ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 8, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF ANTIDIABETIC AND ANTIOXIDANT EFFECTS OF ETHANOLIC LEAF EXTRACT OF *ERYTHRINA ABBYSINICA* LAM, EX DC

DAVIE REXON KAMADYAAPA[1]*, MAVUTO MASOPERA GONDWE[1], MATHULO SHAULI[1], CONSTANCE SEWANI-RUSIKE[1], BENEDICTA NKEH-CHUNGAG[2]

[1]Department of Human Biology, Faculty of Health Sciences, Walter Sisulu University, Private Bag X1, Mthatha 5117, Republic of South Africa. [2]Department of Biological Sciences, Faculty of Sciences, Walter Sisulu University, Private Bag X1, Mthatha 5117, Republic of South Africa. Email: dkamadyaapa@yahoo.co.uk

Received: 11 December 2017, Revised and Accepted: 03 May 2018

ABSTRACT

Objective: This study was conducted to scientifically evaluate the antidiabetic and antioxidant effects of ethanolic leaf extract of *Erythrina abbysinica* (EEA).

Methods: Acute and sub-chronic effects of EEA at 100, 200, and 400 mg/kg/bwt and glibenclamide (GL) at 5 mg/kg/bwt. were evaluated in both normal and streptozotocin (STZ)-induced diabetic male Wistar rats (250–300 g). The acute studies were performed using oral glucose tolerance test (OGTT). In sub-chronic studies, animals were orally administered with EEA and GL daily for 6 w. Brine shrimp assay was used to determine the toxicity of EEA. 1, 1-diphenyl-2-picrylhydrazyl, ferric reducing capacity of plasma, and thiobarbituric acid reactive substances assays were used to determine antioxidant properties of EEA.

Results: Following OGTT, EEA significantly ($p<0.05$) and dose-dependently (100, 200, and 400 mg/kg/bwt) decreased blood glucose levels in both normal and STZ-induced diabetic rats when compared with positive and negative control counterparts at all-time points, whereas GL significantly ($p<0.05$) decreased blood glucose only in normal rats but not in diabetic rats. Daily, oral administration of EEA for 6 w significantly ($p<0.05$) and dose-dependently (100, 200, and 400 mg/kg/bwt) decreased blood glucose levels in STZ-induced diabetic rats when compared with the diabetic control group. EEA revealed weak toxicity with a lethal concentration$_{50}$ value of 997 µg/ml). Furthermore, EEA showed significant free radical scavenging, total antioxidant, and anti-lipid peroxidative capacities.

Conclusion: The study has shed more light on the scientific basis for the use of *E. abbysinica* in management of diabetes in some communities of Eastern Cape of South Africa.

Keywords: *Erythrina abbysinica*, Antidiabetic, Antioxidant, Acute, Sub-chronic, Brine shrimp.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i8.24207

INTRODUCTION

Diabetes mellitus is a noncommunicable disease and is considered to be one of the five leading causes of death worldwide [1]. Diabetes mellitus describes a group of chronic metabolic disorders characterized by persistent hyperglycemia and glucose intolerance, and is due to insulin deficit, resistance or to a combination of these two [2]. The world has experienced a dramatic increase in the prevalence of diabetes mellitus among all age groups. For instance, it was estimated that over 387 million of the world's population is affected by diabetes and this figure is projected to increase to 552 million by the year 2030 [3]. In Sub-Saharan Africa, it was estimated that 12.1 million people were living with diabetes mellitus in 2010, and this figure is projected to increase to 23.9 million by 2030 [4]. This steady increase in the prevalence of diabetes is largely attributable to urbanization with increased associated changes in lifestyle risk factor levels such as sedentary lifestyle, obesity, smoking, excessive intake of alcohol and unhealthy diets [5]. Diabetes causes significant morbidity and mortality due to complications that are associated with this disease. Diabetic-related morbidity and mortality are caused by microvascular (peripheral neuropathy retinopathy and nephropathy) and macrovascular (atherosclerosis and myocardial infarction) disorders [6]. It is, therefore, clear that diabetes continues to present a major challenge to health-care systems worldwide.

Much emphasis on diabetes mellitus care and management is on optimal blood glucose control, and this is fundamental to delaying its progression and averting adverse metabolic outcomes [7]. Massive efforts have been made during the past decades to combat this predicament by identifying drugs that can treat this disease and promoting some non-pharmacological treatments such as lifestyle modification. However, despite the technological and scientific advances made toward the treatment of diabetes, the challenge remains overwhelming, as some of the available synthetic remedies have adverse side effects such as causing hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhea while some are beyond the reach of the poor communities due to high costs [8-10]. In addition, there are relatively high levels of nonadherence in all areas of non-pharmacological treatments, since this usually requires changes in the patient's daily lifestyle [11]. Alternative methods of lowering blood glucose are, therefore, urgently needed. Medicinal plants with antidiabetic properties play an important role in the management of diabetes mellitus by delaying the development of complications associated with diabetes and also by correcting metabolic abnormalities [12]. Traditional herbal remedies have been used and continue to be a reliable source of treatment of diabetes worldwide, and many scientific studies have reported the benefits of traditional medicinal plants with anti-hyperglycemic properties [13-15]. Moreover, some of the newly discovered bioactive drugs derived from anti-hyperglycemic plants have revealed to be more potent than conventional oral hypoglycemic agents [16]. There is, therefore, a need to intensify the search for more affordable and effective classes of compounds from medicinal plants with little or no adverse effects.

E. abbysinica (Ea) is one of the most popularly and commonly used medicinal plants in the traditional medicine by several communities

in the management of many diseases including diabetes mellitus. Ea is a flowering plant belonging to the genus Erythrina in the pea family, Fabaceae. *Ea* is common and native to South Africa, especially in the Eastern Cape. The plant grows in wooded grassland as well as open-wooded and rocky hillsides at an altitude between 800 and 1750 m and flowers between the month of July and October [17].

