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In high-fat, streptozotocin-induced type 2 diabetic Wistar rats, the effect of a phenolic and alkaloid extract from Senna Occidentalis leaves on the heart's oxidative status was studied.

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Abstract: The purpose of this study was to investigate the impact of Senna occidentalis leaf extracts (alkaloid and phenolic) on the oxidative state of the heart in Streptozotocin-induced Wistar rats that were given a high-fat diet. The rats were inoculated intraperitoneally with 35 mg/kg of streptozotocin to induce type 2 diabetes. Research Tools and Procedures: Group 1 served as the control group and included seven rats out of a total of 43 rats that were randomly assigned to one of seven groups of six. The other groups were as follows: Diabetes rats that were not treated, rats that were treated with metformin, rats that were treated with phenolic extract, rats that were treated with alkaloids extract, rats that were treated with high doses of phenolic extract, and rats that were treated with high doses of alkaloids extract. Antioxidant status was determined by measuring the activity levels of many biochemical variables, including catalase, superoxide dismutase, glutathione-S-transferase, glutathione peroxidase, glutathione, and malondialdehyde. The results of this investigation suggested that S. occidentalis phenolic and alkaloid extracts might be useful in the treatment of type 2 diabetes due to their antioxidant characteristics.

Keywords: Senna occidentalis extract, streptozotocin, hypoglycemia, phenolics

INTRODUCTION

Traditional medicine has relied on plants for illness treatment since ancient times. One benefit of medicinal plants is the abundance of plant-based therapeutic chemicals that have been developed for use in modern medicine. [2] In low- and middle-income nations, where around 80% of the population resides, people often use plant resources for their main healthcare. There is a great deal of unrealized potential in medicinal plants, and they are already playing an important role in scientific progress. There are a number of phytochemicals in medicinal plants that have the potential to cure infectious and chronic illnesses in the near and distant future. When it comes to treating a wide range of ailments, people living in tropical and subtropical climates often turn to herbal plants like Senna occidentalis (S. occidentalis). According to traditional medicine, it has a number of medicinal applications [5]. 6, 7 The Hausa people of Nigeria call this plant Sanga-sanga or Rai dore; the Igbo people call it Akidi agbara; and the Yoruba people call it Abo rere. Tables 8–10. Antimicrobial activity has been shown by previous researchers in extracts of S. occidentalis, [11] beneficial for the liver and antioxidant prospects,[12] antimalarial characteristics,[13] anxiety- and depression-reducing abilities,[14] anodyne effects,[7] and anti-diabetic capabilities. in references [15,16] When the pace of reactive oxygen species (ROS) formation exceeds the body's ability to neutralize them, oxidative stress ensues. Enhanced reactive oxygen species (ROS) production and/or diminished ROS scavenging capacities lead to elevated oxidative stress, which in turn damages tissues. [17] Both hereditary and environmental factors contribute to the complexity of type 2 diabetes mellitus (T2DM). New evidence reveals that genetic information greatly assists in diabetes risk prediction and therapy personalization.

Although there are over 70 genes that have been shown to be associated with type 2 diabetes, they only account for around 10% of the total heredity of the disease. Twin studies have shown a strong genetic impact on the development of type 2 diabetes, suggesting a stronger relationship to family history and lineage than type 1. A person's ethnic background may also have a role. It is impossible to completely rule out environmental factors, however. Even while there may be a hereditary predisposition to obesity and common patterns of eating and exercising, a person's lifestyle choices greatly affect the likelihood that they will acquire type 2 diabetes. A lack of proper insulin responses is a hallmark of the pathophysiological changes caused by diabetes, which include dysfunctional peripheral βcells and reactive oxygen species (ROS) caused by inflammation or cells interacting with the insulin receptor or downstream signaling pathways. Microvascular and macrovascular complications, as well as impaired blood glucose management, are the cumulative effects of chronic inflammation and insulin resistance, to which all of these causes contribute. Antioxidants may lessen the effects of oxidative stress. Enzymes that neutralize free radicals, such as catalase, glutathione peroxidase, glutathione transferase, and reduced glutathione (GSH) are examples of endogenic antioxidants; vitamins A, C, and E are examples of exogenic antioxidants. By either scavenging free radicals or increasing their catabolism, both classes of antioxidants prevent free radical formation. [18] in Additionally, antioxidants have the ability to deoxygenate oxidized compounds. The year 19 Natural chemicals derived from medicinal plants have developed into successful treatments to fight oxidative stress, since phytochemicals are a major source of antioxidants. In addition to improving insulin secretion and glycogen storage in the liver, these antioxidants reduce oxidative stress. In [20],

