Effect of Ethanolic Leaf Extract of *Senna Fistula* on some Haematological Parameters, Lipid Profile and Oxidative Stress in Alloxan-induced Diabetic Rats

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Summary: Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. The disease is also known to adversely affect some haematological parameters and cause dyslipidemia. This study was designed to investigate the effect of chronic administration of ethanolic leave extract of Senna fistula on haematological values, oxidative stress and dyslipidemia in experimental diabetic rats. Twenty-four albino rats weighing 120-150 g were divided into 4 experimental groups of six rats each; control, diabetic untreated, diabetic treated with glibenclamide and diabetic treated with 100 mg/kg b.w of Senna fistula. Diabetes was induced by 100 mg/kg b.w. of alloxan monohydrates. The control and diabetic groups received normal saline while the diabetic treated groups were administered with 5mg/kg and 100mg/kg body weight of glibenclamide and ethanolic leaves extract of Senna fistula respectively for 28 days. At the end of experimental period blood samples were taken from the animals for the determination of Red blood cells (RBC), packed cell volume (PCV), Haemoglobin concentration (Hb), total cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and malondialdehyde (MDA), marker of lipid peroxidation. The result showed that in diabetic rats, PCV, RBC and Hb were decreased but the application of the extract increased the parameters (P<0.05, n=6). Similarly, the result showed a significant increase in total cholesterol, TG and LDL level of the diabetic group when compared with the control, glibenclamide and extract treated diabetic groups, however, there was no significant difference in HDL level in all the groups. The result also showed a significant decrease in elevated MDA (P<0.05, n=6) of diabetic treated rats. These findings suggest that ethanolic leaves extract of Senna fistula might improve the diabetic induced disturbances of some haematological parameters, reduces the plasma lipid imbalances and decreases the production of free radicals associated with diabetes.

Keywords*:* Glibenclamide, Senna Fistula, Diabetes Mellitus, Packed Cell Volume, Malondialdehyde

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INTRODUCTION

Diabetes is a disorder in the metabolism of protein, carbohydrates, and fat resulting from absolute or relative deficiency in insulin secretion without/with varying level of insulin resistance (Devlin, 1997; Barar, 2000). It may also be defined as a disease where the body either produces little insulin/cease to produce insulin or progressively resistant to its action (Ranjan, 2002).

Patients with diabetes experience significant morbidity and mortality from macrovascular and microvascular complications. The macrovascular complications are due to accelerated atherosclerosis resulting from increased plasma low density lipoprotein- cholesterol (LDL-C), which leads to increased incidence of stroke and myocardiac infarction. The microvascular abnormalities include renal disease (diabetic nephropathy) leading to anaemia and renal failure, proliferative scaring of the retinal (diabetic retinopathy) resulting into blindness

(Halder *et al.,* 2003; Merlin *et al.,* 2005). Diabetic neuropathy involves abnormalities in the autonomic nervous system and peripheral nerves. The neuropathy and atherosclerotic circulatory insufficiency in addition to reduced resistance to infection in the extremities especially in the feet can lead to chronic ulceration and gangrene. The cost of treating diabetes and associated complications exceed $100 billion per year worldwide (Jarald *et al.,* 2008). The complications are less common and less severe in people who have well controlled blood glucose level (Andrew, 2000)

Increased oxidative stress is a well-known factor in the development and progression of diabetes and its complications. (Baynes, 1991; Baynes and Thorpe, 1999; Ceriello, 2000). The disease is usually associated with increased production of free radicals (Baynes and Thorpe, 1999; Baynes, 1991; Chang *et al.,* 1993; Young *et al.,* 1995) or reduced antioxidant defenses (Halliwell and Gutteridge, 1990; Saxena *et*

al., 1993; McLennan *et al.,* 1991). The end result is oxidative stress.

Diabetes has now become an epidemic with a worldwide incidence of about 9% in the general population (WHO, 2012), making it one of the most common non-communicable diseases (Jarald *et al.,* 2008). In the year 2012, about 1.5 million deaths were caused by diabetes directly and 80% of these deaths occur in low and middle income countries (WHO, 2014). Diabetes is projected to be the 7th cause of death by 2030 (Mathers and Loncar, 2006).

