Research Article

Evaluation of the anti-diabetic potentials of *Albiza zygia* (DC.) stem barks in Alloxan-induced diabetic rats

J. O. Okafoanyali[1*], P. A. Akah[2], M. A. Omoirri[2], O. Onyekwere[3], C. Odiaka[1]

[1]*Biotechnology Research Centre, Nnamdi Azikiwe University, Awka, Anambra State*

[2]*Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*

[3]*National Biotechnology Development Agency, Abuja, Nigeria*

Received: 8 January 2020 Revised: 4 February 2020 Accepted: 28 February 2020

Abstract

Background: To date, there is paucity of data on the antidiabetic potentials of *Albiza zygia*. Though it has been reported to possess antidiabetic properties and is used locally in the management of diabetes. **Objective:** In view of this knowledge gap, the present study assessed the methanolic stem bark extract of *Albiza zygia* to evaluate its antidiabetic potentials in rat models. **Materials and Methods:** Methanolic extract of *Albizia zygia* (stem bark) was prepared by cold maceration for 48 hours and the crude extract was fractionated to obtain n-hexane, n-butanol and diethylether soluble fractions (HF, BF and DF) respectively. The crude extract and its fractions were screened to unveil the resident phytochemical constituents. The fractions (HF, BF and DF) and methanol extract were tested for antidiabetic effect on alloxan monohydrate induced diabetic rats. **Results and conclusion**: The results revealed the presence of alkaloid, tannin, saponin, flavonoid, terpenoids, cardiac glycoside, carbohydrate and reducing sugars in the extract while the oral LD_{50} was above 5000 mg/kg. The methanol extract was significantly ($P<0.05$) reduced the blood glucose level of diabetic rats in a dose dependent manner after 22 days treatment. The fractions at 800 mg/kg were significantly ($P<0.05$) reduced blood glucose after 6 hours. The potency of the fraction in reducing the blood glucose was in the order BF > HF > DF. The effect of the extract on the lipid profile, liver and kidney function of the diabetic rats was investigated. The extract was significantly ($P<0.05$) reduce the serum LDL, triglyceride and total cholesterol concentration and a significant ($p < 0.05$) increase in the serum HDL concentration. The serum AST, ALP and ALT concentration was significantly ($P<0.05$) reduced in all the groups. The dose of 800 mg/kg treated group was significantly ($P>0.05$) reduced the Urea and Creatinine concentration. Thus, the study lends support to the ethnomedicinal use of the plant in the management of diabetes.

***Keywords*:** Diabetes mellitus, hyperglycemia, *Albizia zygia*, alloxan monohydrate

Introduction

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). These complications can be acute or chronic in nature. Acute complications include diabetic ketoacidosis, coma or death. Chronic complications include chronic kidney failure, heart disease, stroke, foot ulcers and damage of the eyes. In 2004, an estimated 3.4 million people died from consequences of high fasting blood sugar (WHO, 2009) and more than 80% of diabetes deaths occur in low and middle-income countries (Mathars and Loncar, 2006). Basically, the amount of glucose in the blood is regulated by insulin and glucagon. The hormone insulin is produced in the beta cells of the pancreas and helps the body cells especially liver, muscle and adipose tissue, to take up glucose in the blood for conversion/storage into other forms of energy for metabolic processes. Plants form the main ingredients of medicines in traditional systems of healing and have been the source for several major pharmaceutical drugs. More than 80% of the world's rural

Address for Corresponding Author:
Ogochukwu James Okafoanyali
Biotechnology Research Centre, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
E-mail: oj.okafoanyali@unizik.edu.ng

DOI: https://doi.org/10.31024/apj.2020.5.1.1

settlers use traditional medicine in their primary healthcare needs, most of which use plants or their active principles (WHO, 2002). In Nigeria the use of plants resources mainly as herbal medicine, food, forage etc. has been a common practice and the *in vitro* and *in vivo* properties to microbial pathogens have been widely reported (Okafor, 2001; Iwalokun et al., 2004; Hashish and Gomaa, 2003).

