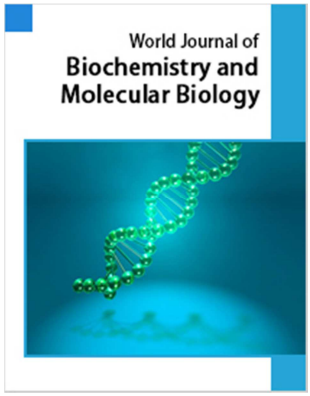
World Journal of Biochemistry and Molecular Biology
2017; 2(4): 16-19
http://www.aascit.org/journal/wjbmb

American Association for Science and Technology



Keywords
Senna occidentalis,
Biochemical Complications,
Diabetes

Received: May 19, 2017
Accepted: August 1, 2017
Published: September 14, 2017

Changes in Haematological Parameters Arising from Alloxan-Induced Diabetes in Rats Treated with Aqueous and Ethanol Extracts *Senna occidentalis* Leaves

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Citation

Yakubu Ojochenemi Ejeh, Nwodo Okwesili Fred Chiletugo. Changes in Haematological Parameters Arising from Alloxan-Induced Diabetes in Rats Treated with Aqueous and Ethanol Extracts *Senna occidentalis* Leaves. *World Journal of Biochemistry and Molecular Biology.* Vol. 2, No. 4, 2017, pp. 16-19.

Abstract

The present study was designed to evaluate the effects of *Senna occidentalis* in alloxan-induced diabetic and its complications in Wistar rats. Thirty male Wistar rats with body weight ranging from 180–250 g were selected for the study. Diabetes was induced by single intraperitoneal dose of alloxan injection (150 mg/ kg body weight). Treatment was carried out orally using aqueous and ethanol extracts of *Senna occidentalis* leaves at 200 mg/kg body weight once daily for 21-days. Serum bilirubin, platelets (PLT) and white blood count (WBC), lymphocytes (LYM) and red blood count (RBC) were evaluated. Diabetic condition caused significant increase ($p<0.05$) in WBC and PLT. Treatment of diabetic rats with aqueous extract and glibenclamide had no significant difference ($p<0.05$) from the diabetic control group, except the group treated with ethanol extract for WBC, but PLT was significantly lower ($p<0.05$) in aqueous extract and glibenclamide. Unlike WBC, LYM and RBC levels were significantly lower ($p<0.05$) in diabetic control. Hence treatment with extracts as well as glibenclamide caused significant increase in diabetic control in LYM and RBC levels. Serum bilirubin, PCV and Hb levels were significantly lower ($p<0.05$) in diabetic control compared to the normal control. Hence treatment with extracts caused significant increase bilirubin, PCV and Hb levels. The result of study showed to a large extent the effects of *Senna occidentalis* in the management of some complications resulting from diabetes and its complications.

1. Introduction

The use of herbal medicines in human and animal health care systems is well documented in ancient literature. In many parts of the World, ethno therapies are no longer seen as myth, superstition, witchcraft or ungodly practices and indeed it is gaining popularity, with the belief that “natural is better” [1]. 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 [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 [4, 5].

S. occidentalis 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

Fresh leaves of *Senna occidentalis* 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 followed by an electric blender.

2.2. Extraction

About 500g of finely ground powder of *Senna occidentalis* was weighed into a plastic rubber and filled with 2500ml 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 experiment.

2.4. Induction of Diabetes

Diabetes was induced by subjecting the animals to over-night fasting, followed by intraperitoneal injection of alloxan with a single dose of 150mg/kg body weight. Alloxan is capable of inducing hyperglycermia as a result of massive pancreative insulin release, after 72 hrs the animal were tested and confirmed to be diabeteic. Animals with FBS 200mg/dl and above were selected for the study. The blood glucose concentrations of the animals were determined weekly using a glucometer (Accu-Check Active).

2.5. Experimental Design

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 and treatment was made orally for 21 days as described below.

Group 1: Normal Control (Non diabetic, no treatment).

Group 2: Diabetic control, (Diabetic, no treatment).

Group 3: Diabetic + *Senna occidentalis* aqueous extract 200mg/kg

Group 4: Diabetic + *Senna occidentalis* ethanolic extract 200mg/kg

Group 5: Diabetic + Glibenclamide

2.6. Sample Collection

At the end of administration, the animals 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.

2.7. Determination of Haematological Parameters

The total Red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), lymphocyte concentration (LYM), White blood cell count (WBC) and Platelet count were determined using Abacus 280 auto haematology analyzer.

2.8. Serum Bilirubin

This was determined colorimetrically according to the method described by Jendrassic and Grof [7], using assay kits (Agape Laboratories Ltd, UK).

