

Anti-diabetic Activity of Aqueous Extract of *Senna alata* (Fabacea) Flower in Alloxan-induced Diabetic Male Rats

Activité anti-diabétique de l'extrait aqueux des fleurs de *Senna alata* (Fabacées) sur le diabète alloxane-induit chez les rats mâles

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ABSTRACT

This study investigated the acclaimed anti-diabetic potential of aqueous extract of *Senna alata* flower in alloxan-induced diabetic rats. Forty-two, male Wistar rats were randomized into six groups (I-VI) such that animals in group I which were non-diabetic received 0.5ml each of distilled water while those in groups II-IV which were induced into diabetes (intraperitoneal administration of 150 mg/kg body weight of alloxan) received orally 0.5ml each of distilled water, glibenclamide (2.5 mg/kg body weight), 25, 50 and 75mg/kg body weight of the extract respectively once daily for 13 days. The secondary metabolites in decreasing order of abundance were phenolics, flavonoids, cardiac glycosides, steroids, saponins, anthraquinones, tannins and alkaloids. The elevated ($P < 0.05$) blood glucose level, total cholesterol, triglycerides, low density lipoprotein-cholesterol, haemoglobin, total bilirubin, serum urea and creatinine as well as the reduced body weight, high density lipoprotein-cholesterol, liver glycogen, packed cell volume, albumin, conjugated bilirubin and hexokinase activity by the alloxan were reversed and/or ameliorated by the extract, similar to the reference drug, glibenclamide. The extract restored the alloxan treatment related distortion and atrophy in the pancreas. The available data in the study suggests that aqueous extract of *Senna alata* flower at the doses of 25, 50 and 75mg/kg body exhibited anti-diabetic activity with the highest dose displaying the most profound activity. The anti-diabetic activity conferred by the secondary metabolite is suggested, among other, to be via enhancing the activity of hexokinase. This study has justified the folkloric use of *S. alata* flower in the management of diabetes.

Key words: Anti-diabetic, *Senna alata*, Pancreas, Fabaceae, Hexokinase.

RESUME

Cette étude a examiné le potentiel antidiabétique acclamé de l'extrait aqueux de *Senna alata* fleur dans les rats diabétiques induite par l'alloxane. Quarante-deux, des rats Wistar mâles ont été randomisés en six groupes (I-VI) telle que les animaux du groupe I qui n'étaient pas diabétiques a reçu 0,5 ml chacune de l'eau distillée, tandis que ceux des groupes II-IV qui ont été induites dans le diabète (l'administration intrapéritonéale de 150 mg/kg de poids corporel de l'alloxane) reçues oralement 0.5ml chaque de dis-Labouré eau, glibenclamide (2,5 mg/kg de poids corporel), 25, 50 et 75 mg/kg de poids corporel de l'extrait respectivement une fois par jour pendant 13 jours. Les métabolites secondaires dans l'ordre décroissant d'abondance ont été composés phénoliques, flavonoïdes, glycosides cardiaques, stéroïdes, saponines, les anthraquinones, des tannins et alcaloïdes. L'élevé ($P < 0,05$) la glycémie, le cholestérol total, de triglycérides, cholestérol à lipoprotéines de faible densité, hémoglobine, bilirubine totale, sérum-urée et de la créatinine ainsi que la réduction du poids corporel, cholestérol à lipoprotéines de haute densité, le glycogène hépatique, l'hématocrite, de l'albumine, la bilirubine conjuguée et de l'activité de l'hexokinase par l'alloxane étaient inversés et/ou améliorés par l'extrait, similaire à la drogue de référence, glibenclamide. L'extrait restauré l'alloxane distorsion lié au traitement et de l'atrophie dans le pancréas. Les données disponibles dans l'étude suggère que l'extrait aqueux de *Senna alata* fleur à des doses de 25, 50 et 75 mg/kg corps présentait l'activité anti-diabétiques avec la plus forte dose affichant le plus profond de l'activité. L'anti-activité diabétique conférés par le métabolite secondaire est suggéré, entre autres, à être via l'amélioration de l'activité de l'hexokinase. Cette étude a justifié l'utilisation de *S. alata* fleur dans la gestion du diabète.

Mots clés : Anti-diabétique, *Senna alata*, pancréas, Fabaceae, de l'hexokinase.

1. Introduction

Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism arising from defects in insulin secretion, insulin action, or both (Nayak and Roberts 2006). It is a global epidemic with an estimated worldwide prevalence of 246 million people in 2007 which is projected to rise to 300 million by 2025 (Whiting et al. 2011); thus, a major challenge to healthcare systems around the world. A chronic hyperglycemic condition in diabetes is associated with long-term damage, dysfunction, and failure of several organs which include the eyes, kidneys, nerves, heart, and blood vessels. As the disease progresses, tissue or vascular damage ensues leading to severe diabetic complications like retinopathy, neuropathy, nephropathy cardiovascular complications and ulceration (Huang et al. 2002, Wallace et al. 2002, Hove et al. 2004, Saely et al. 2004). Several management options (insulin injections, oral anti-diabetic agents, sulfonylureas, biguanides, alpha-glucosidase inhibitors, troglitazones) for diabetes are not without their undesirable side effects such as worsening of hearing, increase in body weight and hypoglycemia (Dey et al. 2003). Therefore, it is imperative to search for affordable natural alternatives in medicinal plants with high efficacy and reduced or complete absence of side effects. In Nigeria, a number of plants have been mentioned in various literatures for the management of diabetes mellitus without any scientific evidence that have buttressed/supported their uses. One of such plant is *Senna alata* flower.