Albizia zygia is a fast-growing, medium-sized deciduous tree with a spreading crown and a graceful architectural form. Its height ranges from 9 to 30 metres, and when growing in the forest, can produce a clean bole up to 15 metres tall. It is used locally in traditional medicine, and is also sometimes used for food and other commodities (Protabase, 2017). Albizia species are used in Indian folk medicine for treatment of several pathological conditions such as asthma, arthritis, antiseptic, burns, antidysentric, allergic rhinitis, bronchitis, paralysis and helminth infections. With the available hypoglycemic agents from plants and synthetic sources, diabetes mellitus and its further complications has continued to be a major ailment ravaging the world. The use of diabetic therapies to control hyperglycemia has limitation in terms of efficacy and tolerability. Most of these therapies have the tendency to cause increase in body weight, hypoglyceamia and only very few addresses the underlying defects such as obesity and insulin resistance. In Nigeria, the use of herbal medicine is in the increase and this can be attributed to its effectiveness, cost, availability and the fewer side effects compared to other forms of diabetic therapy. The aim of this study is to evaluate the antidiabetic properties of *Albizia zygia* stem bark.

Materials and Methods

Collection and identification of plant material

Fresh stem bark of the *A. zygia* was collected from a wood in Nsukka community and was identified and authenticated by Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka with reference number PCG/UNN/0302. The fresh stem bark was dried under shade for one month and was milled into coarse powder using a locally fabricated milling machine.

Sample preparation and extraction

About 2 kg of the powder was extracted with 5 L of methanol by cold maceration for 48 hours and filtered. The filtrate was concentrated in rotary evaporator to obtain the methanol extract (ME). The ME was further fractionated, using chromatographic techniques, with the following solvents in the order of increasing polarity viz n-hexane, diethylether and butanol.

Phytochemical analysis

The whole methanol extract and the individual solvent fractions were subjected to phytochemical investigation, using the method described by Harbone (1989). The test unveiled the resident Phytochemical such as alkaloids, saponins flavonoids, tannins, glycosides, resins, triterpenes, steroids, carbohydrates, fats and oil, and reducing sugars in the plant extract.

Experimental design

Male and female albino rats (100-130) g and mice were used for the study. The rats were purchased from the animal house of the Department of Pharmacology and Toxicology UNN. They had free access to food and water during the period of experimentation.

Determination of LD_{50}

The acute toxicity (LD_{50}) of the extract was determined in order to define the range of the lethal dose and the safe range for the extract. The methanol extract was administered in normal saline. The test was carried out in two phases as described by Lorke (1983) using a total of 13 mice of weight 25-32 g. In the first phase, the animals were divided into 3 groups of 3 mice each, and the extract was administered at three dose level (10, 100 and 1000 mg/kg) body weight. The animals were then monitored for 24 hours. Based on the results obtained from this first phase, the remaining animals were then grouped into 4 groups of 1 animal each for second phase of the test. In the second phase, 4 dose range were also used 800, 1600, 2900 and 5000 mg/kg body weight. Each dose was administered to one specific group only and the animals were examined again for another 24 hours.

Induction of diabetes mellitus

Alloxan monohydrate was used to induce diabetes in rats. Alloxan was first weighed and then solubilized with distilled water just prior to injection. Diabetes was induced by injecting a dose of 120mg/kg body weight intraperitonially (Szkudelski, 2001). The alloxanized rats were kept for 3 days for hyperglycemia to develop with free access to food and water. The rats were fasted on the 4th day for 12 hours and their blood glucose levels were determine using Accu check Glucometer (Model, ZH-G01). Rats with glucose levels of 200 mg/dl and above were recruited for the study.

Study of methanol extract on alloxanized rats for 22 days (three weeks)

Glycemic rats (blood glucose level >200mg/dl) were fasted for 12 hours but had access to water ad libitum. Five groups of 5 rats per group were used and were treated as follows

Group 1 200mg/kg

Group 2 400mg/kg

Group 3 800mg/kg

Group 4 2mg/kg

Group 5 2ml/kg distilled water

Blood samples were collected from the tail vein on the early hours of the 1st, 4th, 7th 10th 13th, 16th, 19th and 22nd day after 12 hours fasting and the blood glucose levels determine as well. The weights of the animals were also monitored alongside the blood glucose.

