Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

ANTIDIABETIC EFFECT OF *HELIANTHUS ANNUUS* L., SEEDS ETHANOLIC EXTRACT IN STREPTOZOTOCIN- NICOTINAMIDE INDUCED TYPE 2 DIABETES MELLITUS

SHIVANI SAINI*, SUNIL SHARMA

Guru Jambheshwar University of Science And Technology, Hisar, India, 125001, Email: shivanisaini88@gmail.com

Received: 27 Jan 2013, Revised and Accepted: 13 Mar 2013

ABSTRACT

Aim: *Helianthus annuus* L., commonly known as sunflower, have a long history for use in traditional system of medicine. Seeds are claimed to be antihyperglycemic but no scientific data is available to validate the folklore claim In present study, ethanolic extract of sunflower seeds and its fractions are studied for their antidiabetic potential. Phytochemical screening is done to know the various chemical constituents present in extract. We hypothesized that the oral administration of ethanolic extract containing polyphenols from *Helianthus annuus* L. cause a reduction in diabetes and its complications.

Materials and methods: The antihyperglycemic effect of ethanol extract at 250mg/kg and 500mg/kg is studied in normal, glucose loaded hyperglycemic and streptozotocin (STZ) induced Type2 diabetic rats.

Results: Administration of alcoholic extract of *Helianthus annuus* L. at two dosage 250mg/kg and 500 mg/kg, p.o. respectively showed less significant changes in blood glucose level of normoglycemic rats ($P<0.05$), whereas, oral glucose tolerance test in diabetic rats depicted much reduction in blood glucose levels ($P<0.01$). Administration of extract in streptozotocin-nicotinamide induced diabetic rats, significantly decreased the blood glucose level ($P<0.001$) comparable to Glibenclamide (600 µg/kg), restored the lipid profile, showed improvement in body weight, liver glycogen content, glycosylated haemoglobin, plasma malondialdehyde, glutathione level and serum insulin levels in diabetic rats.

Conclusion: The study demonstrated the potential antidiabetic property of ethanolic extract of *Helianthus annuus* L. seeds in Type2 diabetes mellitus, thus justifying the traditional usage. Chlorogenic acid present in seeds is reported to have antidiabetic effect.

Keywords: *Helianthus annuus* L., antidiabetic, streptozotocin, oral glucose tolerance test, streptozotocin induced diabetes.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Hyperglycemia is associated with disturbances in carbohydrate, fat and protein metabolism due to defects in insulin secretion and insulin action [2-3] In addition to hyperglycemia, it leads to many other complications such as hyperlipidemia, hypertension, arthrosclerosis etc. [4]. It is estimated that there are 171 million people in the world with diabetes in year 2000 and this is likely to increase up to 366 million by 2030 [5]. There are various herbs which are used for the treatment of diabetes mellitus. Large number of plants have been explored for their antidiabetic potential, for e.g *Cyompsis tetragonoloba* [6], *Cichorum intybus* [7], *Ficus carica* [8], *Picralima nitida* [9], *Phyllanthus amarus* [10], *Abutilon indicum* [11], *Clausena Anisata,* [12], *Alpinia galangal* [13] etc.

At present, nearly 222 clinical trials investigating the effects of antidiabetic plants on diabetic patients are undergoing [13]. However from 1950 to 1970, only five drugs of plant origin were successfully tested in clinical phases and came in to market [15]. Therefore, it is necessary to search for new drugs and interventions that can be used to manage this metabolic disorder.

Helianthus annuus L. is a coarse, stout and erect annual plant 1-3 meters high. It produces greyish green or black seeds encased in tear-dropped shaped grey or black shells that often times feature black and white strips. **Seeds** encased in plant contains monoterpenes (α-pinene, Sabinene) [16-17], diterpenes (Helikauranoside) [18], oleic acid, triacyl glycerol, alkaloids, cyanogenic glycosides, saponins, cardiac glycosides, tannins, fixed oils, flavanoids [19], sesquiterpenes lactones [18], alkaloids [20]. **Flowers** contain quercimeritrin, anthocyanin, abundant amount of cholin and betain, triterpene [21], saponins [22]. Seeds contain 45 to 48 percent fixed oil, tannins [23], polyphenols [24]. *Helianthus annuus* L. is a folk remedy for bronchiectasis, bronchitis, carbuncles, catarrh, cold, colic, cough, diarrhoea, dysentery, dysuria, epistaxis, eyes, fever, flu, fractures, inflammations, laryngitis, lungs, malaria, menorrhagia, pleuritis, rheumatism, scorpion stings, snakebite, splenitis, urogenital ailments, whitlow, and wounds [25].