Several biological activities of this plant have been reported. For instance, extracts obtained from this plant are used for the treatment of several diseases such as meningitis, malaria, allergy [18] elephantiasis, trachoma, syphilis, burns, and swellings [19]. Despite its long traditional use in diabetes, there is very little scientific information regarding its potential antidiabetic properties. One isolated study reported the blood glucose-lowering effect of aqueous stem-bark extract of Ea in alloxan-induced diabetic mice and the phytochemical studies of this aqueous stem bark extract revealed the presence of saponins, flavanols, flavones, flavonoids, chalcones, and tannins, which may be responsible for the antihyperglycemic effect of this plant [20]. However, the antidiabetic activity of the leafy parts of Ea. has not been reported before. Therefore, the present study investigated the antidiabetic effect of 70% ethanolic leaf extract of Ea (EEA) in normal and streptozotocin (STZ)-induced diabetic rats. Oxidative stress, an imbalance between free radical generating system and natural antioxidant defense system, is intimately associated with diabetes and is responsible for the development of a myriad of complications of this chronic disease [21]. With this in mind, the study also investigated the potential antioxidant properties of EEA.

METHODS

Plant material

Fresh mature leaves of Ea were collected from within Walter Sisulu University, Mthatha Campus. The plant was identified and authenticated by a taxonomist of the herbarium section in the Department of Biological Science, Walter Sisulu University, Mthatha Campus.

Preparation of EEA

The leaves of the plant were air-dried under shade at room temperature. The dried leaves were milled to a powder using a commercial blender. A total of 832.63 g of the powdered material was mixed with 5 l of 70% ethanol in a conical flask. The mixture was allowed to stand for 2 d at room temperature with constant stirring using (labcon) platform shaker machine. After 2 d, the mixture was filtered using Whatman no.1 filter paper in *Büchner* funnel. The filtrate was concentrated under reduced pressure using rotary evaporator at 50 rpm and a bath temperature of 55°C. The semi-solid concentrated extract was subsequently evaporated to dryness in an oven at 40°C to obtain a sticky, green crude extract which was designated to be EEA and was kept in a refrigerator until the day of experiments.

Animals

Male Wistar rats (250–300 g) supplied by Shalom laboratories and housed in the storage facility in the Department of Physiology, Walter Sisulu University were used in the study. The animals were acclimatized to the laboratory conditions for 2 w, before the commencement of the experiment and were maintained at an ambient temperature of 25±2°C and 12 h light/12 h dark regime. Animals were given standard rat pellet chow (SafMed, SA) and water *adilibitum* and were kept under standard environmental conditions. The experimental protocol was approved by the Faculty of Health Sciences Research Ethics Committee, Walter Sisulu University. Animals were handled in compliance with the Guide for the care and use of Laboratory Animals (NIP Publication No. 85(23), revised in 1996).

Induction of diabetes mellitus

Groups of diabetic rats were rendered diabetic by a single intraperitoneal injection of (STZ, 60 mg/kg bwt) freshly prepared in citrate buffer at pH 4.5 after base-line blood glucose estimation was performed. Control groups were treated with the equivalent volume of vehicle, citrate buffer. After 72 h, animals with blood glucose concentration of >150 mg/dl were considered diabetic and selected for the study.

Experimental design

The toxicity test was done using Brine Shrimp lethality bioassay. Acute studies of EEA were carried out in both normal and STZ-induced diabetic rats while the sub-chronic studies of EEA were performed in STZ-induced diabetic rats. The antioxidant effect of EEA was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing capacity of plasma (FRAP), thiobarbituric acid (TBA) reactive substances (TBARS) assays.

Acute effects of EEA on blood glucose in normal rats

A total of 30 rats were randomly allocated into five groups with six rats per group as follows:

Group 1: Nondiabetic control treated with vehicle (1 ml).
Group 2: Nondiabetic treated with EEA (100 mg/kg bwt).
Group 3: Nondiabetic treated with EEA (200 mg/kg bwt).
Group 4: Nondiabetic treated with EEA (400 mg/kg bwt).
Group 5: Nondiabetic treated with glibenclamide (GL) (5 mg/kg bwt).

Acute effects of EEA on blood glucose in STZ-induced diabetic rats

30 rats were randomly allocated into five groups with 6 rats per group as follows:

Group 1: Diabetic-control treated with vehicle (1 ml).
Group 2: Diabetic treated with EEA (100 mg/kg bwt).
Group 3: Diabetic treated with EEA (200 mg/kg bwt).
Group 4: Diabetic treated with GL (400 mg/kg bwt).
Group 5: Diabetic treated with GL (5 mg/kg bwt).

Sub-chronic effects of EEA on blood glucose in STZ-induced diabetic rats

36 rats were randomly allocated into 6 groups with 6 rats per group as follows:

Group 1: Normal-control treated with vehicle (1 ml).
Group 2: Diabetic-control treated with vehicle (1 ml).
Group 3: Diabetic treated with EEA (100 mg/kg bwt).
Group 4: Diabetic treated with EEA (200 mg/kg bwt).
Group 5: Diabetic treated with GL (400 mg/kg bwt).
Group 6: Diabetic treated with GL (5 mg/kg bwt).

Oral glucose tolerance test (OGTT) protocol for acute studies

Animals were fasted for 18 h and initial blood glucose measurements were done at time 0 min to establish the baseline. After blood glucose, baseline values were determined, the animals were administered with various doses of EEA (100, 200, and 400 mg/kg. bwt). 30 min after administration of various doses of EEA, the animals were subsequently challenged with an oral glucose load at 0.86 g/kg bwt. Blood glucose measurements were taken at a 30 min interval for 6 h. Separate groups of nondiabetic and STZ-induced diabetic rats were treated with GL at a dose of 5 mg/kg bwt. to compare with the synthetic antidiabetic drugs already in clinical use.

Determination of blood glucose concentrations

Blood samples were collected from the tail veins of the animals and blood glucose was measured using Bayer's Glucometer Elites (Elite (Pty) Ltd., Health Care Division, Isando, South Africa).

Determination of cytotoxicity of EEA

The cytotoxic effect of EEA was conducted using Brine shrimp lethality bioassay according to Meyer *et al.* with slight modification [22]. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a rectangular vessel, containing 2 l of sterile artificial seawater (prepared using NaCl 38 g/L) under constant aeration for 48 h, with artificial light shone on the opposite side of the rectangular hatchery. After hatching was achieved, active *nauplii* were collected from a brighter portion of the hatchery and used for the assay. Ten *nauplii* were drawn through a glass capillary and placed into vials containing 4 ml of brine solution for the control, and for the experimental groups, 10 *nauplii* were placed in 4 ml of brine solution. The vial for the control group contained brine solution only, whereas the vials for experimental groups contained brine solution and EEA of various concentrations (1000 µg/ml, 500 µg/ml,

250 µg/ml, 125 µg/ml, 62.5 µg/ml, and 31.25 µg/ml) with 6 duplicates per concentration. The mixtures were maintained at room temperature under constant light, and a 24 h survival larvae count was recorded. The mortality rate was determined as follows: % deaths = [(Total nauplii – Alive nauplii) × 100%]/Total nauplii.