Study of different fractions on alloxanized rats for 6 hours

The glycemic rats were fasted for 12 hours but had access to water ad libitum throughout the experiment. In this section six groups of 5 rats per group were used and were treated as follows:

Group 1 800mg/kg of n-hexane fraction (HF)

Group 2 800 mg/kg of butanol fraction (BF)

Group 3 800 mg/kg diethyletherfraction (DF)

Group 4 800 mg/kg of methanol fraction (MF)

Group 4 2mg/kg Glibenclamide

Group 5 2ml/kg 10% Tween 80

Blood samples were collected from the tail vein at fixed time intervals of 0, 1, 2, 4, and 6 hours after the administration of the respective drugs and the blood glucose levels determine as well.

Determination of body weight

The body weights of the rats were recorded before treatment twice in a week.

Evaluation of biochemical parameters

On the 22nd day blood sample was collected from the orbital sinus of the rats into labeled blood sample bottles and were left to stand for about 30 minutes. The serum was carefully harvested after centrifuging for 10 minutes at 4000 rpm. ALT, AST, ALP, triglyceride, total cholesterol, HDL, LDL, urea and creatinine were determined enzymatically by standard methods using their specific RANDOX kits and measurement of optical density at the corresponding wavelength with a UV spectrophotometer (PEC MEDICAL)

Statistical analysis

Results are given as mean SEM (standard error of mean). One way ANOVA with post hoc LSD multiple comparison tests. P values of 0.05 and less were taken to imply statistical significance between the means. Analysis was done using statistical package for social sciences (SPSS) version 23.

Results

Acute toxicity (LD_{50}) of *A. zygia* (stem bark)

The LD_{50} of the extract was above 5000mg/kg as no death was recorded at the highest dose after 24hours monitoring. There was no sign of weakness, anorexia or restlessness observed within 24 hours monitoring.

Phytochemical screening of *A. zygia* (stem bark)

Phytochemical screening revealed the abundant presence of alkaloids, tannins, terpenoids, cardiac glycosides as well as reducing sugar in the methanol extract, with moderate presence of saponin in both methanol and n-butanol fractions (Table 1).

Effect on blood glucose

After 22 days treatment, the methanol extract 400 mg/kg and 800 mg/kg caused a significant ($P<0.05$) decrease in the blood glucose level when compared to the untreated control group. The percentage reduction of blood glucose of the treated rats after 22 days was 73 % for 800 mg/kg body weight and 53 % for 400 mg/kg body weight, while the standard drug gave a 76 % blood glucose reduction (Figure 1). The mean percentage reduction was dose dependent as can be seen in figure 2.

Table 1. Phytochemical constituent of *A. zygia* (stem bark)

Metabolites	Methanol	n-Butanol	n-Hexane	Diethylether
Alkaloids	+++	+++	-	++
Tannins	+++	-	++	-
Saponins	++	++	-	-
Flavonoids	+	+	-	-
Steroids	-	-	-	-
Terpenoids	+++	+++	+++	+++
Cardiac glycosides	+++	++	++	+++
Carbohydrates	+++	++	++	+++
Reducing sugars	+++	-	+	-

(-) = Not Present. (+) = Present in small concentration. (++) = Present in moderately high concentration. (+++) = Abundantly present