To our knowledge, there are no available reports on the antidiabetic effect of the seeds of this plant. Hence, the present study was carried out to determine the effect of ethanolic seed extract of *Helianthus annuus* L. on blood glucose level in STZ-induced diabetic rats. In this investigation, Glibenclamide is used as the reference drug.

MATERIALS AND METHODS

Chemicals and standard drugs

Steptozotocin (Sigma-Aldrich Co., Bangalore), Glibenclamide, heparin, EDTA, n-butanol, acetic acid, n-hexane, petroleum ether, ethyl acetate, glucose standard, citric acid, sodium citrate, tris hydrochloride, buffer tablet, sodium lauryl sulphate, thiobarbituric acid, trichloroacetic acid, triton-X, glycogen, ethanol, Tween 80, carboxy methyl cellulose, Ellman's reagent (5,5'-dithiobis-(2-nitro-benzoic acid); DTNB), sodium sulphate, methanol, pyridine, anthrone, thiourea, benzoic acid. Solvents were purchased from SD Fine Chemicals Ltd., Mumbai, India. All the chemicals used were of analytical grade.

Plant material

Helianthus annuus L. seeds were purchased in November, 2011 from the local market of Hisar, Haryana. Identified by Dr. H.B. Singh, Chief Scientist & Head, Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, vide reference no. NISCAIR/RHMD/Consult/-2011-12/1896/196. Dated : November 29, 2011.

Preparation of extract

Helianthus annuus L. seeds were dehulled in a centrifugal disc huller after drying at 50°C for 2 h. The kernels were flaked and defatted with n-hexane and the residual solvent was removed by air drying for 4-6 h at 30°C. The defatted sunflower meal (5g) of 10 mesh sieve size were mixed with solvent (ethanol) in the ratio of 1 of flour to 10 of solvent (w/v) and stirred for 2h at room temperature (about 28°C). The slurry was filtered and the residue on the filter paper was dried at 55-60°C in an oven till constant weight was obtained (5-6h) [26].

Preliminary phytochemical screening

Helianthus annuus L., was subjected to qualitative chemical screening to identify the various major classes of active chemical constituents, namely tannins, steroid, terpenoids, saponins, flavonoids, and alkaloid [27-28].

Animals

This study was carried out in Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India. Healthy adult male Albino Wistar rats (150–200 g), in-house bred at the Lala Lajpat Rai University of Veterinary and Agricultural sciences, Hisar, India; were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature 25 ± 2°C, relative humidity 55 ± 10% and 12:12 light:dark cycle). The rats were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) *ad libitum* and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

Acute toxicity study

The doses for the study were fixed based on Irwin test for the extracts at 1, 2, 3, 4 and 5g/kg [29]. The extracts were dissolved in a vehicle containing 4% Tween-80**.** Non-diabetic, male rats weighing 150 ± 5 g were used in this study. Three rats were used for each group. On the day preceding the experiment, the rats were appropriately grouped and placed in the experiment room for acclimatization. On the morning of the experiment day, food and water were removed from the cages. Then the rats were treated orally with the vehicle or the extracts. At 0, 15, 30, 60, 120, 180 min and 24 h after treatment of the extracts behavioural alterations were observed. 1/10th–1/20th of the dose in which no behavioural alterations were observed was considered safe for further assays. Hence, only ethanol extract at 250mg/kg and 500mg/kg was selected for further studies [30].

Experimental design

Antidiabetic activity of *Helianthus annuus* L. extract (HAE) was assessed in normal, glucose-loaded hyperglycemic and streptozotocin-induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

Induction of experimental diabetes

Experimental diabetes was induced by single intraperitoneal injection of 60mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5 [31-32] after 15 min of i.p. injection of nicotinamide (110 mg/kg) prepared in normal saline. Rats with marked hyperglycemia (fasted blood glucose level greater than 200 mg/dL) after one week of administration of STZ were used for the study.