Senna alata (Linn) Roxb (fabaceae) also known as candle bush, Ringworm bush (English), Asunwon oyinbo (Yoruba-Western Nigeria) and Nelkhi (Igbo-Eastern Nigeria) is an annual, ornamental flowering plant. It is an erect shrub of 2-5 m high with bright coloured yellow flowers. The golden yellow flowers of about 4 cm in diameter is arranged in an inflorescence column that resembles yellow candlestick. The flower is a perfect and complete with 2 long banana-shaped anthers, 3 projections from its stigma, 4 stomiums that serve as the point of pollen, a nectar, and a long pointed stalk that will eventually become the pod of the fruit seeds (Taylor 2005). The plant is used locally for the treatment of ringworm and parasitic skin disease, haemorrhoids, constipation, inguinal hernia, intestinal parasite, blennorrhagia, syphilis, convulsion, heart failure, pain, oedema and diabetes (Palanichamy and Nagarajan 1990, Ogunti and Elujobi 1993, Abo et al. 1998). Traditionally, *S. alata*

flower is well known for the management of problems related to liver, kidney, intestine, vision, syphilis and diabetes (Abdulwaliyu et al 2013). The flowers have been reported to contain secondary metabolites like saponins, tannins, flavonoids, and cardiac glycosides while alkaloids were absent (Essiet and Bassey 2013). Most of the pharmacological studies on *S. alata* flower in the open scientific literature include the antibacterial potency of some crude and purified fractions of *S. alata* flower against 22 bacterial strains and concluded that the purified extract was nearly as potent as standard antibiotics, exhibiting zones of bacterial growth inhibition ranging from 10 to 25 mm, even against multiple antibiotic resistant local isolates that were not susceptible to methicillin, penicillin and streptomycin (Adedayo et al 2001). Other pharmacological studies reported earlier was the antifungal activity of some components of *Senna alata* flower against five fungi which concluded that the partially purified *Senna alata* extracts exhibited a relatively high antifungal activity against mycelial growth with total suppression of sporulation for four days at a concentration of 2 mg/mL, while preventing fungal growth after the seventh day (Adedayo et al 1999). Another *in vitro* antifungal activity of *Senna alata* flower reported by Abubacker et al (2008) concluded that the flower extract can be used as a potential antifungal agent for aflatoxin producing fungi, *Aspergillus flavus* (NCBT 101) and *A. parasiticus* (NCBT 128); plant pathogenic fungi, *Fusarium oxysporum* (NCBT 156) and *Helminthosporium oryzae* (NCBT 165); and human pathogenic fungi, *Candida albicans* (NCBT 140) and *Microsporum audouinii*. Although, the flowers of *S. alata* have been acclaimed to be used in the management of several diseases, unfortunately however, very few of these uses that have been substantiated with scientific experiments are limited to antimicrobial potency/activity. The increasing rate of diabetes and the need to search for alternatives in medicinal plants coupled with the acclaimed use of the flowers of *S. alata* have necessitated the present study. Hence, the study was aimed at evaluating the anti-diabetic activity of aqueous extract of *Senna alata* flower in alloxan-induced diabetic rats with a view to providing scientific data to justify the folkloric use as an anti-diabetic agent and more importantly as a preliminary step towards the identification or discovery of a cheaper and readily available drug lead for the management of the chronic disease.

2. Materials and Methods

2.1. Plant material and authentication

Fresh flowers of the plant was collected during the raining season on 4th May, 2013 at about 9:00 hrs from a farmland at Ogbomoso, Oyo-State, Nigeria. The flowers were au-

thenticated in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria where a voucher specimen

assigned with UIH 1069 was deposited in the Herbarium

Unit of the Department.

2.2. Glucometer, Assay kits, Drugs and Chemicals

Bayer contourTM TS blood glucose meter and the strips were products of Bayer Consumer Care AG, Postfach, Basel, Switzerland. The assay kits for total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglyceride, urea, creatinine, albumin, total and conjugated bilirubin were manufactured by

Randox Laboratory Ltd., Co-Atrim, UK. Alloxan was a product of May and Baker Ltd, Dagenham, England, while glibenclamide and ATP were manufactured by Hoechst Marion Roussel Limited, Mumbai, India and Sigma-Aldrich CHEME GmbH, Steinheim, Germany, respectively.