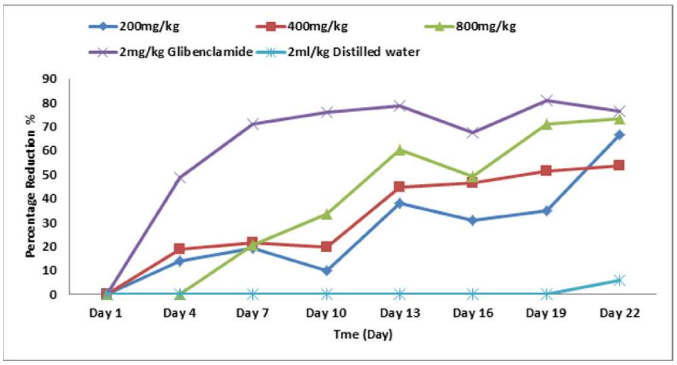

Figure 1. Effect of methanol extract on blood glucose of diabetic rats

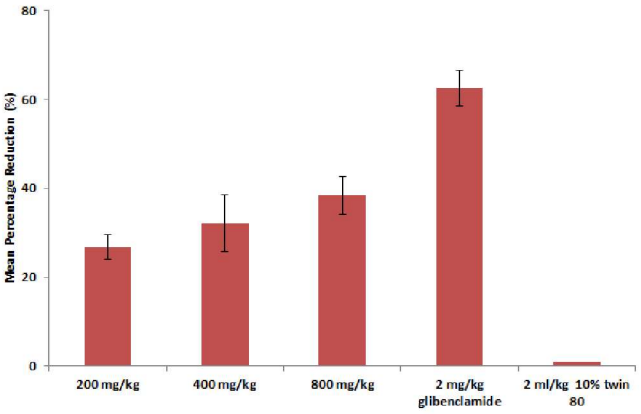

Figure 2. Mean percentage reduction of blood glucose of diabetic rats after 22 days. NB: Results are expressed as Mean±SD (n=5)

Chromatographic fractionation of the methanol extract afforded three fractions (butanol, diethylether and n-hexane fractions). These fractions and the methanol extract were subjected to 6 hours hypoglycemic test on the diabetic induced rats. The butanol fraction (Figure 3) significantly ($p<0.01$) reduced the blood glucose by 54.73 %, the hexane fraction and methanol

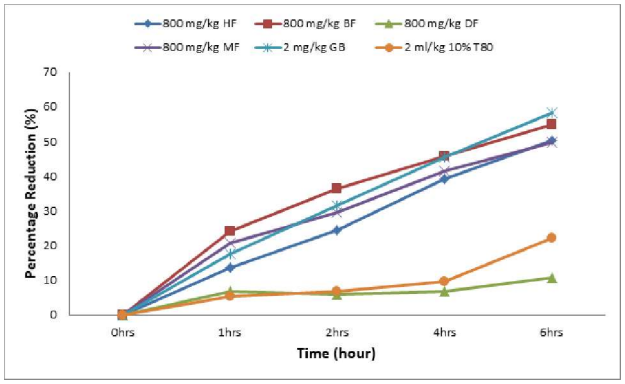

Figure 3. Effect of fractions of *A. zygia* on blood glucose of diabetic rats. NB: HF= Hexane fraction, BF=Butanol fraction, DF=Diethylether fraction, MF=Methanol fraction, GB: Glibenclamide, T80=Tween 80

extract significantly ($p<0.05$) reduced the blood glucose by 50.24 % and 49.81 % respectively after 6 hours.

Effect of methanol extract on body weight

The weight of the untreated control group steadily reduced, while the methanol extract (ME) treated groups maintained body weight (Table 2).

Effect of methanol extract on lipid profile

The serum levels of total cholesterol (TC), triglycerides (TGs), high density lipids (HDL) and low density lipid (LDL) of the control and treated rats are presented in table 3. The 800 mg/kg group significantly ($p < 0.05$) reduced the total cholesterol, triglyceride and LDL-cholesterol. There was also a significant ($p < 0.05$) increase in the HDL-cholesterol across all groups (Table 3).

