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 other complications such as hyperlipidemia, hypertension, arteriosclerosis, 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. Gymnopsis tetragonoloba [6], Chichorium 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. Its large flowers (5-10 cm) are yellow and highly attractive. 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 contain monoterpenes (α-pinene, Sabinene) [16-17], diterpenes (Helikaurasenoside) [18], oleic acid, triacyl glycerol, alkaldoids, cyanogenic glycosides, saponins, cardiac glycosides, tannins, fixed oils, flavonoids [19], sesquiterpenes lactones [18], alkaldoids [20].

Flowers contain querimetinrin, 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, cataract, 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, glycerol standard, citric acid, sodium citrate, tris hydrochloride, buffer tablet, sodium lauryl sulphate, thioarbituric acid, trichloroacetic acid, triton-X, glycogen, ethanol, Tween 80, carboxy methyl cellulose, Eillman’s reagent (5,5’dithiobis–2-nitrobenzoic acid); DTNB), sodium sulphate, methanol, pyridine, antrhone, 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 1:1 of flour to 10 of solvent (w/v) and stirred for 2 h 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].

REFERENCES
[1] Guru Jambheshwar University of Science And Technology, Hisar, India, 20125001, Email: shivansaini88@gmail.com
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. E/436).

**Acute toxicity study**

The doses for the study were fixed based on Irwin test for the extracts at 1, 2, 3, 4 and 5 g/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 250 mg/kg and 500 mg/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 60 μg/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 (600 µg/kg). Glucose (2 g/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 [35].

**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 (600 µg/kg). Glucose (2 g/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 [35].

**Oral glucose tolerance 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 (2 g/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 (600 µg/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, 1 ml per orally); Group 2 (DC): Diabetic rats treated with vehicle alone (1% Tween80, 1 ml 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. Group 5 (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, 7th, 14th and 21st day with commercially available biochemical kit. Body weight of rats was taken on day 0th (day when diabetes is induced), 10th, 20th and 28th 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-analysers (Erba Mannheim, 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-analysers (Erba Mannheim, 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. [37]. Glutathione level was measured by method of Sedlak and Lindsay [38]. 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 500 mg/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.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean blood glucose concentration (mg/dl) ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Normal control</td>
<td>72.43±3.80</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>76.14±2.20</td>
</tr>
<tr>
<td>HAE250mg/kg</td>
<td>75.31±3.94</td>
</tr>
<tr>
<td>HAE500mg/kg</td>
<td>73.12±2.89</td>
</tr>
</tbody>
</table>

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](image1.png)

**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.

![Fig. 2](image2.png)

**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:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Changes in body weight (gm) at (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>15.67±0.76</td>
</tr>
<tr>
<td>Control</td>
<td>15.87±0.70</td>
</tr>
<tr>
<td>Diabetic+ Glibenclamide</td>
<td>15.80±0.77</td>
</tr>
<tr>
<td>Diabetic+HAE (250mg/kg)</td>
<td>16.33±5.04</td>
</tr>
<tr>
<td>Diabetic+HAE (500mg/kg)</td>
<td>15.86±7.49</td>
</tr>
</tbody>
</table>

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.

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 secretory 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 vitiolate glucose oxidation and reduction of insulin biosynthesis and secretion. The toxicity of STZ is due to DNA alkylation of its methyl nitrosourea moiety, mainly 0 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 on day 21 in 45.79% with 250mg/kg and 61.42% with 500mg/kg. HAE at 500mg/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.

**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 500mg/kg) caused significant (P<0.01) increase in serum 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).

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

<table>
<thead>
<tr>
<th>Group treatment</th>
<th>Glycosylated haemoglobin (mg/g)</th>
<th>Serum insulin (µU/ml)</th>
<th>Liver glycogen (mg/g)</th>
<th>Plasma malondialdehyde (nmol/ml)</th>
<th>Plasma glutathione (mg/ml)</th>
<th>Total cholesterol (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.24±0.008</td>
<td>19.01±0.45</td>
<td>15.45±0.82</td>
<td>1.91±0.30</td>
<td>37.45±1.31</td>
<td>65.98±0.78</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.62±0.007</td>
<td>8.41±0.42</td>
<td>8.3±0.74</td>
<td>5.2±0.82</td>
<td>31.88±2.13</td>
<td>94.18±0.67</td>
</tr>
<tr>
<td>Diabetic+</td>
<td>0.23±0.006**</td>
<td>17.66±0.33**</td>
<td>14.05±0.67**</td>
<td>2.5±0.33**</td>
<td>36.65±1.28**</td>
<td>68.17±0.56**</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.32±0.004**</td>
<td>14.33±0.33**</td>
<td>12.65±0.32**</td>
<td>2.45±0.76**</td>
<td>23.65±1.35**</td>
<td>76.89±0.67**</td>
</tr>
<tr>
<td>Diabetic+HAE (250mg/kg)</td>
<td>0.26±0.004**</td>
<td>15.33±0.33**</td>
<td>13.32±0.28**</td>
<td>2.17±0.45**</td>
<td>27.75±1.45**</td>
<td>69.14±0.83**</td>
</tr>
<tr>
<td>Diabetic+HAE (500mg/kg)</td>
<td>0.26±0.004**</td>
<td>15.33±0.33**</td>
<td>13.32±0.28**</td>
<td>2.17±0.45**</td>
<td>27.75±1.45**</td>
<td>69.14±0.83**</td>
</tr>
</tbody>
</table>

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.
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 glycolysis [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 also occurs in presence of enzyme glycogen synthase and glycogen phosphorylase. 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 a 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 diabetes. The hypocholesterolemic activity of HAE fractions after subchronic administration may be due to a number of mechanisms, including a) stimulation of cholesterol-7-α-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 anti diabetic 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 homestatic regulation of blood glucose. It is responsible for the formation of endogenous glucose originate from gluconeogenesis and glycolysis. Recently cholesteric 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 cholesteric 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, blood glucose content etc. The antidiabetic effects of HAE and its fractions may be mediated through an increase in insulin secretion, the inhibition of gluconemias and glycolysis and/or protection of pancreatic β-cells from streptozocin and glucose-induced oxidative stress. This summed effect seems to have a promising value for the development of a potent phyto medicine for diabetes.

**ACKNOWLEDGEMENTS**

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