Several oral anti-diabetic agents are presently available to reduce hyperglycaemia including sulfonylurea and biguanides. Unfortunately, even with intensive use of current antidiabetic agents more than 50% of diabetic patients still suffer poor glycemic control and some even develop serious complication within six years of diagnosis (Jarald *et al.,* 2008). Moreover, none of the glucose lowering agents control hyperlipidemia adequately and oxidative stress that is frequently associated with the disease (Derek, 2001). Clearly, there is a need for new anti-diabetic agents which will be more effective, cheap, readily available and of natural (plant) origin. One plant claimed to be used in the management of diabetes mellitus in folk medicine of Nigeria is *Senna fistula*

Senna fistula Linn. (Senna) belongs to the family leguminosae, is otherwise referred to as "Golden shower", (English) and *Aidantoro* (Yoruba), is a deciduous and mixed- monsoon forests tree that originates from India and Sri-Lanka but is now cultivated in tropical countries of the World from West indies to Indian, South Africa, East Africa and West Africa especially Nigeria (South-West), (Trease and Evans, 1985). The plant grows to about 15m tall with greenish grey bark, compound leaves with 3-7 pairs of leaflets each 5-12 cm long (Gupta, 2010)

Senna fistula plant has been claimed to be used as a laxative and antibiotic (pods, fruit) (Akanmu *et al., 2004;* Kasugo and Nagaye, 1951), methanolic extract of buds of *S. fistula* has been shown to have antipyretic, analgelsic and anti-inflammatory effect (buds) (Bhakta *et al.,* 1999b; Ilavarasan *et al.,* 2005), the leaf, stem bark, pulp and flower of *Senna fistula* have been shown to have antioxidant activity (Siddhuraju *et* al.,2002), antitumor (seed) (Gupta *et al.,* 2000), also the petroleum ether extract of seeds of *Senna fistula* has been shown to have antifertility effect (Yadav and Jain, 1999) others include antidiabetic, antihypercholesterolemic effects (Nirmala *et al.,* 2008) and also ethanolic leave extract of *Senna fistula* has hepatoprotective effects in diethyl nitrosamine (DEN) induced hepatic injury (Kannampalli, *et al,* 2007; Bhakta *et al.,* 2001).

Studies have shown that diabetes is associated with increased oxidative stress, alteration of some haematological parameters, lipid abnormality and most importantly increased blood glucose. There is dearth of information on studies that evaluated the effect of ethanolic leaf extract on diabetes, therefore the present study was undertaken to evaluate the effect of repeated oral administration of ethanolic leaf extract of *Senna fistula on* hyperglycaemia, hyperlipidemia, haematological and oxidative disturbances associated with diabetes.

MATERIALS AND METHODS

Plant materials and authentication
The plant material was obtained from the herb sellers at Oja-Tuntun market, Ilorin, Nigeria, and was authenticated at the Plant Biology Department of University of Ilorin. It was identified with a voucher specimen UIH 1020 earlier deposited in the herbarium.

Glucometer and Assay Kit
One touch ultra® glucometer was a product of lifeScan, Inc. Milpitas, USA. Assay kits for cholesterol, triglyceride were product of Randox Laboratories, Co-Antrim, UK.

Drugs and chemicals
Glibenclamide was a product of HOVID Bhd, Ipoh, Malaysia, alloxan monohydrate and all other chemicals were products of Sigma-Aldrich Cheme GmbH, Steinheim, Germany.

Laboratory animals
Male albino rats *(Rattus norvergicus)* of Wistar strain, weighing between 120- 150 g were obtained from the animal holding unit of the Department of Biochemistry, University of Ilorin. The animals were fed on rat pellet (Premier feed Ltd Ibadan) and water *ad libitum,* they were maintained under standard laboratory conditions and were subjected to natural photoperiod of 12h light; dark cycle; temperature:28-31^{0}C; humidity: 50-55%

Preparation of the extract.
Fresh leaves of *Senna fistula* were air dried at room temperature for about two weeks. The dried materials were pulverized using an electric blender. A known weight of the powder (158.7g) was extracted in 2 litres of ethanol for 24 hours. The extract was then filtered. The filtrate was evaporated to dryness using water bath which yielded 31.42 g. The calculated amount of the extract was weighed and dissolved in normal saline to give the required dose of 100 mg/kg body weight. 24 albino male rats were randomly assigned into four groups of 6 rats each:
- Group A- (control) normal and received 0.5ml of normal saline
- Group B-(untreated) diabetic, received 0.5ml of normal saline

- Group C-(treated) diabetic, received glibenclamide (5 mg/kg b.w.)
- Group D-(treated) diabetic, received 100 mg/kg b.w. ethanolic extract of *S. fistula*

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared alloxan monohydrate (100 mg/kg b.w) in sterile physiological saline. 1 hour after alloxan injection the animals were given their pellet and 5% dextrose saline to overcome initial hypoglycemic phase (Sikarwar and Patil, 2010). Diabetes was confirmed by glucose oxidase method using one touch ultra glucometer, 72 hours after alloxan injection. Only animals with blood glucose level higher than or equal to 200 mg/dl were used for the study. (Yakubu *et al.,* 2010; Meral *et al.,* 2004). The blood glucose levels of the animals were also determined before the administration of alloxan using the blood samples that were drawn from the tail vein.