Acute hypoglycemic effect of *Helianthus annuus* L. ethanolic extract on normoglycemic rats

Acute hypoglycemic studies were performed in overnight fasted normal rats. Normal rats were divided into four groups, each consisting of six rats. Animals in group second and third were treated orally with ethanolic extract of seeds of *Helianthus annuus* L. at a dose of 250 and 500 mg/kg, p.o. and group fourth (positive control) treated with Glibenclamide (600µg/kg). Control animals were administered with equal volume of water. Blood was withdrawn from the retro orbital plexus at 0, 30, 60, 90 and 120 min of glucose administration and glucose levels were estimated within 1 h, by GOD-POD method. [33]

Oral glucose tolerance test (OGTT) in normal rats

Oral glucose tolerance test was performed in overnight fasted normal rats. Normal rats were divided into four groups, each consisting of six rats. Animals in group second and third were treated orally with ethanolic extract of seeds of *Helianthus annuus* L. at a dose of 250 and 500 mg/kg, p.o. and group fourth (positive control) treated with Glibenclamide (600µg/kg). Glucose (2g/kg) was fed 30 min after the administration of extracts [34]. Control animals were administered with equal volume of water. Blood was withdrawn from the retro orbital plexus at 0, 30, 60, 90 and 120 min of glucose administration and glucose levels were estimated within 1 h, by GOD-POD method.

Oral glucose tolarence test (OGTT) in diabetic rats

Overnight fasted diabetic rats were separated in 4 groups of 6 rats each. Animals of all groups were administered with glucose (2g/kg) orally by means of gastric intubation. Animal in group second and third were treated orally with ethanolic extract at a dose of 250 and 500 mg/kg, p.o. and group fourth (positive control) treated with Glibenclamide (600µg/kg), 30 min before the oral administration of glucose orally. Control animals were administered with equal volume of water only. Blood samples were withdrawn from the retro orbital plexus of eye of each animal just after oral glucose administration at 0, 30, 60, 90 and 120 min for the assay of glucose [35].

Evaluation of extract in streptozotocin induced diabetic rats

The rats were divided into five groups of six rats in each group: Group 1 (NC): Normal rats treated with vehicle alone (1% tween80, 1ml per orally); Group 2 (DC): Diabetic rats treated with vehicle alone (1% tween80, 1ml per orally); Group 3 (D+HAE 250): Diabetic rats treated with *Helianthus annuus* L. extract (HAE) at the dose 250mg/kg. Group 4 (D+HAE 500): Diabetic rats treated with *Helianthus annuus* L. extract (HAE) at the dose of 500mg/kg. Group5 (D+Glibenclamide): Diabetic rats treated with Glibenclamide at the dose of 600µg/kg [36].

All rats except normal and diabetic control groups were administered single dose of drug (orally) daily for 21 days. Normal and diabetic control group rat received equal volume of vehicle only. The day of administration of first dose was considered the zero day of treatment. Blood samples were collected by retro-orbital plexus of eye under and fasting blood glucose levels were determined by glucose oxidase method on day 0^{th}, 7^{th}, 14^{th} and 21^{st} day with commercially available biochemical kit. Body weight of rats was taken on day 0^{th} (day when diabetes is induced), 10^{th}, 20^{th} and 28^{th} day. At the end of the experimental period, the animals were deprived of food overnight and then sacrificed by cervical decapitation. Blood was collected in tube containing heparin for the estimation of blood glucose and other parameters.

Biochemical analysis

Blood glucose levels and plasma cholesterol levels were measured by commercial supplied biological kits, Erba Glucose Kit (GOD-POD Method) and Erba Cholesterol Kit (CHOD-PAP Method) respectively using Chem 5 Plus-V2 Auto-analyser (Erba Mannhein, Germany). Glucose and cholesterol values were calculated as mg/dl blood sample. Glycosylated hemoglobin was measured using commercial supplied biological kit (Erba Diagnostic) in plasma sample using Chem 5 Plus-V2 Auto-analyser (Erba Mannhein, Germany). Values are expressed as the percent of total hemoglobin. Malondialdehyde (MDA), an index of free radical generation/lipid peroxidation, was determined as described by Okhawa *et al.* (1979) [37]. Glutathione level was measured by method of Sedlak and Lindsay (1968)**.** Liver was dissected out, washed in ice-cold saline, patted dry, weighed and subjected for bio-chemical estimation using anthrone reagent [38].

Statistical analysis

All values are expressed as mean ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's tests. The results were considered statistically significant if probability factor, $P < 0.05$.

RESULTS

Phytochemical screening of the extracts of *Helianthus annuus* L. showed the presence of various chemical constituents, mainly alkaloids, saponins, polysaccharides, flavonoids, polyphenols. The results obtained were comparable and satisfied the standard literature

Acute hypoglycemic effect of *Helianthus annuus* L. ethanolic extract on normoglycemic rats

The effect of the treatment with *Helianthus annuus* L. extract on the blood glucose level in normal fasted rats is shown in Table 1. In normoglycemic rats, HAE at two doses i.e 250 and 500mg/kg orally did not reduce the plasma glucose in normal rats. However, the rats treated with Glibenclamide showed a marked reduction in blood glucose levels.