Senna occidentalis botanical classification

- Kingdom: *Plantae*
- Phylum: Magnoliophyta
- Class: *Magnoliopsida*
- Subclass: *Rosidae*
- Order: *Fabales*
- Family: *Fabaceae*
- Subfamily: *Caesalpinioideae*
- Tribe: *Cassieae*
- Genus: *Senna*
- Species: *occidentalis*
- Botanical name: *S. occidentalis*.

Materials and Methods

Study area

The samples were obtained in Ilorin, the capital of Kwara State, located in the north-central zone of Nigeria.

Sample collection and authentication

The leaves of *S. occidentalis*, known as "Abo rere" in Yoruba, were collected from a farmland in Ilorin, Kwara State, Nigeria. The plant was identified and authenticated at the Herbarium Unit, Department of Plant Biology, University of Ilorin, Kwara state, Nigeria, and a voucher number (UILH001/1302/2021) was given and deposited for future reference. The leaves were then dried in the open air, away from direct sunlight, for 7 days. Afterward, they were crushed into powder form, resulting in a total of 462 g of pulverized leaves.

Experimental animals

Forty-three Wistar rats weighing between 100 and 120 g were acquired. The rats underwent a 7-day acclimatization period at the room temperature, during which they were provided with rodent feed and had *ad libitum* access to clean distilled water. The Wistar rats were housed at the animal house of the Biochemistry Department, Kwara State University. All animal procedures were carried out in compliance with the guidelines for scientific animal procedures approved by the ethics committee of the Kwara State University.

Reagents and kits

Reagents

The reagents used included acetic acid, ethyl acetate, chloroform, streptozotocin, bromocresol green, atropine, gallic acid, Folin reagent, and ethanol.

Kits

The kits used for the analysis of the experimental animal included the total cholesterol reagent, high-density lipoprotein cholesterol reagent, and triglyceride cholesterol reagent, all manufactured by RANDOX.

Preparation of extracts

Alkaloids extraction

The pulverized sample was mixed with 900 mL of ethanol and 100 ml of acetic acid and allowed to soak for 24 h. The mixture was subsequently filtered using filter paper. A rotary evaporator was used to concentrate the filtrate to obtain the supernatant. The pH of the supernatant was adjusted to approximately 8 by adding NH_4OH solution. The supernatant was then subjected to extraction three times using 50 mL of chloroform each time. A separating funnel was used to separate the chloroform layer from the rest of the solution to obtain the alkaloid extract.

Extraction of phenolic

The powdered sample was mixed with a solvent mixture consisting of 150 mL of ethyl acetate, 30 mL of acetic acid, and 300 mL of water. The mixture was vigorously stirred for 30 min until both phases were fully saturated. The mixture was then transferred to a separating funnel to allow the phases to separate. The phenolic extract was obtained from the organic phase.

Total phenolic content

To determine this, the following steps were undertaken: 7 g of $NaCO_3$ was dissolved in 100 mL of water, and 10 mg of gallic acid was dissolved in 10 mL of methanol. Four concentrations (25, 50, 75, and 100 μg/mL) were prepared. These concentrations involved mixing 5 ml of Folin reagent with 50 mL of water, 0.5 mL of the sample, and 0.5 mL of the gallic acid. Additionally, 12 test tubes were prepared

for gallic acid, and 3 test tubes were prepared for each extract. Water was added accordingly. For the gallic acid concentrations, the following steps were repeated three times: adding 1 mg/mL of the extract to each test tube, adding 2 mL of Folin reagent to each test tube, and adding 4 mL of $NaCO_3$ to each test tube. Finally, the absorbance was measured at 765 nm.