Effect of extract on liver enzymes and kidney functions

The effect of the extract on the liver enzymes is shown in table 4. The extract lowered the AST, ALT and ALP level

Table 2. Effect of extract on body weight of diabetic rats

Treatment	Body Weight (g)							
	Day 1	day 4	Day 7	Day 10	Day 13	Day 16	Day 19	Day 22
ME- 200mg/kg	109.85±4.20	112.18±3.59	113.975±4.07	117.73±5.85	120.83±5.85*	124.65±7.26*	133.10±5.85*	128.08±4.31*
ME- 400mg/kg	108.38±2.94	107.74±3.03	108.64±3.72	110.22±3.60	111.50±4.81	111.10±6.2	118.18±6.10*	118.80±5.50*
ME- 800mg/kg	110.73±4.04	112.53±3.83	115.5±3.81	116.75±3.70	117.35±3.47	120.40±4.09*	125.90±3.11*	128.25±2.62*
Glibenclamide (2mg/kg)	118.80±4.71	121.6±4.27	125.67±5.30	130.57±4.60	135.27±4.51	142.47±7.50	152.67±6.42	165.47±5.64
Distilled water (2 ml/kg)	110.40±4.33	109.13±5.83	108.35±6.82	108.75±8.62	107.45±7.97	106.23±8.96	107.53±9.66	104.55±10.03

Results are expressed as Mean±SD (n=5). Means within respective columns followed by *asterisk* are significantly different from the control at $p < 0.05$. ME: Methanol Extract

Table 3. Effect of methanol extract on lipid profile of diabetic rats

Treatment	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)
ME- 200mg/kg	104.00±1.98	118.40±12.75*	58.23±0.86*	22.17±1.39
ME- 400mg/kg	107.80±4.93	160.00±11.36	61.51±1.96*	20.74±1.53*
ME- 800mg/kg	91.41±4.39*	118.37±9.14*	57.50±1.12*	17.20±1.26*
Glibenclamide (2mg/kg)	97.30±3.20	129.80±6.57	64.25±1.18	12.19±0.98
Distilled water (2 ml/kg)	105.90±2.15	147.80±3.62	51.58±1.01	24.73±1.45

Results are expressed as Mean±SD (n=5). Means within respective columns followed by *asterisk* are significantly different from the control at $p < 0.05$.

Table 4. Effect of extract on liver enzymes levels of diabetic rats

Treatment	AST (U/L)	ALT (U/L)	ALP (IU/L)
ME- 200mg/kg	22.07±1.21*	14.29±1.17*	60.02±3.67*
ME- 400mg/kg	24.86±1.78	18.00±0.59*	61.36±4.47*
ME- 800mg/kg	21.41±0.92*	13.22±0.64*	34.77±3.64*
Glibenclamide (2mg/kg)	21.84±0.98	14.37±1.18	30.00±3.27
Distilled water (2 ml/kg)	25.96±0.68	21.53±1.21	77.46±5.49

Results are expressed as Mean±SD (n=5). Means within respective columns followed by *asterisk* are significantly different from the control at $p < 0.05$.

Table 5. Effect of methanol extract on kidney function of diabetic rats

Treatment	Urea (mg/dl)	Creatinine (mg/dl)
ME- 200mg/kg	87.31±21.66	3.852±0.17*
ME- 400mg/kg	64.03±3.17	6.026±0.49
ME- 800mg/kg	48.4±11.94*	4.70±0.35*
Glibenclamide (2mg/kg)	47.7±7.70	4.79±0.40
Distilled water (2 ml/kg)	68.68±5.01	5.58±0.23

Results are expressed as Mean±SD (n=5). Means within respective columns followed by *asterisk* are significantly different from the control at $p < 0.05$

significantly ($p < 0.05$). There was no significant ($P>0.05$) effect in the levels of urea and creatinine at the doses of 200 mg/kg group, however at 800 mg/kg there was a significant ($p < 0.05$) reduction in the urea and creatinine levels (Table 5).

Discussion

Diabetes is the World's largest growing metabolic disorder, and as the knowledge of the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (Baily and Flat, 1986). People suffering from diabetes are increasing in number due to population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity (King and Rewers, 1993; Ramchnadran et al., 1999). Side effects produced by synthetic anti-diabetic drugs (Larner, 1999) and the high cost of these drugs has created a gap in modern day treatment of the disease, hence the research into finding effective, safe and affordable alternatives in treatment of diabetics mellitus. All over the world, traditional plant medicine has proven to be effective in alienating diabetic complications (Mahamed et al., 2006).