Acute toxicity study
The method described by Lorke (1983) was used for this study. Nine rats were used and were divided into three groups of three rats each, the animals were administered 1000, 2000 and 5000 mg/kg b. w. of ethanolic extract of *Senna fistula* leaves respectively. The animals were observed/monitored closely for 72 hours for symptoms of tiredness and death.

The number of deaths in each group was recorded as percentage of mortality and the LD50 was calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

Determination of biochemical parameters
Plasma triglyceride and total cholesterol levels were measured using enzymatic colorimetric diagnostic kits obtained from Randox Laboratories in which the GPO-PAP method of Trinder (1969) was employed. Absorbance was read at 500nm. The phosphotungstate precipitation method of Richmond (1973) as applied in Randox kit was used for the determination of HDL-Cholesterol. The LDL-Cholesterol was estimated using Friedewald (1972) formula:

$$LDLc = \text{total cholesterol} - HDLc - TG/5$$

Where LDLc = LDL-cholesterol, HDLc= HDL-cholesterol and TG= triglycerides

Determination of haematological parameters
All haematological parameters were determined by an automated haematological analyser, Symex KY-21 (Symex Corporation, Japan) using whole blood sample.

Determination of Malondialdehyde
Plasma MDA was measured by a thiobarbituric acid assay procedure (Albero *et al.*, 1986) which was calibrated using 1,1,3,3 tetraetoxypropane (Sigma chemical, St Louis, Mo USA) as a standard. Results

were expressed as nanomoles of MDA per millitre of serum.

Statistical Analysis
The mean value and standard error of mean (SEM) were calculated. The test for significance was carried out using ANOVA, and Duncan new multiple range test (DMRT). All the results were expressed as mean ± SEM, differences were considered statistically significant at $P < 0.05$.

RESULTS

Table 1 shows the result for the acute toxicity test (LD_{50}). There were no records of death within the first 48 hours in all the groups after oral administration of ethanolic extract of *Senna fistula* leaves. However, there was a record of death in the group given 5000 mg/kg b.w. of the extract 72 hours after administration, which represent about 33.33% mortality. The oral LD_{50} was estimated to be 3.162.28 mg/kg in rats accordingly:

$$LD_{50} = (5000 \times 2000)^{1/2} = 3162.28 \text{ mg/kg}.$$

Table 2 shows the effect of ethanolic extract of *Senna fistula* leaves on blood glucose level of diabetic rats. The diabetic untreated animal had increasing fasting blood glucose (FBG) throughout the period of the experiment rising from 233.80 mg/dl on the first day of the experiment to 262.20 mg/dl on the 28th day. The FBG of all the groups were significantly different from the control on the first day ($P < 0.05$), however, the animals treated with ethanolic extract of *Senna fistula* leaves and glibenclamide treated diabetic rats had reduced FBG value during the experiment and are significantly different from diabetic untreated group ($P < 0.05$).

Table 1: LD_{50} of ethanolic extract of *Senna fistula* leaves in rat

	number of deaths			% mortality		
Dose	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
1000mg/kg	0	0	0	0	0	0
2000mg/kg	0	0	0	0	0	0
5000mg/kg	0	0	0	0	0	33.3

Table 2. Effect of administration of ethanolic leaf extract of *Senna fistula* on the blood glucose level (mg/dl) of alloxan-induced diabetic rats

Group	Initial	After 28 days
A	77.40±7.97[a]	61.80±11.35[a]
B	233.80±26.09[b]	262.20±10.41[c]
C	258.40±10.36[c]	103.40±10.68[b]
D	362.40±50.78[d]	93.20±5.63[b]

Values with different superscript are significantly different, p<0.05. A=Normal saline (0.5 ml), B=Normal saline (0.5 ml), C=Glibenclamide (5 mg/kg), D=*Senna fistula* extract (100 mg/kg)

Table 3: Effect of administration of ethanolic leaf extract of *Senna fistula* on haematological parameters, lipid profile, and serum malondehydehyde level in alloxan induced diabetic rats.