Total alkaloids content

Using atropine as the reference, the total amount of alkaloids was determined using a spectrophotometric technique that depends on the interaction between alkaloids and bromocresol green. The process used is as follows: the plant extract was dissolved in hydrochloric acid solution (2N HCl) at a concentration of 1 mg/mL. A 0.1N sodium hydroxide solution was used to bring a phosphate buffer solution's pH to a neutral range. A 5 mL mixture was then vigorously stirred after 1 ml of this solution was transferred to a separate funnel. Chloroform was used to extract the resultant complex. To achieve the appropriate volume, the extract was diluted with chloroform after being collected in a 10 mL volumetric flask. The complex's chloroform absorbance then measured at 470 nm.

Table 1: Stratification of experimental rats with the corresponding dosage and type of extract administered

Group	Extract administration
Control	Normoglycemic rats orally administered with distilled water
Untreated diabetic rat	Streptozotocin-induced diabetic rats orally administered with distilled water
Diabetic rat	14.3 mg/kg (body weight) of metformin
Diabetic rat	100 mg/kg (body weight) of phenolic extract
Diabetic rat	100 mg/kg (body weight) of phenolic extract
Diabetic rat	100 mg/kg (body weight) of phenolic extract
Diabetic rat	200 mg/kg (body weight) of alkaloid extract

Experimental animal stratification and extract administration

Forty-three Wistar rats were stratified into seven groups: Six groups with six rats each and one control group with seven rats as illustrated in Table 1.

Table 1 illustrates the classification of diabetic rats, the amount and type of extract administered respectively. The protocol utilized in this study complies with the standards of the National Research for the Care and Use of Laboratory Animals and Principles of Good Laboratory Procedure.

Streptozotocin diabetes induction

The induction procedure for Type 2 diabetes was carried out as previously described.[21] Diabetes was induced in the rats using the intraperitoneal method. A solution of 35 mg/kg streptozotocin was prepared in 1.0 M citrate buffer at pH 4.5. Following this, the rats underwent a 12-h fasting period. Fasting blood sugar levels were checked randomly and then checked again after an additional 72 h. It was confirmed that the group subjected to induction displayed diabetic conditions, with glucose levels exceeding 200 mg/dL.[22]

Extraction of serum and tissue homogenates

Jugular puncture was used for this purpose. The rats were anesthetized with diethyl ether, and their fur and skin were shaved to expose the jugular vein. The jugular vein was punctured using a new scalpel to collect blood samples into both ethylenediaminetetraacetic acid (EDTA) and plain bottles. Following blood collection, the animals were immediately dissected, and specific organs such as the liver, kidney, heart, and pancreas were removed. These organs were then placed in different buffers, including sucrose, Tris, and formalin. In addition, the blood samples collected in EDTA bottles were centrifuged at 1500 rpm for 5 min. The resulting serum was carefully extracted from the centrifuged blood using a Pasteur pipette and transferred to clean, dry sample bottles. The serum was stored in the refrigerator overnight before being used for further assays.

Furthermore, 1 g of each organ was weighed and separately homogenized using sucrose and Tris buffers. The homogenates prepared with tris buffer were subsequently centrifuged. The centrifuged homogenates were then transferred to sample bottles and frozen for subsequent laboratory analysis.

Determination of parameters

The following parameters were determined: SOD, CAT, GSH, GPx, malondialdehyde (MDA), and GST.