Alloxan causes tissue injury by inducing free radical production. This alloxan induced free radicals can cause damage to the pancreas (Akah et al., 2011) leading to hyperglycemic condition. Plants rich in flavonoids, terpenoids, alkaloids, and glycosides have antioxidant activity and claimed to possess antidiabetic effect. Flavonoids present in the plant regenerates the damaged beta cells of pancreases, and the polyphenolic compounds and saponin present in the plants inhibit glucose transport by inhibiting sodium glucose co-transporter-1 (S-GLUT-1) in intestine (Hakkim et al., 2001, Tiwari et al., 2007). Phytochemical screening of the methanol extract of *A. zygia* and its fractions revealed the presence of various secondary metabolites, some of which have been reported to possess anti-diabetic properties. Saponins in *Citrullus colocynthis* has been reported to cause a remarkable decrease in blood glucose level in alloxan induced diabetic rats (Abdel-Hassan et al., 2000). Flavonoid fraction isolated from *Pterocarpus marsupium* degranulates pancreatic beta cell, epicatechin, its active principle, has been found to enhance insulin release and converts proinsulin to insulin in vitro

(Modak et al., 2007).

In the present study, the Methanol Extract of the plant reduced elevated blood glucose significantly (P<0.05) when compared to the negative control (Figure 1). This effect represented in the mean percentage reduction value (Figure 2), indicates that the extract acted in a dose dependent manner (200 mg/kg < 400 mg/kg < 800 mg/kg). Ayodhya and Kusum (2010) reported that the possible mechanisms through which the extracts might inhibit blood glucose lowering effect were either by improved glucose homeostasis (increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes, inhibition of intestinal glucose absorption, reduction of glycaemic index of carbohydrates, reduction of the effect of glutathione or by direct stimulation of glucose uptake through increased insulin secretion, it may also be due to the extracts stimulating ß cells in islet of Langerhans, thus increasing serum insulin (Chattopadhyay, 1993), in studying the effect of *Azadirachta indica* on leaf extract on hepatic glycogen in rats suggested that the possible mechanism by which *Azadirachta indica* modulated blood glucose could be to block the inhibitory effect of serotonin on insulin secretion/release in pancreas of rats mediated by glucose. In addition, an increased dosage or consumption of the plant has not been reported to cause hypoglyceamia as compared to administration of insulin or other antidiabetic agents and in this study after 22 days of administration, none of the rats were hypoglycemic.

Alloxan-induced diabetes is associated with the characteristic loss of body weight which is due to increased muscle wasting and due to loss of tissue proteins (Swaston-Flat, 1990). The loss in body weight was prevented by the extract, which indicates the prevention of muscle tissue damage due to hyperglycemic condition (Table 2). This suggests that *A. zygia* was able to suppress the body weight loss due to muscles wasting. This finding is similar to previous studies involving the use of glibenclamide in the treatment of diabetes (Akbarzadeh et al., 2007; Daye et al., 2013; Muhammad and Syed, 2010).

The liver plays an important role in xenobiotic function and the kidneys are the main organs involved in drugs elimination and are therefore particularly exposed to the toxic effects of exogenous compounds (Bidhe and Ghosh, 2004). The transaminases (AST and ALT) are useful enzymes as biomarkers predicting possible liver toxicity (Rahman et al., 2001). Any damage to the parenchymal liver cells will result in elevations in both transaminases (Wolf et al., 1972). The increased AST and ALT activities could also be attributed to the abnormality in protein and carbohydrate metabolism and formation of urea in diabetic rats. Moreover, loss of insulin effect on the liver leads to glycogenolysis with high hepatic glucose production, which may enhance the increase in AST and ALT (Begum and Shanmugasundaram, 1978). Therefore, the toxicity of an extract can be interpreted according to the increase/decrease in AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and total bilirubin levels (Sahil et al., 2013). The extract significantly ($p < 0.05$) reduced the AST, ALT and ALP levels (Table 4). This result is similar to the findings of Ali et al., (2017), in antidiabetic properties of *Garcinia pedunculata* in Rats, suggesting that the plant may possess hepatoprotective properties.