Effect of *Helianthus annuus* L. on oral glucose tolerance test (OGTT) in normal rats

In oral glucose tolerance test, the treatment with the *Helianthus annuus* L. extract at 250 and 500 mg/kg showed significant reduction of 17.78% and 24.83% in plasma glucose levels, respectively after 30 min of glucose administration. The plasma glucose levels of the normal rats reached a peak at 30 minutes after the oral administration of glucose and gradually decreased to the pre-prandial level. Glibenclamide (600µg/kg) produced a significant reduction in the plasma glucose level at 0, 30, 60, 90 and 120 min after oral glucose administration.

Table 1: It shows the acute hypoglycemic effect of *Helianthus annuus* L. ethanolic extract on normoglycemic rats

Treatment	Mean blood glucose concentration (mg/dl) ± S.E.M				
	0 hr	1hr	2hr	3hr	4hr
Normal control	72.43±3.80	73.12±3.18	73.16±3.04	72.98±2.93	73.14±3.16
Glibenclamide	76.14±2.20	69.67±1.15*	64.13±2.07*	60.98±1.86*	69.17±1.98
HAE250mg/kg	75.31±3.94	71.12±2.50	68.12±3.21	65.14±2.89	71.43±2.15
HAE500mg/kg	73.12±2.89	70.84±2.07	65.14±2.07	63.45±1.93	70.17±1.05

The values are expressed as mean ± SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

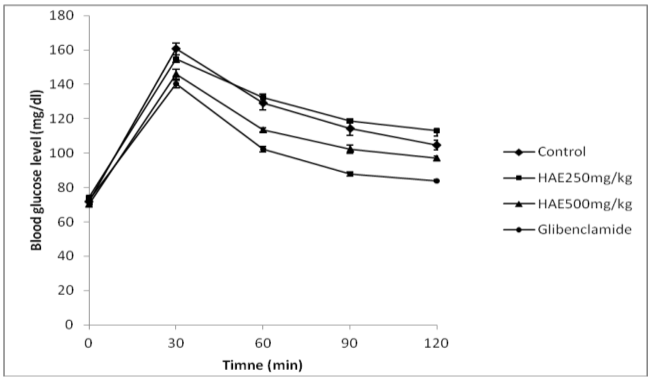

Fig. 1: It shows the effect of *Helianthus annuus* L. on oral glucose tolerance test (OGTT) in normal rats:

The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

Effect of *Helianthus annuus* L. extract on oral glucose tolerance (OGTT) in diabetic rats

In the diabetic rats ethanol extract of HAE at dose of 250mg/kg and 500mg/kg produced significant reduction of 22.03% and 27.31% respectively in plasma glucose levels compared with those of the controls at 0, 30, 60, 90 and 120 min after oral administration. Higher reduction in blood glucose is observed in hyperglycaemic rats than normal rats which shows antidiabetic effect of extract.

Effect of *Helianthus annuus* L. on fasting body weight and blood glucose in STZ-induced diabetic rats

STZ induced diabetic rats produced significant loss in body weight as compared to normal rats during the study. Diabetic control continued to lose weight till the end of the study while HAE two doses (250mg/kg and 500mg/kg b.w) showed significant improvement in body weight compared to diabetic control group. There was no significant difference between the HAE 500mg/kg and Glibenclamide treated groups.

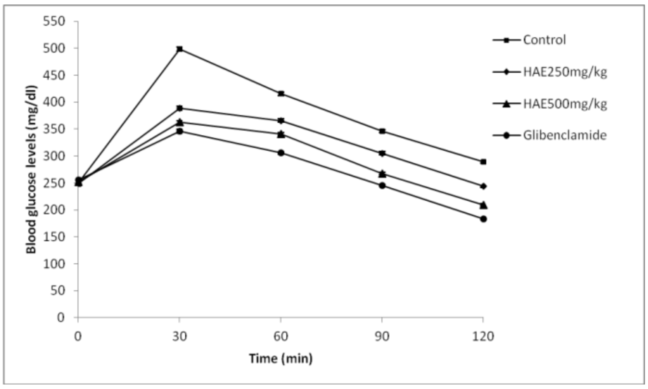

Fig. 2: It shows the effect of *Helianthus annuus* L. extract on oral glucose tolerance (OGTT) in diabetic rats.