Statistical analysis

All the data were expressed as mean ± standard error of the mean for rats in each group. All group data were statistically assessed using the Statistical Package for the Social Sciences (SPSS) 16.0 software (IBM, Armonk, New York, United States). Data acquired were analyzed using the one-way analysis of variance, and subsequent contrasts among groups were done using Duncan Multiple Range Test. The mean difference was statistically significant

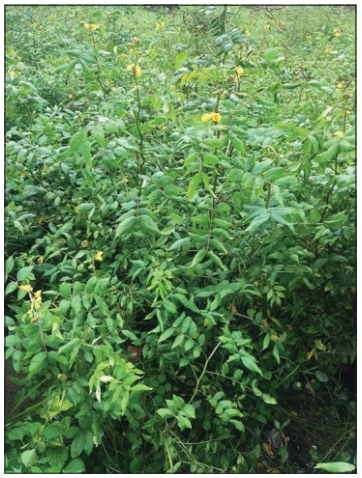

Figure 1: *Senna occidentalis* leaf on harvested farmland in Ilorin, Kwara State

when $P < 0.05$. Results are represented graphically using Graphpad 8.0.

Results

Figure 1 describes the physical appearance of *Senna occidentalis* leaf planted on a farmland in Ilorin, Kwara State, Nigeria.

The effect of *S. occidentalis* on GST activity in the heart of Wistar rats is depicted in Figure 2. Metformin-treated rats showed increased levels of GST activity compared to the control group, while all other treated groups had lower levels of GST activity than the control group ($P < 0.05$).

Figure 3 demonstrates that the levels of glutathione activity were lowered in all treatment groups except for the metformin-treated group, which shared the same level of activity as the control group ($P < 0.05$).

Figure 4 depicts the GPx activity levels in all groups. Compared to the other groups, the metformin-treated group has higher levels of GPx activity. The rats treated with the extracts had significantly decreased GPx activity compared to the control group. When comparing the groups, the diabetes untreated group exhibits a substantial decrease in activity levels when compared to the other groups ($P < 0.05$).

Figure 5 depicts the levels of MDA activity in each treatment groups. Reduced levels were found in all groups except for the untreated diabetic group, which showed a statistically significant rise compared to the other groups ($P < 0.05$).

All treated groups had lower levels of cardiac SOD activity compared to the control group, as shown in Figure 6. However, the untreated diabetic group exhibited the lowest level ($P < 0.05$).

Figure 7 displays the CAT activity levels in each group. Each group had an increased level of CAT activity except for the untreated diabetic group when compared to the control group ($P < 0.05$).

Discussion

T2DM is characterized by hyperglycemia, which significantly increases the production of ROS, oxidative stress, and numerous cellular and molecular modifications, such as mitochondrial dysfunction, that affect the body's normal physiological processes.[23]

ROS is produced by normal cellular metabolism and carry out crucial biological tasks. Although ROS is essential for life, they can harm macromolecules such as lipids, proteins, and nucleic acids due to their strong chemical reactivity. Therefore, cellular defense systems are activated to control ROS generation and prevent oxidative damage. Most ROS defense systems consist of scavenging enzymes, including CAT, GPxs, and SODs, etc., Antioxidant enzymes are the

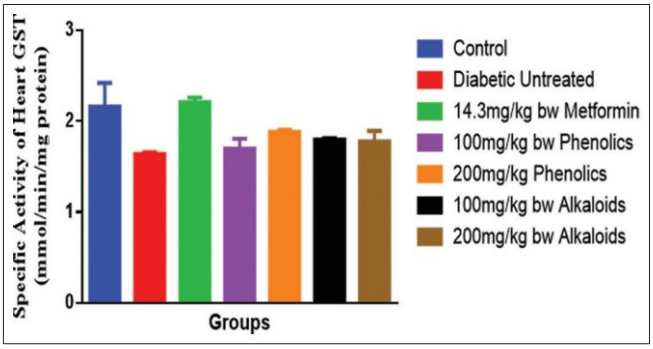

Figure 2: Effect of *Senna occidentalis* extract on glutathione transferase activity in the heart of Wistar rats. GST: Glutathione transferase