2.3. Experimental animals

A total of forty-two male albino rats (*Rattus norvegicus*) of Wistar strain (aged, 12 weeks) with average weight of 141.24 ± 0.32 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats contained in aluminum cages were placed in the animal house with the following housing conditions: temperature $28 \pm 2^\circ\text{C}$, about 12-h light/dark cycle and relative humidity 40-45%. The ani-

mals were allowed free access to rat feed (Premier Feeds, Ibadan, Nigeria) and contaminant free tap water. The experiment commenced after two weeks of acclimatization and animals were handled following the Guidelines of Helsinki Declarations of 1975 as revised in 1983. The protocol of this study complied with the Guide on the Care and Uses of Laboratory Animals of National Research Council (NRC, 2011).

2.4. Preparation of extract

A known weight (230.00 g) of fresh flowers of *Senna alata* were washed clean with distilled water and oven-dried at 40°C . The dried plants were pulverized with an electric blender (Masterchef and Crownstar MC-BL 1664, China) after which 200 g was extracted in 1 litre of distilled water at room temperature for 48 hours. The filtrate

was lyophilized to give 26.60 g corresponding to a percentage yield of 13.30%. The lyophilized sample was reconstituted in distilled water to give the required doses of 25, 50 and 75 mg/kg body weight used in the present study.

2.5. Screening for secondary metabolites

The aqueous extract of *Senna alata* flower was screened for the presence of alkaloids, tannins, saponins, flavonoids, steroids, cardiac glycosides, anthraquinones and phenolics using the procedures described by Harborne (1976) and Awe and Sodipo (2001). The detected secondary metabolites were further quantified as described

for alkaloids (Harborne 1976), saponins (Hudson and El-Difrawi 1979), flavonoids (Lamaison and Carnet 1990), phenolics (Ragazzi and Veronese 1973), tannins (Siddhuraju and Becker 2003), cardiac glycosides (Trease and Evans 1996), anthraquinones (El-Olemy et al. 1994), steroids (Edeoga et al. 2005).

2.6. Induction and confirmation of diabetes

Forty-two male Wistar rats were fasted for 12 hours without food but water. The fasting blood glucose level was determined in the blood that was drawn from the tail of the animals as previously reported by Yakubu et al. (2012). Thereafter, thirty-five rats were induced into diabetes by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight, prepared in sterile physiological saline). The animals were immediately maintained on 5% glucose solution with the aid of feeding

bottles to overcome the initial hypoglycaemic phase normally associated with the induction of chemical diabetes (Sikarwar and Patil 2010). Rat feed (Premier Feeds, Ibadan, Nigeria) was however returned to the animals after the administration of alloxan. The determination of fasting blood glucose was again repeated 24 hours after alloxan administration and only animals with blood glucose level higher than 250 mg/dl were declared diabetic and assigned to various groups.

2.7. Animal grouping and extract administration

The animals were assigned into six groups (I-VI) of seven rats as follows:

Group I: Non-diabetic rats that received 0.5ml of distilled water.

Group II: Diabetic rats that received 0.5ml of distilled water.

Group III: Diabetic rats that received 0.5 ml corresponding to 25 mg/kg body weight of aqueous extract of *Senna alata* flower.

Group IV: Diabetic rats that were administered 0.5 ml corresponding to 50 mg/kg body weight of aqueous extract of *Senna alata* flower.

Group V: Diabetic rats that were administered 0.5 ml corresponding to 75 mg/kg body weight of aqueous extract of *Senna alata* flower.

Group VI: Diabetic rats that received 0.5 ml corresponding to 2.5 mg/kg body weight of glibenclamide (reference drug)

2.8. Preparation of serum and liver supernatant

The procedure described by Yakubu et al. (2012) was used for the preparation of serum and liver supernatant. Briefly, under ether anaesthesia, the neck area was quickly cleared of fur and skin to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were sharply cut with a sterile scalpel blade; the dripping blood was collected into clean, dry centrifuge tubes and left to clot for 20 minutes. This was then centrifuged at $685 \times g$ for 15 minutes using Uniscope Laboratory Centrifuge (model SM90-1, Surgifriend Medicals, Essex, England). The serum was there-

All the administration was done orally once daily between 08:00-08:30 hours using metal oropharyngeal canula. The animals were given rat pellets and tap water *ad libitum* throughout the experiment.

after aspirated with Pasteur pipettes into clean, dry, sample bottles and stored overnight at 4°C before being used for the biochemical assay. The animals were quickly dissected after which the liver and pancreas were excised. The organs were blotted in blotting paper. The liver was weighed and homogenized in Tris buffered 0.25M sucrose solution (1:5^{v/v}) while the pancreas was prepared for histopathological examinations. The homogenates were centrifuged at $1340 \times g$ for 15 minutes to obtain the supernatant that was used for the biochemical analysis

2.9. Determination of other biochemical parameters

The biochemical parameters determined in the serum of the animals were as described for albumin (Doumas et al. 1971), creatinine (Bartels et al. 1972), urea (Weatherburn 1967), total cholesterol (Fredrickson et al. 1967), low density lipoprotein-cholesterol (Friedwald et al. 1972), high density lipoprotein-cholesterol (Lopes-Virella et al 1977), triglycerides (Fossati and

Prencipe 1982), total and conjugated bilirubin (Jendrassik and Grof 1938) while those determined in the liver included glycogen (Kemp and van Heijningen 1954) and activity of hexokinase (EC 2.7.1.1) (Brandstrup et al. 1957). The haemoglobin and PCV levels were determined using the procedure described by Dacie and Lewis (1991).