Lethal concentration (LC_{50}) determinations

The median LC_{50} in µg/ml, 95% confidence interval and the slope was determined after the 24 h count using the probit statistical analysis method [23]. LC_{50} is indicative of the toxicity level of a given compound to the brine shrimp. LC_{50} value of >1000 µg/ml is considered nontoxic, value ≥500 µg/ml and ≤1000 µg/ml is considered weak toxic and value <500 µg/ml is considered toxic [24].

Determination relative organ weights

At the end of 6 w of subchronic studies, rats from each group were sacrificed by cervical dislocation. The abdomen was opened using dissecting kits, and the liver and pancreas were removed, cleaned, and weighed and their relative body weights determined [25].

Blood sample collection for biochemical analysis

At the end of 6 w of sub-chronic studies, rats from each group were sacrificed by cervical dislocation after a 24 h fasting. Blood samples were collected by cardiac puncture technique. The blood samples were centrifuged at 3000 rpm for 15 min (Eppendorf® centrifuge 5804), thereafter serum was collected and stored at −76°C (Skadi® Green Line) until the day of biochemical analysis.

Determination of free radical scavenging activity of EEA

The potential free radical scavenging activity of EEA was determined according to the method described by Turkoglu *et al.* with a slight modification [26]. In brief, 1 ml of 0.2 mM DPPH solution in methanol was added to test tubes containing 1 ml of the prepared EEA at various concentrations ranging from 10 to 320 µg/ml. The test tubes covered in aluminum foil were then shaken vigorously before incubated in the dark for 30 min at room temperature. Ascorbic acid was used under similar conditions as a standard control antioxidant. The absorbance was measured against blank at 517 nm using a spectrophotometer. The free radical scavenging activity was calculated according to the following formula:

% radical scavenging activity = [(Absorbance of control - absorbance of sample)/Absorbance of control] × 100.

Determination of total antioxidant capacity

The antioxidant capacity of EEA was determined in serum by using the FRAP assay according to the method described by Nair *et al.* [27]. This assay measures the ability of an antioxidant to reduce the Fe^{3+} complex of tripyridyltrazine Fe (TPTZ) $^{3+}$ in acidic medium, to an intense blue colored ferrous complex $Fe(TPTZ)^{2+}$ [28]. A spectrophotometer was used to read the absorbance at 593 nm. The results were expressed as micromolar relative antioxidant standard (ascorbic acid) equivalents [29]. A standard curve was plotted, and a value on the straight portion of this curve was used in extrapolating antioxidant activity of EEA.

Determination of Lipid peroxidation

TBARS assay was used to spectrophotometrically measure malondialdehyde (MDA) in serum. MDA is formed as the split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of a lipid substrate. MDA was reacted with TBA at 100°C to form a pink viscous solution which was then centrifuged for 10 min at 3000 rpm to give a clear solution. The absorbance was measured at 532 nm using a spectrophotometer. MDA is formed as a secondary product of lipid peroxidation, and it is postulated that increased MDA concentration in the serum, signifies increased lipid peroxidation [30]. Lipid peroxidation was calculated using a molar extinction coefficient of 1.56 $\times 10^5$ M^{-1} cm^{-1}, MDA concentration (µM)=Absorbance at 532/0.156.

Data analysis

All data collected were presented as means (±standard error the mean). Statistical significance was assessed through one-way analysis of variances followed by Dunnett multiple comparison test to compare the means. A value of $p<0.05$ was considered statistically significant. Graphs were plotted and statistical analysis performed using GraphPad Prism version 5.00 for Windows (Graphpad software, San Diego, CA, USA).

RESULTS

Cytotoxicity of EEA

The median LC_{50} after the 24 h count using the probit statistical analysis method was found to be 997 µg/ml.

Acute effects of EEA and GL treatment on blood glucose

Figs. 1 and 2 show the acute effects of EEA and GL on fasting blood glucose in normal and STZ-induced diabetic rats, respectively. Fasting blood glucose concentration of STZ-induced diabetic rats was significantly ($p<0.05$) higher as compared to non-diabetic control rats at time 0. Following the oral glucose challenge, blood glucose concentration of both nondiabetic and STZ-induced diabetic rats increased to the peak at the 30th min. The blood glucose levels in nondiabetic control group gradually decreased to pre-prandial values after 4 h. However, blood glucose levels in the STZ-diabetic group did not decrease significantly after 4 h.

On the other hand, oral administration of EEA at doses of 100, 200, and 400 mg/kg bwt significantly ($p<0.05–0.01$) and dose-dependently decreased blood glucose concentrations in both non-diabetic and STZ-induced diabetic groups compared with their respective control groups at all-time points that blood glucose was measured following oral glucose challenge. Oral administration of GL at a dose of 5 mg/kg bwt. significantly ($p<0.05$) decreased blood glucose concentration in the non-diabetic group compared with control group at all-time points but did not significantly lower blood glucose in STZ-induced diabetic rats.

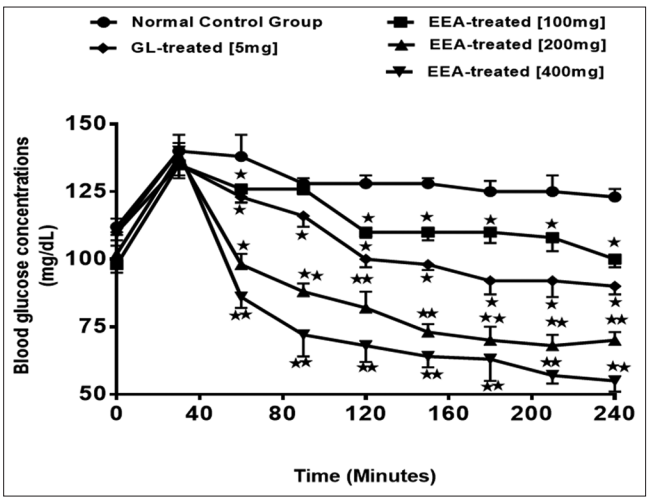

Fig. 1: Acute effect of ethanolic leaf extract of *Erythrina abbysinica* (EEA) and glibenclamide (GL) treatment on blood glucose concentration in normal rats. Oral administration of EEA significantly (p<0.05–0.01) and dose-dependently (100, 200 and 400 mg/kg bwt) decreased blood glucose concentration in treated groups compared with untreated control group at all-time points. GL at a dose of 5 mg/kg bwt. significantly (p<0.05) decreased blood glucose concentration in treated groups compared with untreated control group at all-time points. Values are presented as means±standard error the mean (n=6). *p<0.05; **p<0.01 significantly different compared with untreated control group

