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Amelioration of Some Indices of Diabetic Complications Using *Senna occidentalis* Leaf Extract in Alloxan-Induced Diabetes in Rats

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Abstract
This study was designed to investigate the ameliorative effects of some indices of diabetic complications using *Senna occidentalis* leaf extract in alloxan-induced diabetes in rats. Thirty male Wistar rats with body weight ranging from 150–200 g were selected for the study. Diabetes was induced by single intraperitoneal dose of alloxan injection (150 mg/ kg body weight). Rats were treated orally using aqueous and ethanol extracts of *Senna occidentalis* leaves at 100 mg/kg body weight once daily for 21-days. The fasting blood sugar (FBS), Thiobarbituric acid Reactive Substance (TBARS), alkaline Phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analysed spectrophotometrically. The result of the study showed that treatment of diabetic rats with the extract caused significant ($p<0.05$) decrease in FBS, TBARS, ALP, ALT and AST. The result of study showed to a large extent the effects of *Senna occidentalis* in the amelioration of some complications resulting from diabetes.

1. Introduction

Diabetes Mellitus is a complex metabolic disease with myriads of complications. The causes of diabetes are many and its pathophysiology is multiple and would require more than a single therapeutic agent to reverse majority of its effect. Treatment in diabetes is aimed at achieving good control of blood glucose levels and preventing the development of secondary complications. Many pharmacological therapies have been used to improve the status of diabetes by several mechanisms such as, inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β-cell regeneration and enhancing the insulin releasing activity [1], [2]. At present, drug therapies either alone or in combination cannot restore blood glucose homeostasis and many limitations exist in their use. The management of diabetes without any side effect is still a challenge to the medical system. Many efforts have been made to identify new hypoglycaemic agents obtained from different sources especially medicinal plants because of their effectiveness, fewer side effects and relatively low cost. Several medicinal plants have been investigated for their beneficial use in different types of diabetes in the traditional medicine even though their biologically active compounds and efficacy are unknown [3], [4].

Senna occidentalis is a flowering plant of the Fabaceae family. It is native to the tropical and subtropical regions of America. The common names are coffee weed,

Stinking-weed or mogdad coffee in English. In Hindi it is called Badikanodi, Chakunda or Kasonda, In Hausa, it is known as Tapasa. The leaves and pods (shells) are usually administered in the Ayurvedic and Unani systems of medicine as infusion, and considered a great tonic. The plant is used to cure sore eyes, haematuria, rheumatism, typhoid, asthma and disorders of haemoglobin, it is also reported to cure leprosy [5]. A decoction of the plant is used in hysteria, in dysentery and other stomach troubles, and also as an application to sores, itch and inflammation of the rectum [6]. The plant is employed in dropsy, and as a vermifuge. Along with other plants as, it is made into an ointment used for skin diseases.

2. Materials and Methods

2.1. Collection and Preparation of Plant Materials

The leaves of the plant were collected from its natural habitat in Okuku, Yala L. G. A, Cross River State, Nigeria. It was authenticated in the Department of Biological Sciences, Cross River University of Technology Calabar. The leaves were dried under shade at room temperature and pulverized using mortar and pestle.

2.2. Extraction

Exactly 400g of finely ground powder of the plant sample was weighed into a container and filled with 2000ml of ethanol (1:5 w/v) and was allowed to stand for 24hrs at room temperature, was filtered using Wattman no. 1 filter paper, then the filtrate was concentrated under reducing pressure using rotary evaporator with a vacuum pump. The concentrated extract was weighed. Same procedure was repeated for aqueous extraction substituting ethanol with distilled water.

2.3. Animal Management

Male albino rats were purchased from the animal house of the Department of Medical Biochemistry, Cross River University of Technology, Calabar Nigeria. They were acclimatized for two weeks prior to the commencement of the experiment, kept at a room temperature, and feed using broiler starter. They were weighed prior to the commencement of the experiment.