2.10. Histopathological examination of the pancreas

The procedure described by Krause, (2001) was adopted for the histopathological examination of the pancreas. Briefly, pancreas fixed in 10%^{v/v} buffered formaldehyde were dehydrated through ascending grades of ethanol (70, 90 and 95% ^{v/v}). The pancreas was cleaned in xylene, impregnated and embedded in paraffin wax (melting point 56°C). Thin sections (5 μ m) were floated out on clean microscope slides, which had been albuminized to prevent detachment from slides during staining procedure. These were then air-dried for 2 hours at 37°C (Drury

and Wallington 1973). Thereafter, the slides were passed through ascending concentrations of alcohol (20-100%) for dehydration and cleaned with xylene. A permanent mounting medium (basalm) was put on the tissue section after which a thin glass-covered slip was placed on the covering- mounting medium for the underlying tissue sections to dry. This was later observed using the Leitz, DIALUX research microscope and pictures were snapped in bright field at x100.

2.11. Statistical analysis

All the data were expressed as the mean $\pm$ SEM of seven replicates except otherwise stated. Statistical analyses of the data was performed with SPSS version 20.0 using one

way analysis of variance, followed by Duncan's post hoc test for multiple comparison. Values were considered statistically significant at $P < 0.05$.

3. Results

Aqueous extract of *Senna alata* flower contained alkaloids, tannins, saponins, phenolics, flavonoids, anthraquinones, cardiac glycosides and steroids. Phenolics were relatively the most abundant of the secondary metabolites whereas alkaloids occurred as the least (Table 1).

Table 1: Secondary metabolite constituents of the aqueous extract of *Senna alata* flower

Secondary metabolites	Concentration (mg/100mg)
Anthraquinones	12.53 $\pm$ 0.24
Flavonoids	106.68 $\pm$ 0.18
Phenolics	122.52 $\pm$ 0.31
Alkaloids	0.21 $\pm$ 0.12
Tannins	6.30 $\pm$ 0.10
Saponins	50.22 $\pm$ 0.06
Steroids	71.00 $\pm$ 0.32
Cardiac glycosides	105.99 $\pm$ 0.80

n=3 $\pm$ SEM

Table 2: Fasting blood glucose level of alloxan-induced diabetic male Wistar rats following oral administration of aqueous extract of *Senna alata* flower

Treatment Groups	Fasting blood glucose level (mg/dl)							
	Before induction	Days after induction						
		1	3	5	7	9	11	13
Non-Diabetic rats + Distilled water	61.00 ± 2.04 ^a	61.70 ± 2.50 ^a	60.00 ± 2.94 ^a	60.00 ± 2.00 ^a	61.75 ± 1.64 ^a	61.00 ± 2.38 ^a	62.25 ± 3.07 ^a	61.05 ± 2.75 ^a
Diabetic rats + Distilled water	61.50 ± 1.44 ^a	436.00 ± 4.62 ^c	424.00 ± 2.20 ^d	430.00 ± 3.20 ^d	425.01 ± 3.09 ^d	450.25 ± 1.18 ^e	460.21 ± 1.11 ^e	461.25 ± 3.92 ^d
Diabetic rats +25 mg/kg body weight of extract	61.25 ± 3.39 ^a	439.50 ± 1.32 ^c	302.00 ± 2.78 ^c	285.05 ± 1.46 ^c	269.50 ± 3.22 ^c	200.75 ± 1.01 ^d	195.21 ± 2.20 ^d	125.50 ± 6.01 ^c
Diabetic rats+ 50 mg/kg body weight of extract	62.00 ± 1.96 ^a	476.25 ± 4.99 ^d	423.75 ± 2.03 ^d	283.02 ± 1.06 ^c	262.75 ± 4.82 ^c	154.75 ± 2.73 ^c	128.03 ± 3.63 ^c	101.75 ± 7.49 ^b
Diabetic rats + 75 mg/kg body weight of extract	60.75 ± 1.13 ^a	441.00 ± 2.21 ^c	203.25 ± 4.57 ^b	106.75 ± 5.53 ^b	104.25 ± 2.99 ^b	78.25 ± 3.99 ^b	71.50 ± 2.40 ^b	61.75 ± 1.28 ^a
Diabetic rats + 2.5 mg/kg body weight of Glibenclamide	61.50 ± 1.93 ^a	343.50 ± 3.89 ^b	295.00 ± 2.48 ^c	280.50 ± 2.98 ^c	155.75 ± 1.82 ^b	100.00 ± 1.74 ^b	85.10 ± 2.37 ^b	61.50 ± 2.04 ^a

n=7 ± S.D; Mean values for each day of intervention with superscripts, b, c, d and e, different from their respective controls, a, are significantly different (P < 0.05).