Groups/parameters	A	B	C	D
MDA	3.08±0.86[a]	5.00±0.12 [c]	3.44± 0.05[b]	3.58±0.66 [b]
Cholesterol	2.76±0.11 [a]	5.14± 0.21 [d]	3.84±0.21 [c]	3.54±0.19 [b]
TG	0.96±0.09 [a]	2.14±0.16 [c]	1.08±0.12 [a]	1.96±0.51 [b]
HDL-C	1.24±0.15 [a]	1.09±0.15 [a]	1.28 ±0.08[a]	1.12 ±0.06[a]
LDL-C	1.92±0.39 [a]	3.56±0.18 [d]	2.00±0.16 [b]	2.84±0.24 [c]
PCV (%)	37.4±0.2 [b]	27.4±0.2 [a]	41.2±0.5 [c]	42.4±1.0 [c]
Hb (g/dl)	12±0.1 [b]	8.5±0.1 [a]	11.9±0.1 [b]	12.8±0.4 [b]
R BC ($\times 10^6$)	4.4±0.1 [b]	3.4±0.1 [a]	4.7±0.1 [b]	5.0±0.1 [c]

Values with different superscript are significantly different, p<0.05. A=Normal saline (0.5 ml), B=Normal saline (0.5 ml), C=Glibenclamide (5 mg/kg), D=*Senna fistula* extract (100 mg/kg)

In Table 3 there was a significant decrease in Red blood cell count (RBC), packed cell volume (PCV) and haemoglobin (Hb), values in diabetic (untreated) rats, but administration of the ethanolic extract of *Senna fistula* leaves increased significantly the reduced PCV, RBC and Hb values in diabetic rats. However, there was no significantly different in all these parameters between the control, glibenclamide and the extract treated groups (P<0.05).

As shown in Table 3, the mean value of serum malondialdehyde (MDA) was significantlyelevated in alloxan-induced diabetic rats. Treatment with ethanolic extract of *Senna fistula* leaves causes a significant decrease (P<0.05) in MDA level of diabetic rats which compared favourably with the control and and glibenclamide treated rats. The mean value of cholesterol, triglyceride (TG) and low density lipoprotein (LDL) (Table 3) were significantly increased in diabetic untreated group. Administration of the extract causes a significant reduction in total cholesterol, TG and LDL in diabetic rats in a manner similar to control and glibenclamide treated rats, however, there was no significant difference in high density lipoprotein (HDL) level in all the groups.

DISCUSSION

Plants have been a source of medicinal agents for many years, and a remarkable number of modern drugs have been isolated from plants, many of which are based on their use in traditional medicine." These plant-based traditional medicine systems have continued to play a significant role in health care, with about 80% of the world's populations mainly depending on traditional medicines for their primary health care (Owolabi *et al.,* 2007). Plant products also have an important role in the health care systems of the remaining 20%, who live in developed countries like America, Europe. Many studies have revealed that many plants extract effectively lowered blood glucose level in alloxan induced diabetic animals (Owoyele *et al* 2005; Yakubu *et al* 2010). In this study, ethanolic leaf extract of *Senna fistula* significantly reduced the blood glucose level and effectively restored some biochemical and haematological parameters in alloxan induced diabetic rats.

Study on the acute toxicity suggests that the extract could be toxic at a high dose on acute exposure. However, the dose used in this study is less than the calculated LD50 which demonstrated that the extract could be safely consumed at the dose used in this study.

The use of alloxan to induce diabetes in rats represents a well-established animal models of type I insulin dependent diabetes mellitus characteristically similar to type I diabetes in human (Szkudelski, 2001). Glibenclamide was used as a reference drug mimicking several insulin actions in vivo which include suppressing hepatic glucose production, increasing insulin sensitivity of extrapancreatic tissue, stimulation of lipogenesis and inhibition of lipolysis, enhancing peripheral glucose uptake, decreasing hepatic glycogenolysis and gluconeogenesis and as well as absorption of glucose from the gastrointestinal tract (Wadkar *et al.*, 2008; Zeggwagh *et al.,* 2007).

In this present study, intraperitoneal injection of alloxan caused a significant increase in the level of blood glucose indicating establishment of a diabetic state. However, treatment of diabetic animals with 100 mg/kg b.w. of ethanolic leaf extract of *Senna fistula* produced a significant decrease in plasma glucose

level at the end of the experiment (28 days), the decrease in blood glucose level recorded in this study is comparable with that of the reference group (glibenclamide group). The mechanism of hypoglycaemic effect of ethanolic extract of *Senna fistula* leaves is not known yet, but it could be that the extract facilitates glucose utilization by peripheral tissues or by decreasing hepatic glycogenolysis and gluconeogenesis or it could also stimulate increase insulin production from possibly regenerating pancreatic beta cells, however, further study could be carried out to measure insulin level and liver glycogen in order to know its mechanisms of action.