2.4. Induction of Diabetes

Diabetes was induced by intraperitoneal injection of alloxan with a single dose of 150mg/kg body weight. After 72 hours, animals with FBS 240mg/dl and above were selected for the study. The blood glucose concentrations of the animals were determined weekly using Accu-Check Active glucometer

2.5. Animal Grouping

Thirty (30) male albino rats were divided into five groups consisting of six animals each. Out of the five groups four groups were made diabetic as described below.

Group 1: Normal Control

Group 2: Diabetic control

Group 3: Diabetic + *Senna occidentalis* aqueous extract 100mg/kg

Group 4: Diabetic + *Senna occidentalis* ethanolic extract 100mg/kg

Group 5: Diabetic + Glibenclamide

Treatment was made orally for 21 days.

2.6. Sample Processing

At the end of administration, the animals were fasted overnight, they were weighed using a standard weighing balance, anaesthetized under chloroform anaesthesia and quickly brought for sacrifice. Blood samples were collected through cardiac puncture. Blood sample of about 3-4mls was collected via cardiac puncture and separated into sample tube for analysis. The samples were centrifuged at 3500rpm for 10minutes in order to obtain serum from whole blood.

2.7. Fasting Blood Glucose (FBG) Determination

FBG was determined as described by Ibrahim and Rizk, [7]. One drop of rat blood through the tail was collected on a glucose assay strip and read using Accu-Check Active glucometer. This was carried out on a weekly basis for 21 days.

2.8. Determination of Thiobarbituric Acid Reactive Substance (TBARS)

This was carried out according to the method described by Frode and Medeiros [8]. 10 g (10%) of TCA and 0.67 g (0.67%) TBA was weighed and dissolved in to 100ml of distilled water for use. About 100µl of tissue homogenate was added into a test tube containing 2ml of TCA. 2ml of TBA was added and mixed thoroughly. It was incubated at 80^{o}C for 30 minutes in water bath and cooled immediately under ice. The sample mixture was then centrifuged at 3,500rpm for 10 minutes and the absorbance was read immediately at 535mm using a UV/VIS spectrophotometer.

2.9. Assay for Liver Enzymes

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Serum alkaline phosphatase were determined using assay kits (Agape Laboratories Ltd, UK).

2.10. Statistical Analysis

The results were analyzed by one-way ANOVA, using SPSS statistical package version 21. All data were expressed as Mean ± SD and difference between groups considered significant at $p<0.05$.

3. Results and Discussion

3.1. Fasting Blood Sugar (FBS)

Alloxanization of normal rats led to significant increase ($p<0.05$) in FBS as shown in group 2-5 (table 1) compared to normal rats in group 1. There was significant decrease ($p<0.05$) in FBS level across the weeks (wk 1 > wk 2 > wk 3) in group 3-5 treated with aqueous and ethanol extracts as well as glibenclamide. No statistical significance ($p<0.05$) was observed between the effects exhibited by the extract and glibenclamide at week 3.

Table 1. *FBS levels in diabetic and normal rats treated with Senna occidentalis extracts and glibenclamide.*

FASTING BLOOD SUGAR (FBS)mg/dl				
GROUP	**DAY 0**	**WK 1**	**WK 2**	**WK 3**
NORMAL CONTROL	84.08±05.81^{a}	83.96±05.80^{a}	75.04±03.53^{a}	83.16±05.58^{a}
DIABETIC CONTROL	217.84±06.15^{b}	222.64±06.47^{c}	203.68±09.74^{c}	213.36±03.09^{c}
DIABETIC +100g S. O AQ EXT	222.48±02.47^{bc}	172.48±06.49^{b}	122.32±08.21^{b}	118.24±01.05^{b}
DIABETIC +100g S. O ETH EXT.	244.48±08.32^{c}	170.24±09.93^{b}	102.16±05.54^{bc}	108.48±04.70^{b}
DIABETIC+GLIBENCLAMIDE	203.84±05.90^{b}	186.24±02.69^{b}	135.44±07.23^{c}	119.36±05.22^{b}

Values are mean ± SD. All treated groups are compared with control.
Values with different superscript along the groups are statistically significant at $p<0.05$.