In diabetes, elevated levels of serum urea and creatinine are observed, which may be due to renal damage caused by abnormal glucose regulation or elevated glucose and glycosylated protein tissue levels (Lal et al., 2009). However, at 800 mg/kg the extract reduced the level of urea significantly ($p > 0.05$). A significant reduction of creatinine can also be observed in the 800 mg/kg and 200 mg/kg of the extract treated rats (Table 5). A similar result was observed in the study of Antidiabetic and antihyperlipidaemic activity of ethanol extract of *Melastoma malabathricum* Linn. Leaf in alloxan induced diabetic rats (Balamurugan, 2014). This suggests the presence of kidney-protective activities in the plant.

Conclusion

The results of this present research have shown that the stem bark of *A. Zygia* possesses anti-diabetic activity in rat diabetic models. This justifies its use in traditional medicine for the treatment and management of diabetic diseases. The anti-hyperglycemic effects can be attributed to the presence of secondary metabolites (steroids, terpenoids, saponins, flavonoids and alkaloids) in the plant. The Methanol extract possessed some hepatoprotective and nephroprotechtive properties according to the result and can be a good alternative to drugs with adverse effect in management of diabetes. The study also showed that the stem bark of *A. zygia* is safe even at high doses as the LD_{50} was above 5000 mg/kg. The best fraction from the study in lowering of blood glucose is the butanol fraction.

Recommendation

The anti-diabetic properties of the plant's leaves as well as its root should be explored, as well, further studies towards isolating, purifying and characterizing these bio-active phytoconstituents, which could serve as lead molecules for the development of better anti-diabetic agents.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abdel-Hassan IA, Abdel-Bary JA, Tariq, MS. 2000. The hypoglycemic and anti-hyperglycemic effect of Citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. Journal of Ethnopharmacology 71 (1-2): 325-330.

Akah PA, Uzodinma SU and Okolo CE 2011. Antidiabetic activity of aqueous and methanol extract and fractions of Gongronema latifolium (Asclepidaceae) leaves in Alloxan Diabetic Rats. Journal of Applied Pharmaceutical Science 01 (09), 99-102.

Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi SH, Farhangi A, Allah VA, Mofidian SMA, Lame Rad B. 2007. Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry 22(2):60-64.

Ali MY, Paul S, Tanvir EM, Hossen MS, Rumpa NE, Saha M, Khalil MI. 2017. Antihyperglycemic, Antidiabetic, and Antioxidant Effects of Garcinia pedunculata in Rats. Evidence-Based Complementary and Alternative Medicine : eCAM, 2017, 2979760. http://doi.org/10.1155/2017/2979760

Ayodhya S, Kusum S, Anjali S. 2010. Hypoglycaemic activity of different extracts of various herbal plants. International Journal of Research in Ayurveda and Pharmacy, 1(1): 242 – 224.

Baily CJ, Flatt PR. 1986. Antidiabetic drugs, new development. Indian Journal of Biotechnology, 6: 139-142.

Balamurugan K, Nishanthini A, Mohan VR. 2014. Antidiabetic and antihyperlipidaemic activity of ethanol extract of Melastoma malabathricumLinn. Leaf in alloxan induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine 4 (Suppl 1), S442–S448. http://doi.org/10.12980/APJTB.4.2014C122

Begum N, Shanmugasundaram KR. 1978. Transaminases in experimental diabetes. Arogya Journal of Health Sciences 4:116-22.

Bidhe RM, Ghosh S. 2004. Acute and sub chronic (28-Day) oral toxicity study in rats fed with novel surfactants. AAPS Pharmacological Sciences 6 (2): 1-10.

Chattopadhyay RR, Chattopadhyay RN, Maitra SK. 1993. Effect of Azadirachta indica leaf extract on hepatic glycogen in rats. Indian Journal of Pharmacology 25: 174-175.

