which showed no significant ($p>0.05$) change relative to the fractions. The result equally showed that the effects on the crude ethanol root extract were dose dependent and the value of the standard control is quite similar to that of the negative control. The results of this study imply that crude ethanol root extract and fractions of *Sphenocentrum jollyanum* are harmless sources for obtaining the natural antioxidants that may not only anticarcinogenic but may also be protective against cardiovascular diseases and diabetes.

**Keywords:** *Sphenocentrum jollyanum, in vivo* antioxident, oxidative stress, albino rats

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin action, insulin secretion or both [1, 2, 3,4]. Insulin deficiency leads to chronic hyperglycemia with disturbances in fat, carbohydrate and protein metabolism. It is a global disease, prevailing throughout the world, although the prevalence rate differs from country to country [5, 6, 7, 8]. India, China and United States of America (USA) are the top three countries in terms of number of hyperglycemic patients [10, 11, 12, 13]. The increasing ageing population, consumption of calorie-rich diet, obesity and sedentary lifestyle has led to tremendous increase in the number of hyperglycemics worldwide [14,15, 16,17,18,19]. DM is known to comprise of two major types: type 1 and type 2, each with distinct pathogenesis [20, 21, 22, 23, 24, 25, 26, 28]. Common to both, however, is hyperglycemic and various life threatening complications [29, 30, 31, 32].

Medicinal plants such as *Sphenocentrum jollyanum*called "Ezeogwu" in Igbo, "AduroKoroo" or "Okramankote" in the Akan Language in Ghana [31] has been shown to have antihypertensive, antioxidant, antinociceptive, antiviral and anti-angiogenic effects in animals [18,23]. *Sphenocentrum jollyanum* is an erect shrub that belongs to the family Menispermacea [30]. The plant is also documented for its use against chronic coughs, worms and other inflammatory conditions as well as tumors [23]. Most medicinal plants presently employed by local herbalists are used without much scientific information. It is therefore important to access and document the ethno-medical claims of these medicinal plants.

Streptozotocin (STZ) is widely used to induce diabetes in various laboratory animals as it is particularly toxic to the pancreatic insulin-producing beta cells in mammals . Aloxan and streptozotocin are the most prominent diabetogenic chemicals in diabetic research. Both are cytotoxic glucose analogues and their mechanism of beta cell selective action is identical.

Antioxidants are molecules that inhibit the oxidation of other molecules. They are group of compounds that are characterized by their ability to be oxidized in the place of other compounds [5]. Antioxidant defense mechanism involves both enzymatic and non-enzymatic strategies.

Oxidative stress represents an imbalance in the production and clearance of reactive oxygen species/free radicals in biological systems [15]. Disturbances in the normal redox state of tissues can cause toxic effects through the production of peroxides and other free radicals that damage components of the cell, including proteins, lipids and DNA, hence in humans, oxidative stress has been identified as one of the aetiological factors in many diseases [2]. Reactive oxygen species may be beneficial as they are used by the immune system as a way to attract and kill pathogens [1].

## MATERIALS AND METHODS

### Collection of Biological Materials

The present study was carried out using the roots of *Sphenocentrum jollyanum* and albino rats and mice. Fresh roots of *Sphenocentrum jollyanum* were collected from Ovoko in Igbo-Eze South Local Government Area of Enugu State, Nigeria and was authenticated in the

*Herbarium* Unit of Department of Botany, University of Nigeria, Nsukka by Mr O. Onyeukwu. Part of the authenticated plant was deposited in the *herbarium* for reference purposes.

Fourty eight albino *wistar* rats were purchased from the Department of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria. They were acclimatized for a period of two weeks at the animal house of the Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria prior to commencement of experiment. They were maintained at room temperature, 12hr day/night period and fed *ad libitum* on water and growers mash; weighed prior to commencement of experiment and daily till the end of the experiment.

**Preparation of the Plant Extract**

The roots of *Sphenocentrum jollyanum* were harvested and washed under tap water to remove contaminants and air dried under shade. They were pulverized using laboratory milling machine and sifted using 0.25 mm sieve. One thousand five hundred gram (1,500g) of the powdered root sample of *Sphenocentrum jollyanum* was soaked in 7500 ml of ethanol for 48 hours with agitation. The resulting ethanol root extract was filtered using muslin cloth and evaporated to dryness using rotary evaporator at a temperature of 45$^0$C. The concentrated ethanol root extract of *Sphenocentrum jollyanum*was used for subsequent analyses.

