Effect of germinated *Glycine max* seeds on glycemic control in STZ+NAD induced type 2 diabetic models: a preliminary study

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**Summary**

**Objective:** *Glycine max* (soybean) is a legume (Fabaceae) native to East Asia. *Glycine max* seeds are a powerful stimulant of immune system, used to decrease menopause symptoms, estrogen, treat certain types of cancers including that of the breast, lungs, prostate. It is also used for treating diabetes, liver and kidney disorders. The study aimed to investigate the effect of germinated *Glycine max* seeds on glycemic control in Streptozotocin (STZ) plus Nicotinamide (NAD) induced type 2 diabetic rats.

**Methods:** The effect of *Glycine max* seed extract at the dose of 100, 200, 400 mg/kg b.w, respectively, germinated at different time intervals was investigated in STZ+NAD induced diabetic rats. The study was conducted for 21 days to investigate the effect of extract on blood biochemical parameters indicative of hyperglycemia; fasting blood glucose (FBG), oral glucose tolerance test (OGTT), glycosylated hemoglobin (GHb) and lipid profile, *i.e.* total cholesterol (TC), low and high density lipoprotein cholesterol (LDL-c and HDL-c) and triacylglycerol (triglycerides, TG) levels.

**Results:** Aqueous extract (200 mg/kg b.w) of 12 h soaked and germinated *Glycine max* seeds produced significant reduction in FBG after 90 min (9.6%) and 3 h (15.6%) of administration. When the extract (200 mg/kg) was administered daily for three weeks to diabetic rats, significant fall in FBG (48.6%) and GHb (30.2%) was observed. Glucose tolerance was also found to be improved. Extract also showed significant hypohipolipidemic effect in diabetic rats as apparent from fall in TG, TC and LDL-c, and significant improvement on HDL-c.

**Conclusion:** The results suggest that aqueous extract of 12 h germinated *Glycine max* seeds possess antihyperglycemic as well as hypolipidemic activity.

**Key words:** Germination; *Glycine max*; Nicotinamide; Streptozotocin; Type 2 diabetes

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candidate for anti-cancer drug. According to some previous reports soybean diet, when given to diabetic patients, results in antihyperglycemic activity [9-13]. Antidiabetic potential of dietary fibers from Glycine max seeds is well documented in literature [14, 15]. Germination induced a substantial increase in the content of saponin, oestrogenic compounds and almost all phytosterols of seeds. Recently it has been reported that Glycine max seeds when soaked and germinated regulates hyperglycemia [16].

As of yet the period of germination which results in antihyperglycemic activity is not known and the reports on its effect in type 2 diabetes models are lacking. Therefore, in the present study intensive investigation was done to study the antihyperglycemic as well as hypolipidemic potential of Glycine max seed extract in type 2 diabetic models. The period of germination which produces maximum antihyperglycemic activity is also defined.

Materials and methods
Selection of animals
Male albino Wister rats (weighing 160-200 g) were procured from Central Animal House of University College of Medical Sciences (UCMS), Delhi, India. The animals were housed in standard conditions of temperature (22±2°C) and 12 h light-dark cycle. The rats were fed with commercial diet (Hindustan Unilever Ltd., Mumbai, India) and water ad libitum. The animal experimental protocol was approved by Institutional Animal Ethical Committee of UCMS. All experimental procedures were conducted in accordance to the Ethical Guidelines of International Association for the Study of Pain [17].

Preparation of Glycine max seed extract
Germination of Glycine max seeds
The seeds of Glycine max were procured from local market and identified by taxonomist at University of Delhi. The seeds were soaked for 12 h and germinated in sprout maker for 12, 24 and 72 h respectively at 37°C. The seeds were grounded and formed a fine paste.

Preparation of extract in aqueous and organic solvents
The seed paste underwent sequential extraction with petroleum ether, chloroform, acetone, ethanol and distilled water respectively. Initially, the seed paste (100 g) was suspended in petroleum ether (250 ml), kept for 3 h with shaking and filtered through 5-7 layers of muslin cloth. The residue left after filtration was extracted with other organic solvents. The prepared organic extracts were then evaporated under vacuum at 40°C. For aqueous extract, seed paste was suspended in 500 ml of distilled water overnight and then filtered. The aqueous extract was lyophilized. The yield of aqueous extract was approximately 10% of dried seeds. The extracts prepared were then screened for antihyperglycemic activity in type 2 diabetes models.