The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

Table 2: It shows the effect of *Helianthus annuus* L. on body weight in STZ-induced diabetic rat:

Treatment	Changes in body weight (gm) at (days)			
	0	10	20	28
Normal	155.67±0.76	166.33±0.91	172.00±0.91	185.33±0.87
Control	158.17±0.70	153.50±0.56	148.67 ±0.66	139.00±0.77
Diabetic+ Glibenclamide	158.00±0.77	161.50±1.05**	163.33±0.80**	168.33±1.35**
Diabetic+HAE (250mg/kg)	163.33±5.04	166.83±0.94**	168.50±0.34**	175.17±1.37**
Diabetic+HAE (500mg/kg)	158.67±0.49	165.33±1.70**	167.33±0.55**	173.50±1.14**

The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

The effect of repeated oral administration of HAE on blood glucose levels in STZ-diabetic rats is presented in Table 3. HAE, administered at different doses of 250 and 500 mg/kg to STZ-treated diabetic rats caused significant (P<0.001) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 21 i.e 45.79% with 250mg/kg and 61.42% with 500mg/kg. HAE at 500 mg/kg exhibited maximum glucose lowering effect in diabetic rats compared to 250mg/kg. Glibenclamide exhibited a 62.41% reduction in blood glucose levels at the end of the study when compared to diabetic control.

Table 3: It shows the effect of *Helianthus annuus* L. on fasting blood glucose in STZ-induced diabetic rat:

Treatment	Blood glucose levels (mg/dl)			
	0th	7th	14th	21st
Normal	87.7±2.91	88.8±3.06	85.2±1.02	86.5±0.96
Control	312.96±5.46	328.54±4.89	331.58±6.24	360.1±4.51
Glibenclamide	283.58±5.83**	237.60±3.36 ***	165.72±4.74***	89.51±4.49***
Diabetic+HAE (250mg/kg)	291.12±5.35*	274.64±3.62***	229.62±4.55***	175.24±4.12***
Diabetic+HAE (500mg/kg)	286.15±5.26*	264.16±2.17***	185.08±3.05***	124.65±2.64***

The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

Effect of *Helianthus annuus* L. on serum insulin in STZ-induced diabetic rats

STZ caused a significant decrease in serum insulin levels. Administration of HAE at all the two doses (250mg/kg and 500 mg/kg) caused significant (P < 0.01) increase in insulin levels at the end of the study. Of the two doses, 500mg/kg showed maximum increase which was comparable to Glibenclamide (Table 4).

Effect of *Helianthus annuus* L. on serum lipids in STZ-induced diabetic rats

HAE showed a dose related significant (P < 0.01) reduction in level of total cholesterol. HAE at the doses of 500mg/kg was more effective than 250mg/kg in reducing the cholesterol levels (Table 4).

Effect of *Helianthus annuus* L. on glycogen content in STZ-induced diabetic rats

Administration of HAE at the doses of 250mg/kg and 500 mg/kg for 21 days resulted in significant (P < 0.01) increase in the glycogen levels in the liver . However, with none of the dose levels, the values were restored to normal (Table 4).

Acute oral toxicity study

In acute toxicity study, HAE treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weights and food consumption when compared to the vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded that HAE was safe at 2000 mg/kg.

Table 4: It shows the effect of *Helianthus annuus* L. on various blood parameters.

Group treatment	Glycosylated haemoglobin (mg/g)	Serum insulin (µU/ml)	Liver glycogen (mg/g)	Plasma malondialdehy-de (nmol/ml)	Plasma glutathione (mg/ml)	Total cholesterol
Normal control	0.24±0.008	19.01±0.45	15.45±0.82	1.91±0.30	37.45±1.31	65.98±0.78
Diabetic control	0.62±0.007	8.41±0.42	8.34±0.74	5.23±0.82	13.88±2.13	94.18±0.67
Diabetic+ Glibenclamide	0.23±0.006**	17.66±0.33**	14.05±0.67**	2.53±0.33**	36.65±1.28**	68.17±0.56**
Diabetic+HAE (250mg/kg)	0.32±0.004**	14.33±0.33**	12.65±0.32**	2.45±0.76**	23.65±1.35**	76.89±0.67**
Diabetic+HAE (500mg/kg)	0.26±0.004**	15.33±0.33**	13.32±0.28**	2.17±0.45**	27.75±1.45**	69.14±0.83**