3.2. Liver and Kidneys Thiobarbituric Acid Reactive Substances (TBARS) Levels

Significant increase ($p<0.05$) was observed in the TBARS levels of the diabetic control group compared to normal control (table 2). Treatment of diabetic animals with the extracts and glibenclamide caused significant decrease ($p<0.05$) in TBARS concentration in both the liver and kidneys of diabetic control group.

Table 2. *Liver and kidneys TBARS levels in diabetic and normal rats treated with Senna occidentalis extracts and glibenclamide.*

THIOBARBITURIC ACID REACTIVE SUBSTANCES (nmol/ml)		
GROUP	**TBARS L**	**TBARS K**
NORMAL CONTROL	0.22±0.06^{a}	0.12±0.03^{a}
DIABETIC CONTROL	0.34±0.07^{b}	0.27±0.08^{b}
DIABETIC +100g S. O AQ EXT	0.23±0.02^{a}	0.12±0.02^{a}
DIABETIC +100g S. O ETH EXT.	0.24±0.04^{a}	0.13±0.02^{a}
DIABETIC+ GLIBENCLAMIDE	0.26±0.03^{a}	0.12±0.03^{a}

Values are mean ± SD. All treated groups are compared with control.
Values with different superscript along the groups are statistically Significant at $p<0.05$.
TBARS L = Liver TBARS; TBARS K = Kidney TBARS

3.3. Serum Liver Enzyme Activities

There was significant increase ($p<0.05$) in liver enzyme (ALT, AST, ALP) activities owing to diabetic condition caused by alloxanization (Table 3) compared to normal control group. Treatment of diabetic rats with aqueous and ethanol extracts caused significant decrease ($p<0.05$) in the enzyme activities compared to the diabetic control group. Glibenclamide treatment of diabetic rats also caused significant decrease ($p<0.05$) in the enzyme activities compared with the diabetic control. However, the extracts elicited more effects than the glibenclamide treatment, though not statistically significant ($p<0.05$).

Table 3. *Serum liver enzyme activities in diabetic and normal rats treated with Senna occidentalis leaf extracts and glibenclamide.*

LIVER ENZYMES ACTIVITY (IU/L)			
GROUP	**ALP**	**ALT**	**AST**
NORMAL CONTROL	32.27±3.25^{a}	20.34±0.48^{a}	30.89±1.24^{a}
DIABETIC CONTROL	54.12±4.99^{c}	61.10±1.55^{d}	50.41±0.57^{c}
DIABETIC +100g S. O AQ EXT	34.78±5.86^{b}	31.06±1.35^{b}	31.75±2.53^{b}
DIABETIC +100g S. O ETH EXT.	35.57±7.83^{b}	30.54±0.80^{b}	34.11±0.16^{b}
DIABETIC+ GLIBENCLAMIDE	36.05±7.36^{b}	37.01±0.86^{c}	40.41±0.63^{bc}

Values are mean ± SD. All treated groups are compared with control.
Values with different superscript along the groups are statistically Significant at $p<0.05$.

3.4. Serum Bilirubin Concentration

Treatment of diabetic rats with the extracts caused significant decrease ($p<0.05$) in serum bilirubin concentration compared with the diabetic control (Table 4). The decrease in serum total bilirubin concentration caused by glibenclamide was not statistically significant ($p<0.05$) compared with the diabetic control.