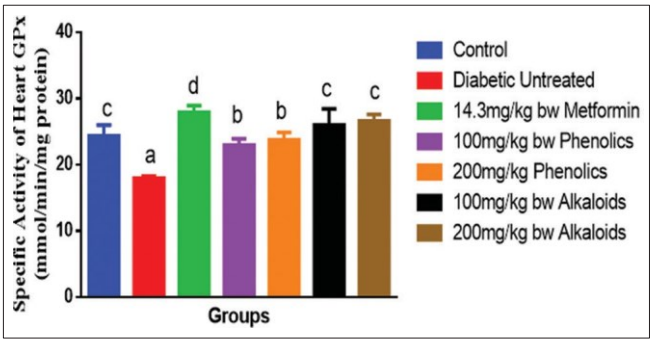

Figure 4: Effect of *Senna occidentalis* on glutathione peroxidase activity in the heart of Wistar rats. GPx: Glutathione peroxidase

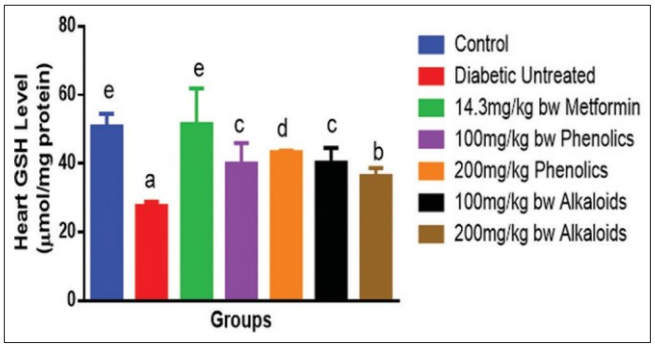

Figure 3: Effect of *Senna occidentalis* extract on glutathione activity in the heart of Wistar rats. GSH: Reduced glutathione

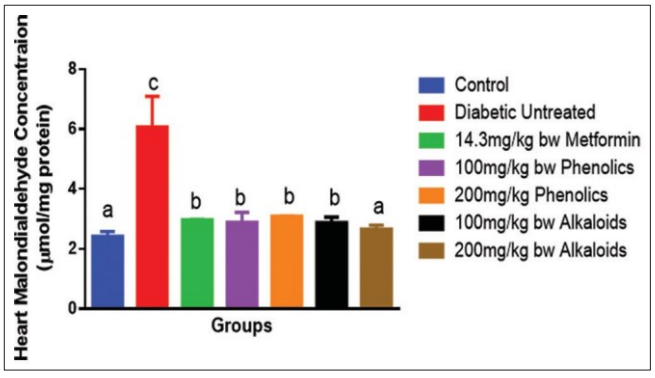

Figure 5: Effect of *Senna occidentalis* extract on malondialdehyde activity in the heart of Wistar rats

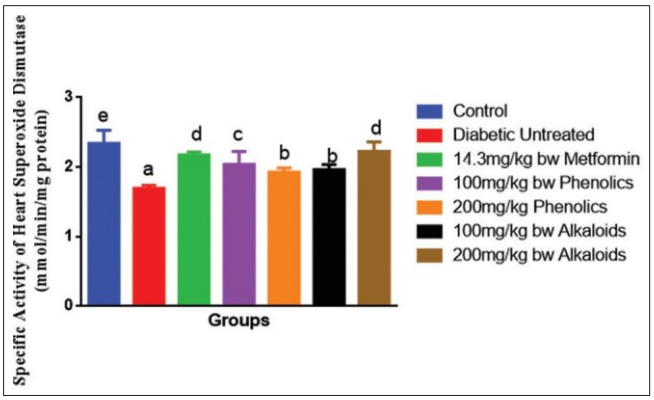

Figure 6: Effect of *Senna occidentalis* extract on superoxide dismutase activity in the heart of Wistar rats. SOD: Superoxide dismutase

good biological indicators of cellular perturbation; they are also known as biomarkers.