Sub-chronic effects of EEA and GL on body weight and relative organ weights of liver and pancreas

Table 1 shows the effect of daily oral administration of EEA and GL for 6 w on body weight and relative organ weights of liver and pancreas. The body weight of the untreated diabetic control group was significantly lower (−11.28% weight loss) compared with the normal and diabetic-treated groups. EEA and GL significantly improved the body weight of the rats in all the treated groups with EEA at 100, 200, and 400 mg/Kg/bwt. and GL causing percent weight increases of 5.38%, 9.68%, 17.16%, and 6.08%, respectively. Furthermore, EEA and GL significantly increased the relative organ weights of liver and pancreas as compared with the diabetic control group.

Sub-chronic effects of EEA and GL on blood glucose

Fig. 3 shows the sub-chronic effects of EEA and GL on blood glucose after 6 w of the experimental period. Daily oral administration of EEA at doses of 100, 200, and 400 mg/kg bwt significantly and dose-dependently lowered blood glucose concentration in streptozotocin (STZ)-induced diabetic rats as compared with the diabetic control group. GL at a daily dose of 5 mg/kg. bwt. slightly, but significantly lowered blood glucose in STZ-induced diabetic rats as compared to untreated-diabetic control group. The effects of GL on blood glucose were comparable to that of the lowest dose of EEA (100 mg/kg bwt).

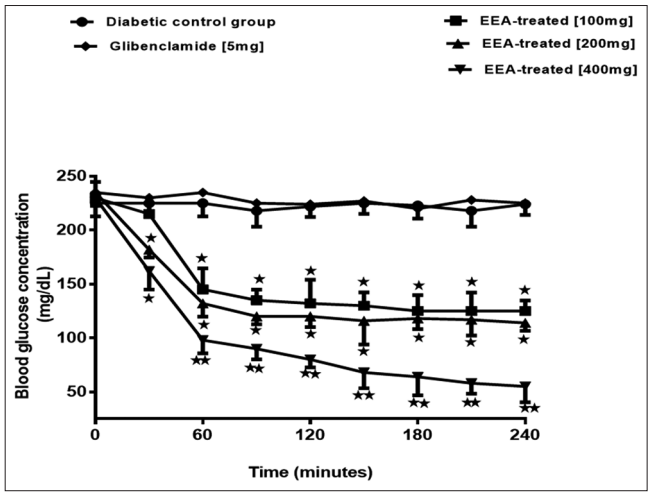

Fig. 2: Acute effect of ethanolic leaf extract of *Erythrina abbysinica* (EEA) and glibenclamide (GL) treatment on blood glucose concentration in streptozotocin -induced diabetic rats. Oral administration of EEA significantly (p<0.05–0.01) and dose-dependently (100, 200 and 400 mg/kg bwt) decreased blood glucose concentration in diabetic treated groups compared with diabetic un-treated control group at all-time points. GL at a dose of 5 mg/kg bwt. did not significantly (p>0.05) decrease blood glucose concentration in diabetic treated group compared with un-treated diabetic control group at all-time points. Values are presented as means ± standard error the mean (n=6). *p<0.05; **p<0.01 significantly different compared with untreated control group

Sub-chronic effects of EEA and GL on serum FRAP activity and MDA values

Table 2 shows the effect of daily oral administration of EEA and GL for 6 w on serum FRAP and MDA values. FRAP and MDA values were significantly higher and lower, respectively in EEA and GL treated groups as compared with the diabetic untreated control group.

Free radical scavenging activity of EEA and ascorbic acid

Fig. 4 shows the free radical scavenging activity of EEA and ascorbic acid. EEA dose-dependently revealed the ability to scavenge DPPH free radicals suggesting its proton-donating potential. The free radical scavenging ability of EEA was comparable to that of ascorbic acid.

DISSCUSSION

Economic problems in the developing nations such as South Africa continue to influence the lower rate of control of diabetes and its complications. It is envisaged that treatment of diabetes in poor African nations would become cheaper and therefore, accessible by using scientifically proven phytotherapy. The process of developing the new generation of drugs by isolation of individual active ingredients from plant material is highly expensive, unsustainable and time-consuming as compared to obtaining a simplified crude extract. Cham *et al.* 2015 emphasized that any standardized plant crude extract that contains target compounds should be acceptable as a potent therapeutic entity [31]. Thus, it would be necessary to establish the mechanism (s)

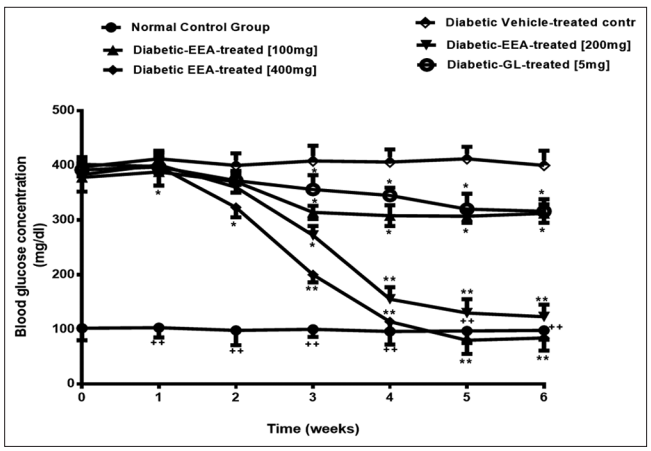

Fig. 3: Sub-chronic effect of ethanolic leaf extract of *Erythrina abbysinica* (EEA) on blood glucose in streptozotocin (STZ)-induced diabetic rats. EEA significantly (p<0.05–0.01) and dose-dependently (100, 200 and 400 mg/kg bwt) lowered blood glucose concentration in STZ-induced diabetic rats as compared with the diabetic control group. GL at a daily dose of 5 mg/kg. bwt. slightly, but significantly lowered blood glucose in STZ-induced diabetic rats as compared with untreated-diabetic control group. Values are expressed as means ± standard error the mean (n=6). *p<0.5; **p<0.01, and ++p<0.01 significantly different when compared with non-treated diabetic control group