Administration of alloxan to the normal rats significantly increased the fasting blood glucose from 61.50 - 436.00 mg/dl after 24 hours. The increase in fasting blood glucose continued throughout the experimental period reaching a peak value of 461.25 mg/dl (Table 2). However, all the doses of the extract significantly and progressively decreased the elevated blood glucose level by the alloxan with the 75 mg/kg body weight producing blood glucose levels that compared favourably with both the non-diabetic distilled water treated control animals and the diabetic animals treated with glibenclamide by the end of the experimental period (Table 2).

Unlike the alloxan-treated diabetic animals where the body weight of the rats decreased progressively throughout the experimental period, the body weight of diabetic animals treated with all the doses of the extract increased (P<0.05) progressively in a manner similar to both the non-diabetic animals that received distilled water and the diabetic animals that was administered glibenclamide (Table 3). By the end of the experimental period, the percentage body weight produced by the highest dose of the extract (75 mg/kg body weight) was 14.90% compared to the 17.45% and 9.17% in the non-diabetic distilled water treated animals and diabetic animals that received 2.5 mg/kg body weight of glibenclamide respectively (Table 3).

Administration of alloxan significantly increased the levels of total cholesterol, triglycerides and low density lipoprotein-cholesterol whereas the serum high density lipoprotein-cholesterol decreased significantly in the serum of the animals (Table 4). In contrast, the extract reversed the levels of total cholesterol, triglycerides, high density lipoprotein-cholesterol and low density lipoprotein-cholesterol in a manner similar to both the non-diabetic animals administered distilled water and dia-

betic animals administered glibenclamide (Table 4).

The extract of *Senna alata* flowers significantly (P<0.05) reversed the elevated levels of urea, creatinine, total bilirubin, haemoglobin, as well as the reduced levels of albumin, conjugated bilirubin, packed cell volume, liver glycogen and hexokinase activity in the liver of the alloxan-induced diabetic rats (Table 5). The trends of reversal of these biomolecules compared favourably (P>0.05) with both the non-diabetic animals that were administered distilled water and the diabetic animals that received glibenclamide (Table 5).

Compared with the histology of the pancreas of non-diabetic rats administered distilled water (Plate A), alloxan administration induced atrophic changes in the histology of the pancreas (Plate B). These atrophic changes were normalized following the administration of the extract (Plates C-E) in a manner similar to the pancreas of diabetic rats treated with glibenclamide (Plate F).

4. Discussion

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for diabetes (Nayak et al. 2011) because they have chemical compounds and biological activities that produce definite physiological action in the body. Some plants have been used traditionally to treat diabetes and some have been proven scientifically and reported to have anti-hyperglycemic effects in chemically-induced diabetes using alloxan and streptozotocin.

Alloxan monohydrate, a beta- cytotoxin causes massive destruction of β -cells of the islets of Langerhans resulting in reduced synthesis and release of insulin (Szkudelski 2001). It is well established that during the evolution of diabetes mellitus in rats, after administration of alloxan, the function of the insulin system is suppressed, which is expressed by the resulting hyperglycemic condition (Szkudelski 2001). Therefore, the elevation of fasting

blood glucose in the animals following the administration of alloxan in the present study suggests a reduction in the synthesis and release of insulin. The reduction in the fasting blood glucose level by the extract which hitherto were progressively elevated by alloxan suggest anti-hyperglycemic effect of the extract. The mode of action of the extract as an anti-hyperglycemic agent might be via an extra pancreatic mechanism, stimulating the residual pancreatic beta-cell by increasing peripheral utilization of glucose, increasing glucose uptake by fat and muscle cells and slowing down glucose absorption from the gut. The exact mechanism of anti-hyperglycemic effects of this plant is largely unknown and remains speculative; therefore, further studies are required to unravel the pathways of its anti-hyperglycemic action. However, the findings in the present study with respect to the reduction in blood glucose level is similar to the previously published one by Shanmugasundaram et al. (2011) following the administration of *Senna auriculata* leaves to alloxan-induced diabetic rats.

Studies on anti-hyperglycemic activity of plants have identified compounds like flavonoids and alkaloids (Karawya and Wahab 1984; Szkudelski 2001), terpenoids

Table 3: Changes in the body weight of alloxan-induced diabetic rats following the administration of aqueous extract of *Senna alata* flower