Free radicals are neutralized by enzyme antioxidants. The antioxidant enzymes convert harmful oxidative products to hydrogen peroxide and subsequently to water via a multi-step process when they are in the presence of cofactors like copper, zinc, manganese, and iron. Free radical chain reactions are disrupted by nonenzymatic antioxidants. [24] The controlled and treated groups showed significantly higher levels of CAT, SOD, GST, GPx, and GSH activity than the diabetic untreated group, according to the results of this study. The fact that these biomarker levels rose in the treated groups suggests that the antioxidant characteristics of S. occidentalis leaf extract aid in the elimination of oxidative stress and the enhancement of appropriate glucose homeostasis.

Metabolic damage assessment (MDA) is an important sign of oxidative stress among the six cardiac function indicators investigated in this research. Stable and very poisonous, MDA is the byproduct of lipid peroxidation. The study found that the untreated diabetes group had a much higher MDA levels. Increased malondialdehyde (MDA) levels in untreated type 2 diabetic rats suggest increased free radical activity and lipid peroxidation. The development of type 2 diabetes and its consequences are linked to oxidative stress, which is in turn caused by lipid peroxidation, according to substantial evidence. S. occidentalis leaf extract lowered MDA levels in rats when administered topically. Evidence suggests that enzymatic and nonenzymatic antioxidant defense systems are weakening as lipid peroxidation levels rise.

According to previous research, diabetes mellitus is a well-known risk factor for cardiovascular disease. Compared to nondiabetic people, those with T2DM have increased cardiovascular morbidity and mortality.[26] Heart antioxidant enzymes such as CAT, SOD, and GPx represent the primary defense opposing oxidative damage by eliminating main ROS.[27] Based on the evidence gathered in this study, it is evident that the administration of *S. occidentalis* leaf extract serves as an antioxidant therapy that can help prevent the

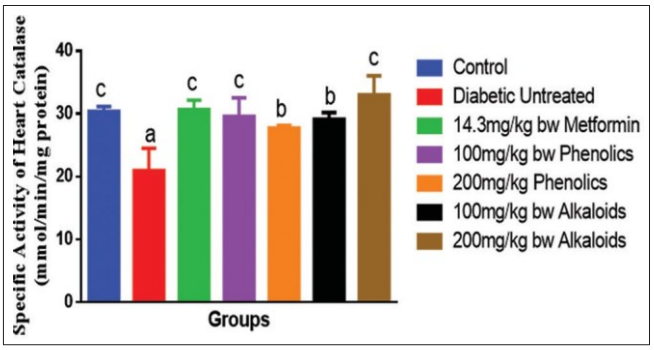

Figure 7: Effect of *Senna occidentalis* extract on catalase activity in the heart of Wistar rats

development of diabetic heart complications by increasing the activity levels of CAT, SOD, and GPx.

The outcome of this research also confirmed the antioxidant potential of metformin. This agrees with previous reports on the efficacy of metformin, which possesses antioxidant properties that reduce ROS by inhibiting mitochondrial oxidative phosphorylation.[28] Evidence from this research shows that metformin heightened the actions of CAT, GSH, SOD, GST, and GPx and decreased the activity of MDA in type 2 diabetic rats.

Conclusions

The therapeutic importance of medicinal plants in illness prevention, treatment, and management has led to their significant attention in recent years. Our research shows that S. occidentalis leaf extract enhances antioxidant enzymes and GPx activity while decreasing malondialdehyde (MDA) activity in the hearts of rats with type 2 diabetes. The findings show that the leaf extract of S. occidentalis has antioxidant properties and aids in the treatment of type 2 diabetes when taken at the recommended dosages. The findings of this study support the use of S. occidentalis leaf extract as a treatment for oxidative stress in the prevention of type 2 diabetes complications. It is also suggested that scientists sequence S. occidentalis leaves so they may learn more about the plant's genetic makeup, whether that's by finding individual genes or variants. Several fields may benefit from this data, such as plant breeding, evolutionary biology, and the study of plant physiology and development.

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