Table 1: Effect of daily oral administration of EEA and GL for 6 weeks on body weight and relative weights of liver and pancreas in STZ-induced diabetic rats

Treatment group	% body weight change	Relative liver weight (mg/g)	Relative pancreas weight (mg/g)
Non-Diabetic control	22.86±3.08**	30.72±1.08**	4.62±1.12*
Diabetic control	−11.28±2.64	11.98±0.64	1.23±0.13
Diabetic+100 EEA	5.38±2.16*	13.38±0.16	1.34±0.17
Diabetic+200 EEA	9.68±3.53*	17.16±0.53*	2.28±0.09*
Diabetic+400 EEA	17.16±3.27**	24.16±1.27**	3.12±0.19*
Diabetic+GL	6.08±4.18*	12.08±0.18	1.31±0.16

EEA and GL significantly improved the body weight of the rats in all the treated groups. EEA at 100, 200, and 400 mg/Kg/bwt. and GL caused percent weight increases of 5.38%, 9.68%, 17.16%, and 6.08%, respectively. EEA and GL significantly increased the relative organ weights of liver and pancreas as compared to the diabetic control group. Values are presented as means±standard error the mean (n=6). *p<0.05; **p<0.01 significantly different when compared with non-treated diabetic control group

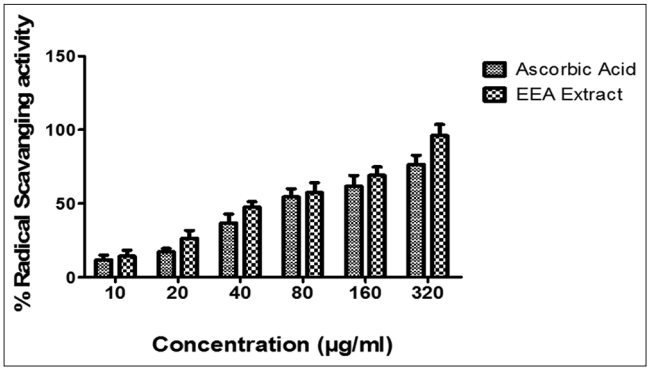

Fig. 4: The free radical scavenging activity of ethanolic leaf extract of *Erythrina abbysinica* (EEA) and ascorbic acid. EEA dose-dependently revealed the ability to scavenge DPPH free radicals suggesting its proton-donating potential. The free radical scavenging ability of EEA was comparable to that of ascorbic acid. Values are presented as means± standard error the mean of triplicate samples (n=3)

Table 2: Effect of daily oral administration of EEA and GL for 6 weeks on the FRAP and MDA values in rat serum

Treatment group	MDA (µmol/g)	FRAP (µmol/g)
Non-diabetic control	0.965±0.08**	143.72±4.28**
Diabetic control	5.98±0.26	68.52±6.81
Diabetic+100 EEA	2.38±0.162*	122.96±9.31**
Diabetic+200 EEA	1.56±0.156**	128.61±6.42**
Diabetic+400 EEA	0.74±0.127**	140.61±8.03**
Diabetic+GL	2.88±0.18*	82.63±5.71*

EEA and GL significantly increased FRAP activity and decreased the MDA levels in treated groups compared with the untreated control group. Values are presented as means±standard error the mean of triplicate samples (n=3). *p<0.05; **p<0.01 significantly different compared with non-treated diabetic control group

of the antidiabetic effects, efficacy, and safety of the traditional herbs and develop them into therapeutic formulations that could readily be available, affordable and accessible to the community. Traditional medicine from readily available medicinal plants offers great potential for the discovery of a new generation of anti-diabetic drugs [32]. Thus, search for more cost-effective and safer anti-diabetic drugs has become the focus point of interest and research. The present study has demonstrated the potential antidiabetic and antioxidant properties of ethanolic crude leaf extract of Ea.

In the present study, EEA has demonstrated potential glucose tolerant activity by preventing the development of acute hyperglycemia, after an oral glucose challenge, as it managed to lower blood glucose to normal values within 4 h of glucose load. Following an oral glucose load, blood glucose levels in both normal-treated and diabetic-treated rats increased rapidly and reached a peak at the 30th min followed by a gradual and progressive decrease towards normal values, suggesting the potent glucose tolerance activity of EEA, which was comparable to that of standard drug, GL. OGTT is a widely acceptable test that is used to diagnose pre-diabetes and diabetes as it assesses the body's potential to effectively utilize plasma glucose. In diabetic conditions, either insulin is not produced, or the body loses its ability to utilize insulin effectively enough to stabilize the blood glucose concentration, as a result fasting blood glucose concentrations are significantly higher than in normal states. Impaired fasting and postprandial glucose tolerance are associated with increased risk of diabetes mellitus and its associated complications. Patients with impaired glucose tolerance or impaired fasting glucose have a significant risk of developing diabetes and thus are an important target group for primary prevention [33]. Moreover, postprandial hyperglycemia is reported to be a major risk factor for complications associated with diabetes resulting in clinical consequences such as erectile dysfunctions, peripheral neuropathy, retinopathy, nephropathy, myocardial infarction, atherosclerosis, and many more [34]. It is reported that about 280 million people have impaired glucose tolerance and that the prevalence of diabetes is estimated to reach 552 million by 2030 globally [35]. Therefore, any form of approach that aims at alleviating acute postprandial hyperglycemia is critical in the control and management of diabetes and its complications [36]. Thus, EEA could be a potentially promising source of novel treatment of diabetes and its associated complications since it has revealed the potential to improve glucose tolerance.

In the present study, EEA was screened against an *in vivo* STZ-induced rat model of diabetes. The use of *in vivo* models provide a tool for understanding the pathological mechanisms of diabetes and are important for screening possible new drugs in management of diabetes [37].