**Fractionation of the Crude Extract of *Sphenocentrum jollyanum* Roots**

The ethanol root extract of *Sphenocentrum jollyanum* (20 g) was fractionated in a glass column (150 cm x 1.5 cm) packed with 200 g of a slurry of silica gel G. (70-230 mesh). The column was eluted in succession with 500 ml ethyl acetate and 500 ml methanol to obtain ethyl acetate (EAF) and methanol (MF) fractions respectively. The resulting fractions were evaporated to dryness using rotary evaporator at a temperature of 45$^0$C. The concentrated ethyl acetate (EAF) and methanol root fractions of *Sphenocentrum jollyanum* were used for subsequent analyses.

**Determination of *In vivo* Antioxidant potential of Ethanol Root Extract of *Sphenocentrum jollyanum***

Malondialdehyde (MDA) Level was determined using the method of Varshney and Kale (1990) [30], Vitamin E was determined using the method of Rutkowski *et al.* (2005) [25], Vitamin C (Ascorbic Acid) was determined using the method of Bajaj and Kaur (1981) [5], Catalase (CAT) activity was determined using the method of Cohen *et al.* (1970) [6], Superoxide dismutase (SOD) activity was determined using the method of Misra and Fridovich(1972) [20], and Glutathione peroxidase (GPx) activity was determined using the method of Sato *et al.*(1978) [26].

**STATISTICAL ANALYSIS**

Results were expressed as mean± standard deviations where applicable. The data were subjected to one-way analysis of variance (ANOVA), followed by Post hoc Duncan multiple comparison test using SPSS software version 21 and $p < 0.05$ was regarded as significant.

## RESULTS

### Effect of Crude Ethanol Root-Extract and Fractions of *Sphenocentrumjollyanum* on Oxidative Stress Indices in STZ-induced Diabetic Albino Rats

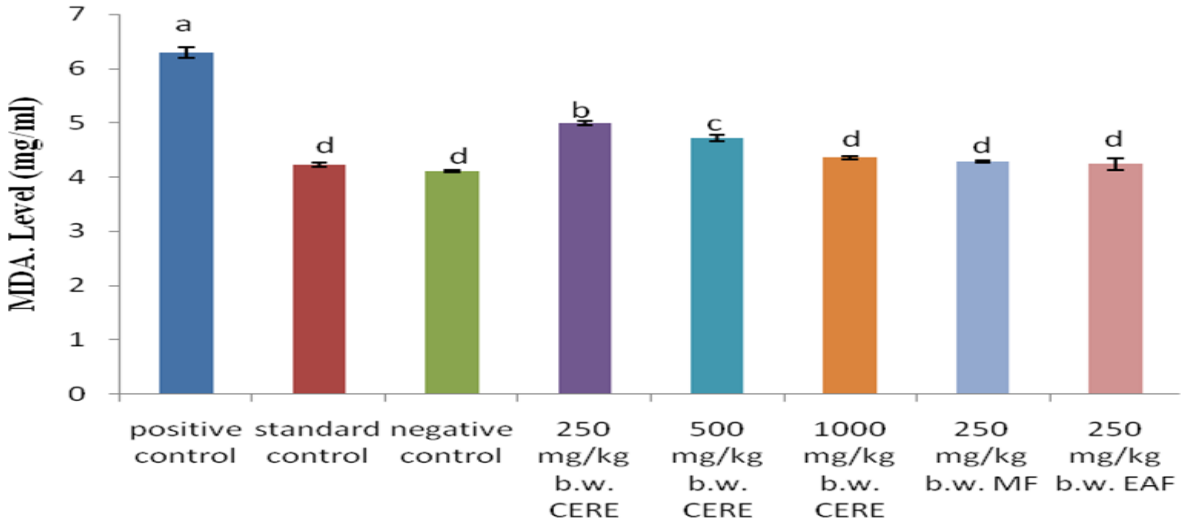


**Figure 1**: MDA levels in STZ induced diabetic wistaralbino rats treated with crude ethanol root-extract and fractions of *Sphenocentrumjollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at p<0.05. **Key**: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