Table 4. *Serum bilirubin concentration in diabetic and normal rats treated with Senna occidentalis leaf extracts and glibenclamide.*

	SERUM BILIRUBIN (mg/dl)	
GROUP	**TOTAL BIL.**	**DIRECT BIL.**
NORMAL CONTROL	0.52 ± 0.03^{a}	0.21 ± 0.01^{a}
DIABETIC CONTROL	1.10 ± 0.03^{b}	0.71 ± 0.02^{b}
DIABETIC +100g S. O AQ EXT	0.60 ± 0.12^{a}	0.24 ± 0.05^{a}
DIABETIC +100g S. O ETH EXT.	0.51 ± 0.13^{a}	0.21 ± 0.01^{a}
DIABETIC+ GLIBENCLAMIDE	0.74 ± 0.02^{ab}	0.30 ± 0.08^{a}

Values are mean ± SD. All treated groups are compared with control.
Values with different superscript along the groups are statistically Significant at $p<0.05$.
BIL = Bilrubin

4. Discussion

Diabetes is currently considered as a vascular disease [9]. It has also been considered by researchers that hyperglycaemia-induced oxidative stress is a critical pathogenic mechanism that initiates a plethora of cascade metabolic and vascular perturbations [10]. Studies have revealed the beneficial effects of some secondary plant metabolites that possess antioxidant activity in diabetes management. Lipid peroxidation was investigated in our study by assessing the hepatic levels of TBARS; a significant increase in TBARS levels of diabetic rats was observed when compared to normal control rats. Numerous studies with human and animal models have also shown increased lipid peroxidative status in membranes of different tissues in diabetes [11, 12, 13]. The extract produced significant decreases in TBARS levels in treated diabetic rats when compared to diabetic control rats. Treatment with glibenclamide also caused a slight decrease in TBARS levels of the treated rats. These reductions could lead to a decrease in oxidative stress and hence a reductions in the rate of progression of diabetic complications in the liver.

Table 3 represents the changes in the activities of aspartate transaminase, alanine transaminase and alkaline phosphatase. In the assessment of liver damage by the determination of enzyme, enzyme levels such as aspartate transaminase and alanine transaminase are largely used. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [14]. Hepatocellular necrosis leads to high level of serum markers in the blood, among these, aspartate transminase, alanine transaminase represents 90% of total enzyme and high level of alanine transminase in the blood is a better index of liver injury, but the elevated levels of enzymes are decreased to normal levels after treatment with the extract. Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure [15]. Increased level was obtained owing to alloxan administration and it was brought to normal level by the extract treatment. Treatment with *S. occidentalis* aqueous and methanol leaf extract decreased the serum levels of aspartate transaminase, alanine transaminase and alkaline phosphatse towards the respective normal value; that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by alloxan. The aforementioned changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchymal cells.

Hyperbilirubinaemia was observed due to excessive haeme destruction and blockage of biliary tract. As a result of blockage of the biliary tract, there was mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes; this is in line with the report given by [16]. Administration of extract decreased the level of bilirubin, suggesting that it offered protection.

The antihyperglycemic effect of the extracts was compared with glibenclamide, a standard hypoglycemic drug. Glibenclamide has long been used to treat diabetes, to stimulate insulin secretion from the pancreatic β-cells. From the results, it appears that still insulin producing β-cells are functioning in alloxan treated diabetic rats and stimulation of insulin release could be responsible for most of the observed metabolic activities. Further, the observed blood glucose-lowering effect in fasted normal and alloxan induced diabetic rats could possibly be due to the increased peripheral glucose utilization. A number of other plants have also been shown to exert hypoglycemic activity through stimulation of insulin release [17]. Our observations are in well agreement with the reports by several workers that alloxan-induced diabetes mellitus and insulin deficiency leads to increased blood glucose. Prolonged administration of extract may stimulate the β-cells of islets of Langerhans to produce insulin.

5. Conclusion

The study showed that the ethanol extract of *S. occidentalis* was potent in managing diabetes and its complications. The possible mechanism by which *Senna occidentalis* leaf extracts bring about its hypoglycaemic action may be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from cells of islets of Langerhans or its release from the bound form.

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