Treatment Groups	Body Weight (g)		
	Before diabetic induction	After diabetic induction Day 7	After diabetic induction Day13
Non-Diabetic rats + Distilled water	141.69 ± 1.52 ^a	153.14 ± 0.58 ^a (8.08%)	166.42 ± 2.99 ^a (17.45%)
Diabetic rats + Distilled water	144.29 ± 0.91 ^a	135.35 ± 1.14 ^b (-6.20%)	122.28 ± 1.28 ^d (-15.25%)
Diabetic rats + 25 mg/kg body weight of extract	138.53 ± 1.82 ^a	134.65 ± 0.91 ^b (-2.80%)	141.64 ± 2.37 ^c (2.25%)
Diabetic rats + 50 mg/kg body weight of extract	140.99 ± 1.27 ^a	136.00 ± 0.64 ^b (-3.54%)	145.9 ± 1.79 ^c (3.48%)
Diabetic rats + 75 mg/kg body weight of extract	141.91 ± 0.96 ^a	152.98 ± 0.46 ^a (7.80%)	163.05 ± 1.65 ^a (14.90%)
Diabetic rats + 2.5 mg/kg body weight of glibenclamide	140.0 ± 0.65 ^a	135.44 ± 0.71 ^b (3.89%)	152.84 ± 2.18 ^b (9.17%)

n=7 ± S.E.M; Mean values down the column with superscripts, b, c and d, different from their respective controls, a, are significantly different (P < 0.05). Values in parentheses represent percentage

and tannins (Reher et al. 1991), steroids (Ivorra et al. 1989), glycoprotein (Hikino et al. 1989) and polypeptide (Khanna and Jain 1981) as the chemical compounds responsible for this action. Saponins stimulate insulin release from the pancreas; flavonoids are known to regenerate the damaged beta cells in the alloxan-induced diabetic rats and acts as insulin secretagogues (Geetha et al. 1994). Hyperglycemia causes oxidative damage by the

generation of reactive oxygen species and results in the development of diabetic complications. Phenolic and flavonoid compounds therefore play a role in reducing the complications of diabetes by exhibiting their antioxidant properties through scavenging radical species such as reactive oxygen species and or/ reactive nitrogen species,

Table 4: Effect of aqueous extract of *Senna alata* on the serum lipid profile of alloxan-induced diabetic rats

Treatment Groups	Serum total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL- (mg/dl)
Non-diabetic rats + Distilled water	169.32 ± 2.45 ^a	102.06 ± 5.00 ^{ab}	109.68 ± 4.31 ^a	49.61 ± 4.33 ^a
Diabetic rats + Distilled water	274.62 ± 3.24 ^b	148.82 ± 6.15 ^c	42.7 ± 1.86 ^b	199.89 ± 1.13 ^b
Diabetic rats + 25 mg/kg body weight of extract	174.66 ± 4.81 ^a	110.68 ± 5.67 ^{ab}	97.54 ± 8.58 ^a	73.51 ± 1.87 ^a
Diabetic rats + 50 mg/kg body weight of extract	170.08 ± 3.26 ^a	94.44 ± 6.30 ^a	99.76 ± 2.47 ^a	55.29 ± 5.65 ^a
Diabetic rats + 75 mg/kg body weight of extract	175.64 ± 4.98 ^a	105.42 ± 1.48 ^b	108.65 ± 6.12 ^a	48.08 ± 3.84 ^a
Diabetic rats + 2.5 mg/kg body weight of glibenclamide	167.90 ± 3.40 ^b	100.93 ± 4.57 ^b	105.27 ± 1.91 ^a	50.80 ± 5.31 ^a

n=7 ± S.E.M; Mean values for each parameter with superscripts different from their respective controls are significantly different (P

suppressing radical species formation by inhibiting some enzymes or chelating trace metals involved in free radical production and up-regulating or protecting antioxidant defense (Cotelle, 2001). Alkaloids on the other hand might exert anti-hyperglycemic activity by an extrapancreatic mechanism independent of insulin secretion (Punitha et al. 2006). Tannins have also been implicated to exhibit anti-diabetic activity through its glucose transport-stimulatory property (Liu et al. 2005). Although, there is the need by way of further experiment to shed more light on the anti-diabetic agent(s) in *S. alata* flower, the presence of flavonoids, alkaloids, saponins, phenolics and tannins among others may confer the anti-diabetic activity on *S. alata* flower unveiled in the present study.

Body weight is determined by the ratio of food intake into the body for the purpose of acquiring energy to energy expenditure. An imbalance between energy intake and energy expenditure results in a change in body weight. Alloxan administration decreased the body weight of the animals. The decrease in body weight in diabetic rats may be a consequence of a loss (or) degradation of structural proteins. Weight loss which is one of the clinical features

of diabetes mellitus may also be due to energy loss from the body due to frequent urination and overt conversion of glycogen, a storage carbohydrate to glucose (Reno and Leland 1999). Therefore, the increase in the body weight of the diabetic animals treated with the extract in the pres-

extract can restore one of the complications of diabetes. The trend of results in the present study with respect to body weight changes is similar to the report by Ahmed et al. (2005) following the administration of *Terminalia catappa* to animals induced into diabetes by alloxan.