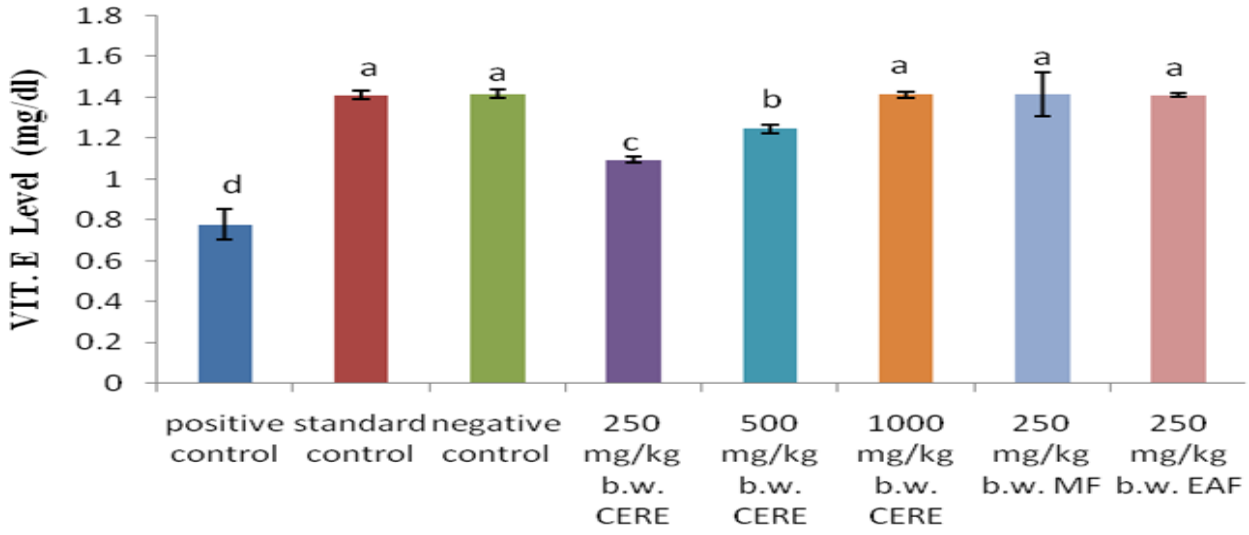


**Figure 2:** Vitamin E Levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrum jollyanum*. Data are shown as mean ± standard deviation (n=4). meanValues with different alphabet showed significant difference at p<0.05. Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

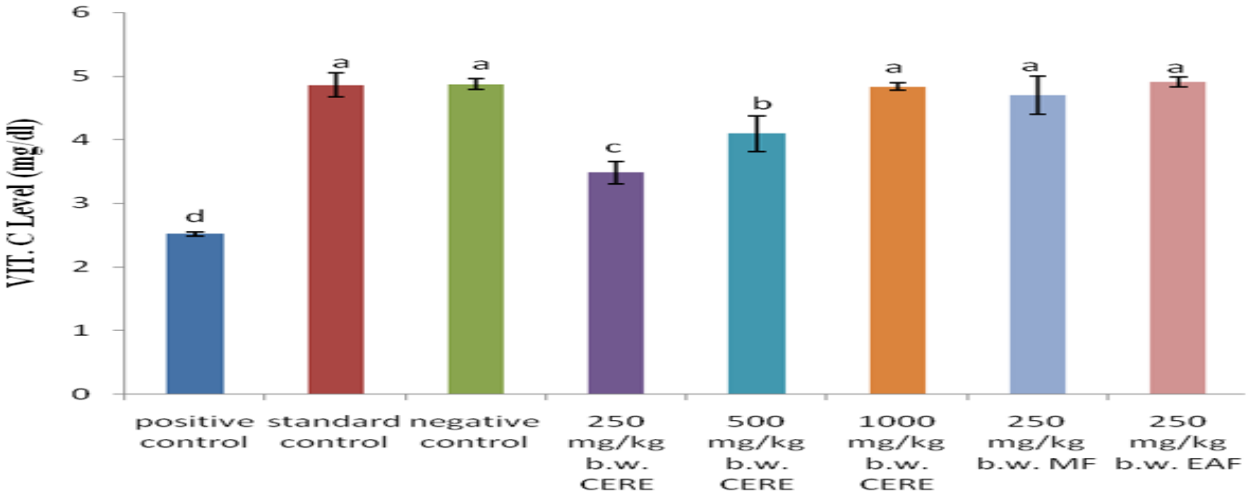


Figure 3: Vitamin C levels in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrumjollyanum*. Data are shown as mean ± standard deviation (n=4). meanvalues with different alphabet showed significant difference at p<0.05.