2.9. 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

3.1. Full Blood Count: White Blood Count (WBC), Lymphocyte (LYM), Red Blood Count (RBC) and Platelets (PLT)

In the full blood count, diabetic condition caused significant increase ($p<0.05$) in WBC and PLT in group 2 (table 1) compared to the normal control in group 1. Treatment of diabetic rats with aqueous extract and glibenclamide in group 3 and 5 had no significant difference ($p<0.05$) from the diabetic control group, except the group treated with ethanol extract i.e for WBC, but PLT was significantly lower ($p<0.05$) in aqueous extract and glibenclamide. Unlike WBC, LYM and RBC levels were significantly lower ($p<0.05$) in diabetic control compared to the normal control. Hence treatment with extracts as well as glibenclamide caused significant increase in diabetic control in LYM and RBC levels compared to the diabetic control group.

Table 1. *Full blood count in diabetic and normal rats treated with Senna occidentalis leaf extracts and glibenclamide.*

	FULL BLOOD COUNT			
GROUP	**WBC**	**LYM**	**RBC**	**PLT**
NORMAL CONTROL	1.31 ± 0.95^{a}	2.57 ± 0.06^{b}	4.88 ± 0.24^{b}	227.36 ± 061.66^{b}
DIABETIC CONTROL	3.58 ± 0.64^{b}	0.54 ± 0.09^{a}	1.41 ± 0.60^{a}	295.44 ± 04.99^{d}
DIABETIC +100g S.O AQ EXT	2.17 ± 0.92^{ab}	2.12 ± 0.37^{b}	5.30 ± 0.52^{b}	251.28 ± 06.92^{c}
DIABETIC +100g S.O ETH EXT.	1.81 ± 0.14^{a}	3.40 ± 0.56^{b}	3.96 ± 0.94^{b}	284.00 ± 65.25^{cd}
DIABETIC+ GLIBENCLAMIDE	2.30 ± 0.64^{ab}	2.88 ± 0.04^{b}	3.97 ± 0.25^{b}	184.64 ± 19.09^{a}

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

3.2. Packed Cell Volume (PCV) and Haemoglobin (Hb) Concentrations

PCV and Hb levels were significantly lower ($p<0.05$) in diabetic control compared to the normal control. Hence treatment with extracts as well as glibenclamide caused significant increase in diabetic control in PCV and Hb levels compared to the diabetic control group.

Table 2. *Packed Cell Volume (PCV) and haemoglobin (Hb) concentrations in diabetic and normal rats treated with Senna occidentalis leaf extracts and glibenclamide.*

PACKED CELL VOLUME AND HAEMOGLOBIN		
GROUP	**HB (g/100ml)**	**PCV (%)**
NORMAL CONTROL	15.44 ± 2.03^{b}	47.86 ± 10.99^{b}
DIABETIC CONTROL	11.41 ± 5.94^{a}	35.67 ± 01.29^{a}
DIABETIC +100g S.O AQ EXT	15.09 ± 3.77^{b}	47.23 ± 02.65^{b}
DIABETIC +100g S.O ETH EXT.	16.62 ± 8.51^{b}	47.44 ± 02.21^{b}
DIABETIC+ GLIBENCLAMIDE	14.77 ± 2.36^{b}	46.83 ± 01.13^{b}

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

3.3. Serum Bilirubin Concentration

Table 3. *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 $p<0.05$. Treatment of diabetic rats with the extracts caused significant decrease ($p<0.05$) in serum bilirubin concentration compared with the diabetic control (table 3). The decrease in serum total bilirubin concentration caused by glibenclamide was not statistically significant ($p<0.05$) compared with the diabetic control.

4. Discussion

Diabetes is currently considered as a vascular disease [8]. 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 [9]. Studies have revealed the beneficial effects of some secondary plant metabolites that possess antioxidant activity in diabetes management.

Haematological and biochemical indices have been reported to be a reliable parameter for assessment of the health status of animals. There was significant decrease ($p<0.005$) in the level of RBC of the healthy rats and the treated rats compared to the untreated animals (Table 1). These may be as a result of anaemia or the onset of glycosylation process in the untreated diabetic rats. Anaemia has been identified as a common complication of chronic kidney disease (CKD), affecting over half of all patients, and the most common cause of CKD in about 2/3 of cases is diabetes mellitus [10]. There was a significant reduction ($P<0.05$) in WBC levels of diabetic rats treated with ethanolic leaf extract of *Senna occidentalis* compared to the untreated animals and those treated with aqueous leaf extract. However, there was significant elevations in platelets levels of group treated with ethanolic extract and slight increase in lymphocytes levels of those treated with aqueous extract compared to the other groups. Aqueous and ethanolic leaf extracts of *Senna occidentalis* treatment may not have adverse effects on the bone marrow, kidney and heamoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and

associated parameters would have effects on the bone marrow, kidney and heamoglobin metabolism [11]. The results obtained from this study showed clearly that Ethanolic leaf extract of *Senna occidentalis* does possess hematopoietic activity and is not hematotoxic.

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 [12]. 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 [13]. 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 chemical constituents of the various plants containing carbohydrates, phenolics, flavonoids, alkaloids, saponins and glycosides gives recovery of islet which gives increase in the pancreatic secretion of insulin from islet of Langerhans. It is no doubt that S. occidentalis extracts have elicited appreciable potency in the treatment/management of oxidative stress-induced haematological disorders. Hence, the leaf of the plant stands a better chance for being processed for drug development.

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