A typical characteristic feature of STZ-induced diabetic rat model is absolute insulin deficiency, due to the extensive pancreatic beta-cell destruction caused by STZ toxicity [38]. The results of the acute/short-term anti-diabetic effect of EEA, suggest that apart from stimulation of insulin synthesis and release from pancreatic beta cells, it may also exert its blood glucose-lowering effects through extrahepatic pathways such as increased cellular utilization of glucose by peripheral tissues, increased glycogenesis, decreased glycogenolysis and decreased intestinal glucose absorption [39]. This conclusion is further supported by the fact that EEA lowered blood glucose levels in both glucose loaded normal and STZ-induced diabetic rats, whereas, GL, a drug which lowers blood glucose by stimulating pancreatic beta cells to secrete and release insulin, only revealed its blood glucose-lowering effect in glucose loaded normal rats but not in glucose loaded STZ-induced diabetic rats, meaning that the pancreatic beta cells in STZ-induced diabetic rats were severely damaged and therefore were unable to synthesize and release adequate amount of insulin.

Evidence suggests that levels of TBARS, a marker of oxidative stress are markedly increased in the liver and pancreas during the progression of diabetes, and that, this plays a major role in pancreatic and hepatocyte damage associated with diabetes [40]. Regeneration of the pancreatic beta cells is one of the proposed mechanisms by which, plant-based drugs are believed to lower blood glucose in STZ-induced diabetic rats because the regenerated beta cells increase the capacity of the endocrine pancreas to synthesize and release insulin, which in turn, promotes cellular glucose utilization [41]. Evaluation of relative vital organs weight showed that EEA-treated diabetic rats had significantly increased liver and pancreatic weights when compared with the diabetic untreated control group. Therefore, the significantly increased relative pancreatic weight, accompanied by decreased blood glucose, observed in EEA-treated animals, after 6 w of daily treatment as compared to their counterpart control untreated diabetic group, would seem to suggest, that the extract, induced progressive regeneration of the pancreatic beta cells, which resulted in the restoration of pancreatic endocrine function, leading to enhanced insulin synthesis and release, with a concomitant reduction in blood glucose levels. A decrease in the relative pancreatic weight of the diabetic control rats, observed in the present study, would be an indication of pancreatic damage, leading to loss of functional pancreatic beta cells, and hence, decreased insulin accompanied by elevated blood glucose levels in this group of rats.

Plant extracts with antidiabetic properties have been reported to possess the capacity to promote hepatic glycogenesis, which restores normal liver weight in diabetic rats. Gondwe *et al.* [42] reported the antihypeglycemic properties of *Sclerocarya birrea* stem-bark ethanolic extract, which was associated with increased hepatic glycogen synthesis. Therefore, it may be argued that increased relative liver weight observed in EEA-treated rats, may appear to suggest, the hepatoprotective activity of EEA against oxidative stress-induced damage, thereby promoting the liver's capacity to synthesize glycogen,

accompanied by a decrease in blood glucose and restoration of normal liver weight.

Loss of body weight is one of the signs manifested in diabetic condition because the body fails to effectively utilize glucose, and as a result, it begins to break down structural proteins, leading to muscle wasting [43]. A daily oral administration of various doses of EEA and GL, resulted in a significant improvement of body weight in treated groups as compared to untreated diabetic control group, suggesting the protective effect of EEA against structural protein catabolism [44]. Increased insulin synthesis and release by regenerated pancreatic beta cells might have potentiated increased cellular glucose uptake and utilization by peripheral tissues, leading to increased synthesis of structural proteins and consequently, to improved body weight and decreased blood glucose [45].

Oxidative stress, an imbalance between free radical generating system and natural antioxidant defense system, is intimately associated with diabetes and is responsible for the development of a myriad of complications of this chronic disease [46]. Antioxidants play a critical protective role against damages caused by reactive oxygen species, thereby maintaining normal cellular functions. Some synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate are available on the market. However, due to the growing concerns about the potential health hazards that are associated with dietary intake of these synthetic antioxidants such as liver toxicity, and carcinogenesis, there has been a dramatic shift toward the use of various natural antioxidants, mainly of herbal origin [47]. Several phytochemicals such as flavonoids saponins, polyphenols, Vitamin C and E, and carotenoids are used as potential antioxidants since they possess various antioxidant mechanisms, which among others, include the ability to scavenge free radicals, chelating metal ions, and inhibiting enzymatic systems which promote the generation of free radicals [48,49]. The present study reports the antioxidant properties of EEA as revealed by its ability to scavenge DPPH free radicals, to increase serum total antioxidant capacity and to inhibit lipid peroxidation. This finding may suggest that EEA contains active phytochemicals with antioxidant properties, and therefore, has the potential to prevent complications associated with diabetes. It may as well be postulated, that one other mechanism by which, EEA exerts its anti-diabetic actions is through its capacity to reduce oxidative stress which is implicated in the pathogenesis of diabetes mellitus.

One attractive feature with use of plant-based therapies is a common belief that they are safe. However, many phytochemicals may have significant adverse effects on human beings. Toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant [50]. It is, therefore, imperative that potential toxicity of medicinal plants be determined before consumption of the plant materials. Brine shrimp toxicity study was conducted to ascertain the safety of EEA. Brine shrimp lethality assay is widely used in toxicological tests involving screening of large numbers of medicinal plant extracts for drug discovery [51-53]. Moreover, some studies have reported a significant correlation between the LC_{50} value obtained with Brine Shrimp lethality assay and the LD_{50} value obtained from the acute oral toxicity assay done in mice [54]. Therefore, this assay could conveniently replace other biological models such as *in vivo* assays involving the use of live laboratory animals which are constantly facing ethical challenges. The high EEA and GL significantly increased FRAP activity and decreased the MDA levels in treated groups compared with the untreated control group. Values are presented as means ± standard error the mean of triplicate samples (n=3). *$p<0.05$; **$p<0.01$ significantly different compared with nontreated diabetic control group value of EEA (997 µg/ml), observed in the present study, may suggest that EEA has slightly weak toxicity and may probably be safe for human consumption. However, the fact that it exhibited a certain degree of toxicity, it may be advisable that consumption of the materials derived from this plant should be done cautiously.

The phytochemical analysis of the stem bark and root bark of Ea, reported the presence of several principle compounds, including flavonoids alkaloids, saponins, terpenoids, tannins, flavones, and chalcones [55,56]. The present study did not conduct the phytochemical analysis of EEA. However, it is most likely that these compounds reported to be available in the stem and root barks of Ea, it may also be richly available in the leafy parts of the plant, and that they are responsible for the antidiabetic and antioxidant activities displayed by EEA in this study. Furthermore, considering the remarkable, therapeutic potential of EEA, it would seem, plausible enough, to use the renewable leafy parts of this plant, as an alternative to stem and root barks, with the aim, to conserve the biodiversity of this miraculous medicinal plant, Ea. Harvesting of roots of medicinal plants for medicinal uses is not sustainable as it threatens the survival of the plants [57].