Key: CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

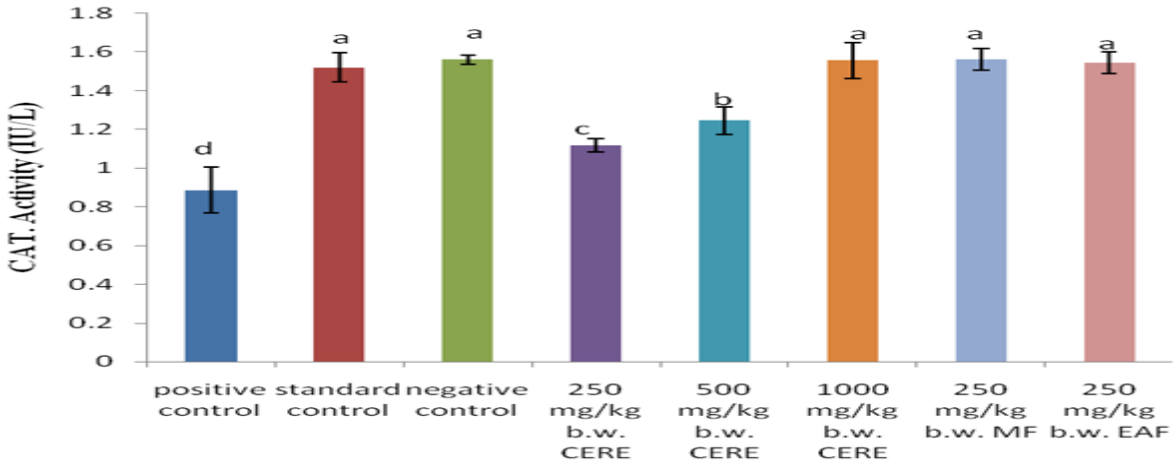


**Figure 4:** Catalase activity in STZ-induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrumjollyanum*. Data are shown as mean ± standard deviation (n=4). meanvalues with different alphabet showed significant difference at p<0.05.

**Key:** CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

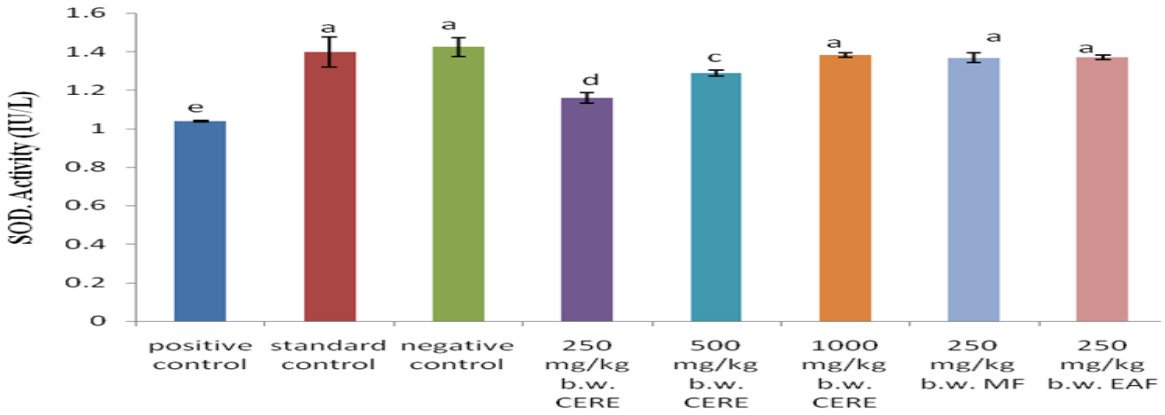


**Figure 5:** SOD activity in STZ-induced diabetic wistaralbino rats treated with crude ethanol root-extract and fractions of *Sphenocentrumjollyanum*. Data are shown as mean ± standard deviation (n=4). mean values with different alphabet showed significant difference at p<0.05.