Daye C, Bin L, Yunhui L. 2013. Antihyperglycemic effect of Ginkgo biloba extract in streptozotocin-induced diabetes in rats. BioMed Research International 162724, p.7.

Hakkim FL, Girija S, Kumar RS, Jalaluddeen MD. 2001. Effect of aqueous and ethanol extracts of Cassia auriculata L. flowers on diabetes using alloxan induced diabetic rats. International Journal Diabetes and Metabolism 15:100–106.

Hashish MN, Gomaa NF. 2003. The inhibitory effects of garlic (Allium sativa) on growth of some microorganisms. Journal of Egyptian Public Health Association 78(5-6): 361-72.

Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi OJ. 2004. In-vitro antimicrobial properties of aqueous garlic extract multi-drug resistant bacteria and Candida species from Nigeria. Journal of Medicinal Food 7(3): 327-333.

King H, Rewers M. 1993. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults: WHO Ad Hoc Diabetes Reporting Group Diabetes Case 16: 157 -177.

Lal SS, Sukla Y, Singh A, Andriyas EA, Lall AM. 2009. Hyperuricemia, high serum urea and hypoproteinemia are the risk factor for diabetes. Asian Journal of Medical Sciences 1:33–34.

Larner J. 1999. Insulin and oral hypoglycaemic drug. Glucogan In: Gilman AG, Goodman LS, Rall IW, Murad F editors. The pharmacological Basis of Therapeutics. 10th ed. New York: Macmillian. pp. 1490-1516.

Mahamed B, Abderrahrim Z, Hassane M, Abdelhafid T, Abdelkhaleq L. 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). International Journal Diabetes and Metabolism 14: 1-25.

Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul A, Devasagayam T. 2007. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of Clinical Biochemistry and Nutrition 40 (3): 163-173.

Muhammad Z, Syed NH. 2010. Effects of STZ-induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. International Journal of Morphology 28(1):135-142.

Rahman MF, Siddiqui MK, Jamil K. 2001. Effects of Vepacide (Azadirachta indica) on aspartate and alanine aminotransferase profiles in a sub chronic study with rats. Journal of Human and Experimental Toxicology 20(5): 243-249.

Sahil T, Hitesh V, Jagan P, Naya G, Nitesh K, Anoop K, Punit B, Rekha RS, Krishnadas N. 2013. Toxicological evaluation of *Terminalia paniculata* bark extract and its protective effect against CCl_4–induced liver injury in rodents. BMC Complementary and Alternative Medicine 13(1): 127.

Tiwari AK, Rao JM. 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Current Science 83:30–38.

WHO. 1999. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications.WHO/NCD/NCS/99.2 pg 2. http://www.who.int/iris/handle/10665/66040. Retrieved: March 2017.

WHO. 2002. Traditional Medicines strategy 2002-2005.WHO/EDM? TRM/2001. http://www.wpro.who.int/health_technology/book_who_traditional_medicine_strategy_2002_2005.pdf.

WHO. 2013. Diabetes Fact sheet N° 312.https://web.archive.org/web/20130826174444/http://www.who.int/mediacentre/factsheets/fs312/en/ Retrieved: June 2017.

Wolf PL, Williams D, Tsudaka T, Acosta L. 1972. Methods and Techniques in clinical chemistry. 605 third ave., New York, NY 10016, pp. 145.

WORLD Health Organization. 2009. Global health risks. Mortality and burden of disease attributable to selected major risks. Geneva.

World Health Organization. 2016. Global report on diabetes.http://www.who.int/diabetes/global-report. Retrieved: June 2017.

World Health Organization. *(2006).* Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF Consultation. *Geneva: p. 21.* ISBN 978-92-4-159493-6.

World Health Organization. Global Physical Activity Questionnaire (GPAQ) Analysis Guide.http://www.who.int/chp/steps/resources/GPAQ_Analysis_Guide.pdf.Retrieved: June 2017.