The phytochemical and mineral composition screening of the leaf extract carried out also revealed the presence of flavonoids, alkaloids, tannins, saponins, Ca, K, Zn, Mn, Mg and vitamin C, this may also be partly responsible for its hypoglycaemic property, because studies have documented that medicinal plants with hypoglycaemic property usually contain Alkaloids, Flavonoids, Tannins and Terpenoids (Oladele *et al.,* 1995; Ojewole, 2005).

Diabetes has been found to adversely affect haematological parameters by decreasing the life span of red blood cells and white blood cell count. (Kamenov *et al.,* 1997). In the present study, some haematological parameters were analysed and the result showed that red blood cell (RBC), packed cell volume (PCV), and haemoglobin (Hb) concentration values were decreased in diabetic rats. This is in line with previous work where occurrence of anaemia in diabetes has been documented (Merlin *et al.,* 2005). Treatment with ethanolic leaf extract of *Senna fistula* increased the reduced RBC, PCV, and Hb concentration values in diabetic rats. The increased blood indices could be related to the mineral composition of the leaf extract of *Senna fistula* which include protein, Zn, Ca, K, Mn, Fe, P, Mg and vitamin C most of these mineral components are well known haematological factors that have influence on the production of blood from the bone marrow (Ganong, 2006).

Diabetes hyperglycemia results in an increase in free radical production through mechanisms involving glucose oxidation, protein glycation and oxidative degeneration (Hunt *et al.,* 1990) which may play a role in increased lipid peroxidation in diabetes mellitus. In this study, the increased levels of MDA (marker of lipid peroxidation) in diabetic rats clearly showed that diabetic rats were exposed to an increased oxidative stress via lipid peroxidation, (Velazquez *et al* 1991, Nacitarhan *et al* 1995, Losada and Alio 1996, Mahboob *et al.,* 2005, Kaji *et al.,* 1985). This is in agreement with previous studies documenting elevated

serum lipid peroxide level in diabetic subjects (Sato and Hotta-Nsoka 1979; Oberley 1988; Halliwell and Gutteridge, 1990). Administration of ethanolic leaf extract of *Senna fistula* decreased the elevated lipid peroxide in alloxan induced diabetic rats in a manner similar to glibenclamide treated group. The decreased level of lipid peroxide in *Senna fistula* treated rats may be due to decreased oxidative load which the extract might have caused by either directly scavenging the reactive oxygen metabolites due to the presence of many antioxidant compounds like flavonoids or by increasing the synthesis of antioxidant molecules. Furthermore, increased production of lipid peroxide in diabetic rats may also explain the reduction in some haematological parameters (RBC, PCV, Hb) found in diabetic rats. Occurrence of anaemia in diabetes mellitus has been linked to increased non-enzymatic glycosylation of red blood cell membrane proteins. Oxidation of the glycosylated membrane protein and hyperglycemia in diabetes causes an increase in the production of lipid peroxide which in turn leads to haemolysis of red blood cells. Thus increased RBC count, packed cell volume and haemoglobin concentration of ethanolic leaf extract of *Senna fistula* treated rats could be due to reduction of lipid peroxide level in RBC membrane, hence a decreased susceptibility of RBC to haemolysis.

In diabetes, there is hyperlipidemia which may be linked to insulin deficiency; in this state (hyperglycemia) fatty acids are mobilized from adipose tissue causing accumulation of excess fatty acids in the liver which are then converted to triglyceride (Velazquez *et al.,* 1991). Administration of ethanolic leaf extract of *Senna fistula* reduced total plasma cholesterol, triglycerides and low density lipoprotein level in diabetic rats. This is in line with previous studies where hypolipidemic activity of some medicinal plants in alloxan induced diabetic rats have been documented (Nirmala *et al.,* 2008; Ayinla *et al.,* 2011). The observed hypolipidemic effect recorded in this study could be due to the presence of some phytochemical compounds which include alkaloids, flavonoids, saponins and tannins. All these compounds are known to reduce serum lipid level in animal especially alkaloid which is known to normalize lipogenesis due to its insulinogenic effect on lipid metabolism while flavonoids cause decrease in the activity of HMG-CoA reductase in the liver.

In conclusion, the significant reduction of the high blood glucose in diabetic extract treated group to the values of the control and reference treated group indicates anti-hyperglycaemic activity of ethanolic leaf extract of *Senna fistula*. This study also revealed that ethanolic leaf extract of *Senna fistula* can

effectively correct dyslipidemia, oxidative stress and some haematological disturbances associated with alloxan induced diabetic rats.

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