The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

DISCUSSION

Diabetic subjects display more subtle changes in the dynamics of insulin secretion, such as blunting of the first phase insulin secretion and disruption of the insulin secretary pulses [39]. STZ [2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose] is an antibiotic that is used to induce experimental diabetes in animals [40]. STZ-induced diabetes may be due to vitiate glucose oxidation and reduction of insulin biosynthesis and secretion. The toxicity of STZ is due to DNA alkylation of its methyl nitrosourea moiety, mainly O at 6 position of guanine [41]. The transfer of methyl group from STZ to the DNA molecule causes damage which results in fragmentation of DNA and functional defects of the beta cells. Moreover, STZ has potential to act as an intracellular nitric oxide (NO) donor and generates reactive oxygen species (ROS). The synergistic action of both NO and ROS may also contribute to DNA fragmentation and other deleterious changes caused by STZ [42-43]. In our study, elevated blood glucose level and decreased insulin level were observed in STZ-induced diabetic rats and it may be due to above stated mechanism of STZ. Oral administration of HAE 250mg/kg, 500mg/kg and Glibenclamide to the diabetic rats significantly reduced blood glucose level from the first week to the fourth week compared to diabetic control rats. Also, the decreased insulin levels were noticed in diabetic rats compared to normal control rats which directly support and represent STZ-mediated beta cell destruction or damage. Hence, the hypoglycemic activity of HAE may be due to its protective action against STZ-mediated damage to the pancreatic beta cells and also possibly because of regeneration of damaged beta cells and also possibly because of inhibition of gluconeogenesis and glycogenesis [43].

STZ-induced diabetes was characterized by severe loss in body weight [44]. The decrease in body weight in diabetic rats showed that the loss or degradation of structural proteins was due to diabetes. Structural proteins are known to contribute to body weight [45]. When diabetic rats were treated with *Helianthus annuus* L. extract, the weight loss was reversed. The capability of *Helianthus annuus* L. to protect the body from weight loss seems to be a result of its ability to reduce hyperglycemia.

Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen. The conversion of glucose into glycogen in liver depends on concentration of glucose and availability of insulin which stimulates glycogen synthesis, which occur in presence of enzyme glycogen synthase and glycogen phophorylase. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and this activation appears to be defective in diabetes [46]. Skeletal muscle is also a major site of insulin-stimulated glucose uptake [47]. Decrease in hepatic glycogen was observed in this study. Treatment with HAE (250mg/kg and 500 mg/kg) for 21 days significantly increased liver glycogen indicating that the defective glycogen storage of the diabetic state was corrected by the extract.

Hypercholesteremia is primary factor involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes [48]. Increased fatty acid concentrations also increased the β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol in diabetics. The hypocholesterolaemic activity of HAE fractions after subchronic administration may be due to a number of mechanisms, including **a)** stimulation of cholesterol-7-alpha-hydroxylase (CYP7A1), which converts cholesterol into bile acids; **b)** inhibition of HMG-CoA reductase; and/or **c)** inhibition of cholesterol absorption from the intestines due to the formation of complexes with compounds such as glycosides and saponins [49-52].

The possible mechanism of antidiabetic action of aqueous extract may be by increasing the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. The enzyme system glucose-6-phosphatase plays a major role in the homeostatic regulation of blood glucose. It is responsible for the formation of endogenous glucose originate from gluconeogenesis and glycogenolysis. Recently chlorogenic acid is identified as a specific inhibitor of the glucose-6-phosphatase translocase component which results in less generation of glucose. *Helianthus annuus* L. contains chlorogenic acid, hence HAE extract shows antidiabetic effect.

CONCLUSION

The findings of the current study showed that *Helianthus annuus* L. and its fractions have a hypoglycemic effect in streptozotocin-induced diabetic rats. In addition, they were highly effective in managing the complications of diabetes mellitus such as hyperlipidemia, weight loss, glycogen content etc. The antidiabetic effects of HAE and its fractions may be mediated through an increase in insulin secretion, the inhibition of gluconeogenises and glycogenolysis and/or protection of pancreatic β-cells from streptozotocin and glucose-induced oxidative stress. This summed effect seems to have a promising value for the development of a potent phytomedicine for diabetes.

ACKNOWELDGEMENTS

I thanks to Guru Jambheshwar University of Science and Technology for providing me the financial assistance related to the research work.