**Key:** CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

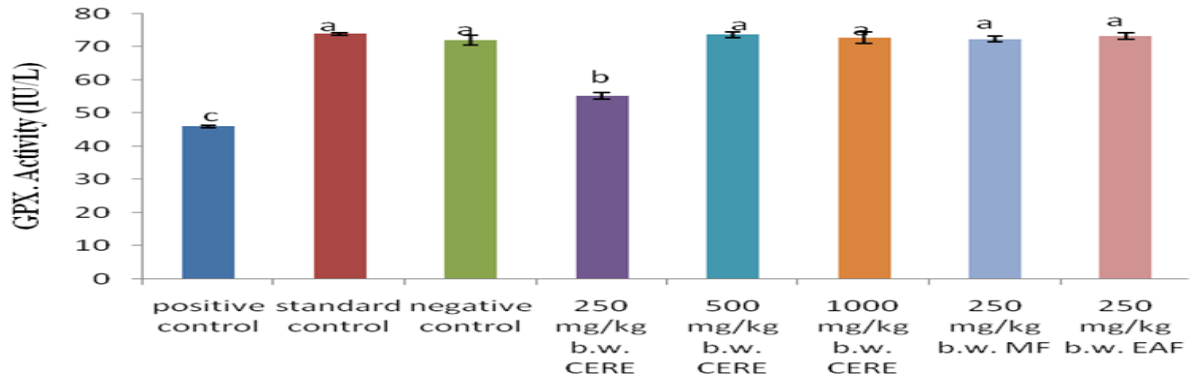


**Figure 6:** GPX activity in STZ induced diabetic wistar albino rats treated with crude ethanol root-extract and fractions of *Sphenocentrumjollyanum*. Data are shown as mean ± standard deviation (n=4). Mean Values with different alphabet showed significant difference at p<0.05.

**Key:** CERE=Crude ethanol root extract, MF= Methanol fraction and EAF Ethylacetate fraction

**Effect of crude ethanol root-extract and fractions of *Sphenocentrum jollyanum* on oxidative stress indices in STZ-induced diabetic albino rats**

The treatment of STZ-induced diabetic albino rats with crude ethanol root-extract of *Sphenocentrum jollyanum* at doses of 250, 500 and 1000 mg/kg body weights and 250 mg/kg body weight of ethylacetate and methanol root fractions of *Sphenocentrum jollyanum* significantly ($p<0.05$) increased the activities of CAT, SOD and GPX as shown in Figures 4, 5 and 6. The levels of serum vitamins C and E were significantly ($p<0.05$) higher in the treated groups as shown in Figures 2 and 3. The crude ethanol root-extract and fractions significantly ($p<0.05$) lowered the level of MDA in the treated groups as shown in Figure 1. The result also showed significant ($p<0.05$) differences in the effect of crude ethanol extract relative to fractions except at the dose of 1000 mg/kg body weight which showed no significant ($p>0.05$) change relative to the fractions. The result equally showed that the effects on the crude ethanol root extract were dose dependent and the value of the standard control is quite similar to that of the negative control as shown in Figures 1-6. The results of this study indicate that the MDA levels in all the STZ-induced diabetic rats treated with the crude ethanol root extract and fractions of *Sphenocentrumjollyanum*were reduced when compared to the MDA level in the diabetic untreated rats (positive control). Malondialdehyde (MDA) is a product of lipid peroxidation that can be easily measured and the results of this research show that the crude ethanol root extract and fractions of *Sphenocentrumjollyanum*reduced lipid peroxidation of the membranes of cells and tissues in the rats. This is an antioxidant effect, meaning that the crude ethanol root extract and fractions have antioxidant constituents. This aligns with the known fact that antioxidant constituents can delay or inhibit the oxidation of lipids and other compounds by inhibiting the propagation of oxidation chain reaction [9,12].

The results of this study also indicate that the vitamins C and E levels of the STZ-induced diabetic rats treated with the crude ethanol root extract and fractions of *Sphenocentrum jollyanum* increased significantly ($P<0.05$) when compared to the diabetic untreated rats(positive control).

Generally the superoxide dismutase, catalase and glutathione peroxidase activities of the STZ-induced diabetic rats treated with the crude extract root extract and fractionsof *Sphenocentrumjollyanum* were significantly ($P<0.05$) increased when compared to the activities of these enzymes in the diabetic untreated rats (positive control). This further confirms the antioxidant improving capacity of the crude ethanol root extract and fractions of *Sphenocentrum jollyanum*. This gives credence that *Sphenocentrum jollyanum* can be antidiabetic as their antioxidant constituents and antioxidant capacities could be associated with antidiabetic properties. The potential of the phytochemicals found in *Sphenocentrum jollyanum* have large scale pharmacological and biological implications, for example its antioxidant constituents (tannins, phenolic acid and flavonoids) have been proven to be effective for the care of health and protection from coronary heart diseases, cancer, anti-carcinogenic and anti-mutagenic effects [9,16] while its antioxidant capacity are protective

against various diseases, particularly cardiovascular diseases and diabetes.

## CONCLUSION

The results of this study imply that crude ethanol root extract and fractions of *Sphenocentrumjollyanum* are harmless sources for obtaining the natural antioxidants that may not only be anticarcinogenic but may also be protective against cardiovascular diseases and diabetes.

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