Table 5: Effect of *Senna alata* flower on some biochemical parameters of alloxan-induced diabetic rats

Treatment Groups	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Serum Total Bilirubin (mg/dl)	Serum Conjugated bilirubin (mg/dl)	PCV (%)	Haemoglobin (g/dl)	Liver glycogen (mg/100mg)	Serum Albumin (mg/dl)	Liver Hexokinase (µg/min protein)
Non-diabetic rats + Distilled water	33.12 ± 2.69 ^a	0.70 ± 0.03 ^a	1.05 ± 0.14 ^a	0.75 ± 0.05 ^a	33.00 ± 0.58 ^a	11.20 ± 0.17 ^b	1.76 ± 0.04 ^a	1.36 ± 0.17 ^a	6.84 ± 0.70 ^a
Diabetic rats + Distilled water	85.88 ± 2.10 ^b	2.29 ± 0.14 ^b	2.45 ± 0.32 ^b	0.28 ± 0.02 ^b	28.10 ± 0.58 ^b	8.23 ± 0.20 ^a	0.42 ± 0.14 ^b	0.71 ± 0.05 ^b	4.40 ± 0.14 ^c
Diabetic rats + 25 mg/kg body weight of extract	36.80 ± 4.08 ^a	0.98 ± 0.05 ^c	1.15 ± 0.17 ^a	0.72 ± 0.07 ^a	32.20 ± 0.88 ^a	12.43 ± 0.29 ^c	1.29 ± 0.37 ^{ab}	0.93 ± 0.02 ^c	5.53 ± 0.41 ^b
Diabetic rats + 50 mg/kg body weight of extract	42.93 ± 6.10 ^a	1.00 ± 0.02 ^c	0.97 ± 0.16 ^a	0.79 ± 0.03 ^a	34.30 ± 1.67 ^a	11.03 ± 0.32 ^b	1.39 ± 0.57 ^{ab}	1.29 ± 0.13 ^a	6.35 ± 0.45 ^a
Diabetic rats + 75 mg/kg body weight of extract	34.05 ± 4.75 ^a	0.77 ± 0.03 ^a	1.17 ± 0.26 ^a	0.75 ± 0.07 ^a	35.30 ± 2.33 ^a	11.40 ± 0.23 ^b	1.28 ± 0.61 ^{ab}	1.49 ± 0.14 ^a	6.72 ± 0.31 ^a
Diabetic rats + 2.5 mg/kg of glibenclamide	30.50 ± 5.67 ^a	0.68 ± 0.03 ^a	1.22 ± 0.12 ^a	0.78 ± 0.02 ^a	34.00 ± 1.06 ^a	10.83 ± 0.53 ^b	1.77 ± 0.29 ^a	1.65 ± 0.06 ^d	6.61 ± 0.26 ^a

n=7 ± S.E.M; Mean values down the column with superscript different from their respective controls are significantly different (P < 0.05).

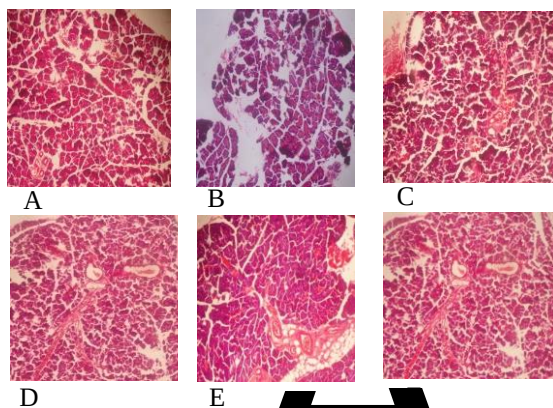


Figure 1: Photomicrograph of the cross section of the pancreas stained with hematoxylin and eosin (×100): A: Non-diabetic distilled water-treated rats; B: Diabetic animals treated with distilled water; C: Diabetic rats treated with 25mg/kg body weight of *S. alata* flower extract; D: Diabetic rats treated with 50 mg/kg body weight of *S. alata* flower extract; E: Diabetic rats treated with 75 mg/kg body weight of *Senna alata* flower extract; and F: Diabetic rats treated with 2.5 mg/kg body weight of Glibenclamide.

ent study suggest restorative effects of the extract on the defective glucose metabolism and impaired tissue breakdown. Such positive effect by the extract on the body weight of the diabetic animals indicates that the

Hypercholesterolemia, elevated level of low density lipoprotein cholesterol, hypertriglyceridemia and reduced level of high-density lipoprotein has been established as independent risk factors for diabetes (Smith 2007). Impaired glucose utilization associated with the administration of alloxan causes hyperglycemia and mobilization of fatty acids from adipose tissue for the production of energy (Jung and Choi 2014). However, treatment of diabetic animals with the aqueous extract of *S. alata* flower reduced the total cholesterol, LDL-cholesterol and triglyceride level in the diabetic animals while the HDL-cholesterol level increased. The findings in the present study with respect to the lipid profile which is similar to that of Sankaran and Vadivel (2011) on *Hibiscus rosasinensis* flower extract also suggests anti-hyperlipidemic potential for the aqueous extract of *S. alata* flower.