CONCLUSION

The present study has revealed the potential acute and sub-chronic blood glucose-lowering properties of EEA in both normal and STZ-induced diabetic rats. Based on the observations made, the study speculates that the extract may exert its antidiabetic effects through both hepatic and extra-hepatic mechanisms. In addition, the extract has exhibited remarkable antioxidant capacity as revealed by its ability to scavenge free radicals, inhibit lipid peroxidation, and reduce reactive oxygen species. Therefore, extracts from Ea, if properly and adequately standardized, may serve as a safe and cost-effective, potential therapeutic agent, with both anti-diabetic as well as antioxidant properties. The study has shed more light on the scientific basis for the use of Ea, in the management and treatment of diabetes by some communities of the Eastern Cape Province of the Republic of South Africa.

Limitations and recommendations

The proposed anti-diabetic mechanisms of EEA in the present study are mainly based on speculations, and therefore, warrant further investigations which should focus at establishment of the actual mechanistic pathways, through which, EEA exerts its anti-diabetic actions. Furthermore, active ingredients in EEA should be identified, isolated and screened for their anti-diabetic and antioxidant properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by the office of the Directorate of Research, Walter Sisulu University, Republic of South Africa.

AUTHOR'S CONTRIBUTIONS

Dr D Kamadyaapa is the corresponding author who has compiled this manuscript and he was very much involved in animal experimentation. Dr MM Gondwe and Dr Sewani-Rusike were involved in animal and brine shrimp toxicity experimentation, while Professor Benedicta Nkeh-Chungag and Miss Mathulo Shauli did the plant collection and extraction.

REFERENCES

1. Loots DT, Pieters M, Islam MS, Botes L. Antidiabetic effects of *Aloe ferox* and *Aloe greatheadii* var. davyana leaf gel extracts in a low-dose streptozotocin diabetes rat model. South Afr J Sci 2011;107:46-51.
2. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology E-Book.: Elsevier Health Sciences; 2017.
3. Aguiree F, Brown A, Cho NH, Dahlquist G, Dodd S, Dunning T, *et al*. IDF Diabetes Atlas. Vol. 4; 2013.
4. Hall V, Thomsen RW, Henriksen O, Lohse N. Diabetes in Sub Saharan Africa 1999-2011: Epidemiology and public health implications. A systematic review. BMC Public Health 2011;11:564.
5. Mayosi BM, Lawn JE, Van Niekerk A, Bradshaw D, Karim SS, Coovadia HM, *et al*. Health in South Africa: Changes and challenges since 2009. Lancet 2012;380:2029-43.
6. Musabayane CT. The effects of medicinal plants on renal function and blood pressure in diabetes mellitus. Cardiovasc J Afr 2012;23:462-8.
7. Ullah R, Tariq SA, Khan N, Sharif N, Din ZU, Mansoor K. Antihyperglycemic effect of methanol extract of *Tamarix aphylla* L.