REFERENCES

1. American Diabetes Association (ADA). Diagnosis and classification of diabetes mellitus. Diabetes care 2012; 35: 64-70.
2. Das AV, Padayutti PS, Paulose CS. Effect of leaf extract of *Aegle marmelose* L. Correaex Roxb. on histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. Indian Journal of Experimental Biology. 1996; 34:341-345.
3. Das SN, Patra VJ, Dinda SC. Diabetes and Indian traditional medicines: an overview. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4: 45-53.
4. Luo Q, Cai Y, Yan J, Sun M, Corke H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. Life Sciences 2004; 76: 137–149.
5. Gerstain HC, Santaguida P, Raina P, Morrison KM. Diabetes Research and Clinical Practice 2007; 78: 305-312
6. Bhandari V, Sharma JN. Effect of petroleum ether extract of guar gum in STZ induced hyperglycaemic rats. Pharmceutical Biology 1999; 37: 248-250.
7. Ki CG, Yim DS, Lee S. Biological activities of the root of *Cichorium intybus*. Natural Product Sciences 1999; 5: 155-158.
8. Perez C, Deminguez E, Canal JR. Hypoglycaemic activity of an aqueous extract from *Ficus carica* leaves in STZ diabetic rats. Pharmaceutical Biology 2000; 38: 181-186.
9. Agawa CN, Ukwe CV, Inya–Agha SI. Antidiabetic effect of *Picracima nitida* aqueous seed extract in experimental rabbit model. Journal of Natural Remedies 2001; volume ½: 135-139.
10. Raphael KR, Sabu MC, Kutttan R. Hypoglycemic effect of methanol extract of *Phyllanthus amarus* on alloxan induced DM in rats and its relation with antioxidant potential. Indian Journal of Experimental Biology 2002; 40: 905-909.
11. Seetharam YN, Setty SR, Chalegeri G. Hypoglycemic activity *Abutilon indicum* leaf extracts in rats. Fitotherapia. 2002; 73: 156-159.
12. Ojenole AO. Hypoglycemic effect of *Clausena anisata* methanolic root extracts in rats. Journal of Ethanopharma-cology. 2002; 81: 231-237.
13. Akhtar MS, Khan MA, Malik MT. Hypoglycemic activity of *Alpinia galangal* rhizome and its extracts in rabbits. Fitotherapia 2002; 73: 623-628.
14. Shojaii A, Goushegir A, Dabaghian FH, Abdollahi M, Huseini HF. Herbs and herbal preparations for glycemic control in diabetes mellitus (a systematic review). Journal of Medicinal Plants Research 2011; 16: 3846-3855.
15. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocrine reviews 2002; 23: 599-622.
16. Ceccarinia L, Macchia M, Flamini G, Cioni PL, Caponi C, Morelli I. Essential oil composition of *Helianthus annuus* L. leaves and heads of two cultivated hybrids “ Carlos” and “Florom 350”. Industrial Crops and Products 2004; 19: 13-17.
17. Verma S, Singh SP, Current and future status of herbal medicines. Veterinary World 2008; 11: 347-350.
18. Francisco AM, Ascensión T, José MG, Molinllo, Rosa MV, Diego C. Ecological biochemistry: Potential allelopathic sesquiterpene lactones from sunflower leaves. Phytochemistry 1996; 43: 1205-1215
19. Bohm, Bruce A, Stuessy TF. Flavanoids of the Sunflower Family (Asteraceae). 1st edition. Austria: Springer; 2001.
20. Javed K, Quantification of alkaloids, phenols and flavonoids in sunflower (*Helianthus annuus* L.). African Journal of Biotechnology 2001; 16: 3149-3151.
21. Ukaya M, Akihisa T, Yasukawa K, Koike K, Takahashi A, Suzuki T, Kimura Y. Triterpene glycosides from the flower petals of sunflower (*Helianthus annuus*) and their anti-inflammatory activity. Journal of Natural Products 2007; 70: 813-816.
22. Chirva V, Chirva Ya, Cheban PL, Lazur`evskii GV. The Saponins of Sunflower. Chemistry of Natural Compounds 1968; 42: 140.
23. Catherine NK, Imungi J K, Okoth M, Momanyi C, Biesalski HK, Vadivel V. Antioxidant and Antidiabetic Properties of Condensed Tannins in Acetonic Extract of Selected Raw and Processed Indigenous Food Ingredients from Kenya, Journal of Food Science 2011; 76: 560-567.