Kidney damage is usually associated with diabetes. In the initial course of the disease, the presence of hypertrophy of the glomeruli and tubular cells, matrix expansion, and enhanced renal flow causes loss of renal function which is evidenced by elevated levels of urea and creatinine (Omara et al. 2012). The increase in the urea and creatinine contents of the animals following the administration of alloxan might have been caused by an increase in the formation of advanced glycation end products accumulation of glycogen granules in the distal tubules (Nishikawa

et al. 2000; Kang et al. 2005). High levels of serum urea and creatinine in the blood are also markers of muscle wastage (Luo and Luo 2009). The reversal of urea and creatinine contents in the serum of diabetic animals by the extract of *S. alata* which compares well with the diabetic animals treated with glibenclamide in the present study suggest ameliorative effect of the extract on some complications of diabetes. All these consistently emphasize the antidiabetic activity of aqueous extract of *S. alata* flower. The findings with respect to urea and creatinine agree with previously published study by Daisy et al. (2007) on *Elephantopus scaber* extract.

The reduction in the serum content of albumin in diabetic animals and increase in bilirubin are not unexpected since hypoalbuminemia and hyperbilirubinemia has been described in several studies as part of the characteristics of diabetes complications in animals (Yakubu et al. 2010). The early sign of ongoing nephropathy is microalbuminuria; the presence of microalbuminuria predicts worsening of renal disease to overt diabetic nephropathy (Krolewski et al. 1995) and the elevated risk of cardiovascular disease in diabetic condition (Nielsen et al. 1997). The elevated levels of bilirubin suggests decreased liver uptake and conjugation or increased bilirubin production. The ability of the extract to progressively restore the levels of albumin and bilirubin in the present study corroborates the earlier position that the extract is capable of ameliorating complications arising from diabetes and can also reverse the progression of diabetic neuropathy.

Haemoglobin values are ubiquitous in clinical practice for the diagnosis of type 1 diabetes and also important for evaluating long-term glycaemic control in patients with type 2 diabetes (Czerwoniuk et al. 2011). During diabetes, the excess glucose in the blood reacts with haemoglobin to form glycosylated haemoglobin. Extracorporeal haemoglobin is thus elevated in diabetes due to increased red blood cell fragility resulting in a shorter red blood cell half-life (Venerando et al. 2002). Therefore, the decreases in the levels of hemoglobin and packed cell volume by the alloxan in the present study may be due to the aforementioned processes. However, the ability of the extract to restore this alloxan treatment related alterations in the haemoglobin and packed cell volume further support the anti-diabetic property of the extract and its tendency to restore diabetic complications with respect to these haematological parameters.

Liver is mainly responsible for maintaining normal concentration of blood glucose by its ability to store glucose as glycogen and to produce glucose from glycogen breakdown or from gluconeogenic precursors (Roden and Bernroider 2003). The selective destruction of pancreatic

β -cells by alloxan during experimental diabetes leads to decreased serum insulin level which results in defective glucose oxidation thereby causing hyperglycemia. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves the overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. These series of events therefore, could have accounted for the reduction in the glycogen content in the liver of diabetic rats. Fortunately however, all the doses of the extract reversed the levels of glycogen with the highest dose restoring it. This may suggest that the extract effectively promoted the synthesis of glycogen probably through modulatory effects on the activities of glycogen phosphorylase and glycogen synthase.

One of the key enzymes in the catabolism of glucose is hexokinase, the first enzyme of glycolysis which phosphorylates glucose by transferring a phosphoryl group from adenosine triphosphate to form glucose-6-phosphate. Hexokinase is both an insulin-dependent and an insulin-sensitive enzyme and is almost completely inhibited or inactivated in the diabetic rat liver in the absence of insulin (Gupta et al. 1997). Hexokinase insufficiency in diabetic rats can cause decreased utilization of glucose for energy production (Vats et al. 2003). The reduction in the activity of hexokinase by the alloxan in the present study suggest that the activity of the enzyme was partly inhibited or inactivated with its attendant consequences of impaired conversion or utilization of glucose by the glycolytic pathway to produce energy. The extract however induced the activity of the enzyme probably by *de novo* synthesis which will enhance further channelization of glucose into the glycolytic pathway. It may be that the extract exhibited a direct stimulation of glycolysis in tissues with enhanced removal of glucose from the blood (Punitha et al. 2006).

Histoarchitectural distortions of the pancreas are consistent with alloxan-induced diabetic rats. Although, many histological alterations might have been inflicted on the pancreas in the present study which were not captured due to the type of histological technique employed, the noticeable atrophic changes in the pancreas of the alloxan-induced diabetic rats could also be a pointer to the effect of alloxan. The absence of these atrophic changes in the alloxan-induced diabetic rats treated with the aqueous extract of *S. alata* flowers suggest a restorative effect of the extract on the architecture of the pancreatic cells. Such extract treatment restorative effects have been widely reported in studies employing alloxan as diabetogenic agent (Kameswararuro et al. 2003; Diasy et al. 2007).

5. Conclusion

The study has provided scientific evidence to the folkloric acclaimed use of aqueous extract of *Senna alata* flower in the management of diabetes. The antidiabetic activity which was most profound at the 75mg/kg body weight of the extract could be due to the presence of flavonoids,

alkaloids, saponins, phenolics and tannins among others acting via various mechanisms. However, work is still ongoing to address the specific bioactive agent(s) of anti-diabetes and its mechanism of action.

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