Karst (Saltcedar) in streptozocin-nicotinamide induced diabetic rats. Asian Pac J Trop Biomed 2017;7:619-23.
8. Zhang F, Lavan BE, Gregoire FM. Selective modulators of PPAR-γ activity: Molecular aspects related to obesity and side-effects. PPAR Res 2007;2007:32696.
9. Mohammed A, Ibrahim MA, Islam MS. African medicinal plants with antidiabetic potentials: A review. Planta Med 2014;80:354-77.
10. Campbell RK, White JR Jr., Saulie BA. Metformin: A new oral biguanide. Clin Ther 1996;18:360-71.
11. DiMatteo MR. Social support and patient adherence to medical treatment: A meta-analysis. Health Psychol 2004;23:207-18.
12. Simmonds MS, Howes MJ. Plants used in the treatment of diabetes. Traditional medicine for modern times-Antidiabetic Plants. Vol. 6. New York: CRC Press; 2006. p. 19-82.
13. Haque N, Salma U, Nurunnabi TR, Uddin MJ, Jahangir MF, Islam SM, *et al.* Management of type 2 diabetes mellitus by lifestyle, diet and medicinal plants. Pak J Biol Sci 2011;14:13-24.
14. Malviya N, Jain S, Malviya SA. Antidiabetic potential of medicinal plants. Acta Pol Pharm 2010;67:113-8.
15. Qi LW, Liu EH, Chu C, Peng YB, Cai HX, Li P. Anti-diabetic agents from natural products-an update from 2004 to 2009. Curr Top Med Chem 2010;10:434-57.
16. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). Int J Diabetes Metab 2006;14:1.
17. Kazembe T, Chikuyu J. *In vitro* efficacy of extracts of *Cantharanthus roseus* and *Erythrina abbysinica* on *Babesia bigemina*. Bull Environ Pharmacol Life Sci 2012;1:32-38.
18. Kareru PG, Kenji GM, Gachanja AN, Keriko JM, Mungai G. Traditional medicines among the Embu and Mbeere people of Kenya. Afri J Tradit Complement Altern Med 2007;4:75-86.
19. Hines DA, Eckman K. Indigenous Multipurpose Trees of Tanzania: Uses and Economic Benefits for People. Rome: FAO; 1993.
20. Piero NM, Joan MN, Kibiti CM, Ngeranwa J, Njue WN, Maina DN, *et al*. Hypoglycemic activity of some Kenyan plants traditionally used to manage diabetes mellitus in Eastern Province. J Diabetes Metab 2011;2:155.
21. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107:1058-70.
22. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982;45:31-4.
23. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kabasa JD, Kiama SG. Evaluation of acute toxicity of crude plant extracts from kenyan biodiversity using brine shrimp, *Artemia salina* L. (*Artemiidae*). In Open Conf Proc J 2012;3:30-4.
24. Bastos ML, Lima MR, Conserva LM, Andrade VS, Rocha EM, Lemos RP. Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (*Bignoniaceae*) extracts and their main constituents. Ann Clin Microbiol Antimicrob 2009;8:16.
25. Olurishe CO, Salawu OA, Zezi AU, Olurishe TO, Bisalla M. Metformin-cefixime co-administration affects glucose regulation and reno-pancreatic histology in alloxan-induced hyperglycemic rats. J Pharm Sci Tech 2013;3:43-50.
26. Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. Food Chem 2007;101:267-73.
27. Nair VD, Dairam A, Agbonon A, Arnason JT, Foster BC, Kanfer I, *et al*. Investigation of the antioxidant activity of African potato (*Hypoxis hemerocallidea*). J Agric Food Chem 2007;55:1707-11.
28. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst 2002;127:183-98.
29. Gohari AR, Hajimehdipoor H, Saeidnia S, Ajani Y, Hadjiakhoondi A. Antioxidant activity of some medicinal species using FRAP assay. J Med Plants 2011;1:54-60.
30. Moukette BM, Pieme CA, Njimou JR, Biapa CP, Marco B, Ngogang JY, *et al. In vitro* antioxidant properties, free radicals scavenging activities of extracts and polyphenol composition of a non-timber forest product used as spice: *Monodora myristica*. Biol Res 2015;48:15.
31. Cham A, Cham K, Chase T, Cham BE. A standardized plant extract containing a target compound is acceptable as a potent therapeutic entity: Relevance to BEC and solamargine, a topical clinical formulation curaderm BEC5. J Cancer Treat Res 2015;3:22-7.
32. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK, *et al.* Antidiabetic agents from medicinal plants. Curr Med Chem 2006;13:1203-18.
33. Basal N. Prediabetes diagnosis and treatment. World J Diabetes 2015;6:296-303.
34. Brogan IJ, Murray IA, Cerillo G, Needham M, White A, Davis JR. Interaction of glucocorticoid receptor isoforms with transcription factors AP-1 and NF-κB: Lack of effect of glucocorticoid receptor β. Mol Cell Endocrinol 1999;157:95-104.
35. Khavandi K, Amer H, Ibrahim B, Brownrigg J. Strategies for preventing type 2 diabetes: An update for clinicians. Ther Adv Chronic Dis 2013;4:242-61.
36. Oguanobi NI, Chijioke CP, Ghasi SI. Effects of aqueous leaf extract of *Ocimum gratissimum* on oral glucose tolerance test in type-2 model diabetic rats. Afri J Pharm Pharmacol 2012;6:630-5.
37. Kavitha S, Rajeshwari S, Ravid D. Antidiabetic activity of *Asystasia gangetica* (L.) T. Anderson flower extract in streptozotocin induced diabetic rats. Int J Pharm Pharm Sci 2016;8:79-84.
38. Deshmukh CD, Jain A. Antidiabetic and antihyperlipidemic effects of methanolic extract of *Citrullus lanatus* seeds in rats. Int J Pharm Pharm Sci 2015;7:232-6.
39. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2012;2:320-30.
40. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clin Sci (Lond) 1998;94:623-32.
41. Eleazu CO, Okafor P. Use of unripe plantain (*Musa paradisiaca*) in the management of diabetes and hepatic dysfunction in streptozotocin induced diabetes in rats. Interv Med Appl Sci 2015;7:9-16.
42. Gondwe M, Kamadyaapa DR, Tufts M, Chuturgoon AA, Musabayane CT. *Sclerocarya birrea* [(A. Rich.) hochst.] [*Anacardiaceae*] stem-bark ethanolic extract (SBE) modulates blood glucose, glomerular filtration rate (GFR) and mean arterial blood pressure (MAP) of STZ-induced diabetic rats. Phytomedicine 2008;15:699-709.
43. Moodley K, Joseph K, Naidoo Y, Islam S, Mackraj I. Antioxidant, antidiabetic and hypolipidemic effects of *Tulbaghia violacea* harv. (wild garlic) rhizome methanolic extract in a diabetic rat model. BMC Complement Altern Med 2015;15:408.
44. Shenoy AG, Goyal RK. Improvement of insulin sensitivity by perindopril in spontaneously hypertensive and streptozotocin-diabetic rats. Indian J Pharmacol 2002;34:156-64.
45. Eleazu CO, Eleazu KC. Ameliorating potentials of 3 medicinal plants on relative pancreatic weights in streptozotocin induced diabetic rats. J Diabetes Metab 2013;4:2.
46. Brownlee M. The pathobiology of diabetic complications. Diabetes 2005;54:1615-25.
47. Gultekin F, Doguc DK. Allergic and immunologic reactions to food additives. Clin Rev Allergy Immunol 2013;45:6-29.
48. Rani JS. The efficacy of aqueous extract of *Talinum triangulare* on carbohydrate metabolic, non-enzymatic antioxidants and antioxidantive enzymes in alloxan induced diabetic rats. Arch Appl Sci Res 2013;5:197202.
49. Olorunnisola OS, Bradley G, Afolayan AJ. Protective effect of T. *Violacea rhizome* extract against hypercholesterolemia-induced oxidative stress in wistar rats. Molecules 2012;17:6033-45.
50. Hamidi M, Jovanova B, Panovska T. Toxicological evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. Macedonian Pharm Bull 2014;60:9-18.
51. Naidu JR, Ismail R, Sasidharan S. Acute oral toxicity and brine shrimp lethality of methanol extract of *Mentha Spicata* L (*Lamiaceae*). Trop J Pharm Res 2014;13:101-7.
52. Kheiri Manjili H, Jafari H, Ramazani A, Davoudi N. Anti-leishmanial and toxicity activities of some selected Iranian medicinal plants. Parasitol Res 2012;111:2115-21.
53. Costa-Lotufo LV, Khan MT, Ather A, Wilke DV, Jimenez PC, Pessoa C, *et al.* Studies of the anticancer potential of plants used in Bangladeshi folk medicine. J Ethnopharmacol 2005;99:21-30.
54. Arslanyolu M, Erdemgil FZ. Evaluation of the antibacterial activity and toxicity of isolated arctiin from the seeds of *Centaurea sclerolepis*. Ankara Ecz Fak Derg 2006;35:103-9.
55. Yenesew A, Induli M, Derese S, Midiwo JO, Heydenreich M, Peter MG, *et al.* Anti-plasmodial flavonoids from the stem bark of *Erythrina abyssinica*. Phytochemistry 2004;65:3029-32.
56. Piero NM, Joan MN, Cromwell KM, Joseph NJ, Wilson NM, Peter KG, *et al*. Hypoglycemic activity of some kenyan plants traditionally used to manage diabetes mellitus in eastern province. J Diabetes Metab 2011;2:155.
57. Maroyi A. Traditional use of medicinal plants in south-central Zimbabwe: Review and perspectives. J Ethnobiol Ethnomed 2013;9:31.