24. Kenneth LM, Cecil Randolph S, Iuan A. Phenolic and sugar components of Armavireo variety sunflower (*Helianthus annuus*) seed meal. Journal of Agricultural Food Chemistry 1970; 18: 27-32.
25. Duke JA, Wain KK. The Medicinal Plants of the World, Computer index with more than 85,000 enteries. London UK: Longman; 1981
26. Sripad G, Prakash V, Narasinga MS. Extractability of polyphenols of sunflower seed in various solvents. Journal of Biosciences 1982; 4: 145-152.
27. Sofowora A. Screening plants for bioactive agents. In: Medicinal plants and traditional medicinal in Africa. 2nd edition. Nigeria: Ibadan; 1993.
28. Trease GE, Evans WC. Trease and Evans Pharmacognosy. 15th edition. W.B. Saunders Edinburgh London, New York: Philadelphia, St. Louis Sydney Toronto. 2002; 42-393.
29. Roux S, Sable E, Porsolt RD. Primary observation (Irwin) test in rodents for assessing acute toxicity of a test agent and its effect on behavior and physiological function. Current Protocols in Pharmacology 2004; 10: 1–23.
30. Sunil C, Ignacimuthu S, Agastian P. Antidiabetic effect of *Symplocos cochinchinensis* (Lour.) *S. Moore*. in type 2 diabetic rats. Journal of Ethanopharmacology 2011; 134: 298-304.
31. Erejuwa OO, Sulaiman SA, Wahab MS, Salam SK, Salleh MS, Gurtu S. Antioxidant protective effect of glibenclamide and metformin in combination with honey in pancreas of streptozotocin-induced diabetic rats. International Journal of Molecular Sciences 2010; 11: 2056–2066.
32. Pandit R, Phadke A, Jagtap A. Antidiabetic effect of *Ficus religiosa* extract in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 2010; 128: 462–466.
33. Daisy P, Feril G.Jeeva Kani. Evaluation of Antidiabetic activity of various extracts of Cassia Auriculata Linn. Bark on Streptozotocin-induced diabetic Wistar Rats. Int J Pharm pharm sci 2012. 4(4): 312-318
34. Shirwaikar A, Rajendran K. Effect of aqueous bark extract of *Garuga pinnata* Roxb. in streptozotocin–nicotinamide induced type-II diabetes mellitus. Journal of Ethnopharmacology 2006; 107: 285-290.
35. Hepcy KD, Dinakar A, Senthil KN. Antidiabetic activity of ethanolic extracts of *Alangium salvifolium* and *Pavonia zeylanica* in streptozotocin induced diabetic rats. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4:337-339
36. Kumar R, Pate DK, Prasad SK, Sairam K, Hemalatha S. Antidiabetic activity of alcoholic leaves extract of *Alangium lamarckii* Thwaites on streptozotocin-nicotinamide induced type 2 diabetic rats. Asian Pacific Journal of Tropical Medicine 2011; 4: 904-909
37. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 1979; 95: 351–358.
38. Vander vies J. Two methods for the determination of glycogen in liver. Biochemical Journal 1953; 57: 410-416.
39. Matthews DR. Physiological implications of pulsatile hormone secretion. Annals of New York Academy Sciences 1991; 618: 28-37.
40. Al-Attar AM, Zari TA. Modulatory effect of ginger and clove oil on physiological response in streptozotocin-induced diabetic rats. International Journal of Pharmacology 2007; 3: 34-40.
41. Szkudelski T, The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001; 50: 536-546.
42. Lenzen S. The mechanism of alloxan and streptozotocin induced diabetes. Diabetologia 2008; 51: 216-226.
43. Ramachandran S, Rajasekaran A, Manisenthilkumar KT. Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. Asian Pacific Journal of Tropical Biomedicine 2012; 2: 262-268
44. Chen V, Ianuzzo CD. Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. Canadian Journal of Physiology and Pharmacology 1982; 60: 1251–1256.
45. Rajkumar L, Govindarajulu P. Increased degradation of dermal collagen in diabetic rats. Indian Journal of Experimental Biology 1991; 29: 1081–1083.
46. Grover J, Vats V, Yadav S. Effect of feeding aqueous extract of *Pterocarpus marsupium* on glycogen content of tissues and the key enzymes of carbohydrate metabolism. Molecular and Cellular Biochemistry 2002; 241: 53–59.
47. Bouche C, Serdy S, Kahn R, Goldfine A. The cellular fate of glucose and its relevance in Type 2 diabetes. Endocrine Reviews 2004; 25: 807–830.
48. Ananthan R, Latha M, Ramkumar K, Pari L, Baskar C, Bai V. Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats. Experimental Diabetes Research 2003; 4: 183–189.
49. Riyad MA, Abdul GAS, Suleiman MS. Effect of fenugreek and lupin seeds on the development of experimental diabetes rats. Planta Medica 1998; 54: 286–290.
50. Yokogoshi H, Oda H. Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high cholesterol-diet. Amino Acids 2002; 23: 433–439.
51. Chen W, Matuda K, Nishimura N, Yokogoshi H. The effect of taurine on cholesterol degradation in mice fed a high-cholesterol diet. Life Sciences 2004; 74: 1889–1898.
52. Mard SA, Jalalvand K, Jafarine JM, Balochi H, Naseri MKG. Evaluation of the antidiabetic and antilipaemic activities of the hydroalcoholic extract of *Phoenix Dactylifera* palm leaves and its fractions in alloxan-induced diabetic rats. The Malaysian Journal of Medical Sciences 2010; 17: 4-13.