Induction of type 2 diabetes
Type 2 diabetes was induced by injection of nicotinamide (NAD; Calbiochem, Merck KGaA, Germany) at the dosage of 230 mg/kg, given intraperitoneally (i.p.) 15 min before streptozotocin (STZ; Sigma Aldrich, USA) administration (65 mg/kg b.w in 0.1 M citrate buffer, pH 4.5, i.p.) to overnight fasted rats [18]. After 48 h of STZ+NAD administration, fasting blood glucose (FBG) levels were measured and again repeated twice at intervals of three days. The animals with blood glucose level above 200 mg/dl were considered to be diabetic.

Experimental diabetes
The rats were divided into four groups (five animals in each group or subgroup) where group I was labeled as normal control. Group II was labeled as diabetic control, group III and IV comprised of diabetic rats: group IV was divided into three subgroups as diabetic rats treated with extract 100 mg/, 200 mg/ and 400 mg/kg b.w respectively; group III rats were treated with glibenclamide (600 μg/kg b.w).

Control rats received vehicle, i.e. distilled water, and treated rats received extract or glibenclamide in 1 ml of distilled water. The vehicle and drugs were orally administered daily for 21 days using a standard orogastric cannula. Blood samples were drawn from overnight fasted rats by retro-orbital venepuncture technique [19]. FBG and serum lipid parameters were measured before the treatment (day 0) and after the treatment on days 7, 14, and 21. Glycosylated hemoglobin (GHB) was measured at the end of the experimental period.

Biochemical investigations
The antihyperglycemic activity was assessed by measuring fall in FBG via the glucose oxidase-peroxidase enzymatic method devised by Barham & Trinder [20]. For oral glucose tolerance test, (OGTT) glucose (2 g/kg) was administered to nondiabetic control rats and the rats treated with glibenclamide (600 μg/kg) and improvement in glucose tolerance was also estimated at an interval of 0, 60 and 120 minutes respectively in rats. Blood samples were collected from overnight fasted rats.

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before giving the drug (shown as 0 h in Table 1) and subsequently after giving the drug (shown as 90 min and 3 h) to determine blood glucose levels. After assessment of antihyperglycemic activity with different doses of extract, maximum fall in blood glucose level was observed with the dose of 200 mg/kg. Therefore, subsequent studies were carried out after giving 200 mg/kg dose for the period of 3 weeks.

Glycosylated hemoglobin was estimated by method of Goldstein et al [21]. All the lipid profile parameters were performed in serum. To obtain clear serum, blood samples were centrifuged at 4000 rpm for 10 min. Triglycerides (TG) were measured as per the method of Fossati & Prencipe [22]; total cholesterol (TC) was assayed as per the method of Allain et al [23]; high-density lipoprotein (HDL) was determined by Burstein et al’s method [24]; low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated by using the formula of Friedwald et al [25].

Statistical analysis
Values are expressed as the mean ± SEM for five animals in each group (experiment done in triplicate). The data was analyzed by using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests. The results were considered significant at p<0.05.

Results
Effect of aqueous and organic extracts of germinated Glycine max seeds
Table 1 shows the preliminary screening of organic as well as aqueous extracts prepared. The data indicates that aqueous extracts of germinated Glycine max seeds produced antihyperglycemic activity, while maximum fall in blood glucose levels was found with aqueous extract of 12 h germinated seeds after 3 h of extract administration (15.6%). The other organic extracts produced no significant fall in FBG.

Effect of seed extract on glycemic control
The ethnopharmacological information about the doses of germinated Glycine max seeds is not previously indicated. Therefore, in this study, the antihyperglycemic effects of graded doses (100, 200 and 400 mg/kg b.w) of aqueous extract is summarized in Table 2 which shows that the dose of 100 mg/kg b.w does not produce significant fall in FBG (5.59%) and dose of 200 mg/kg b.w and 400 mg/kg b.w produced at par reduction of 15.6% and 14.5%, respectively, in blood glucose levels. Therefore, the dose of 200 mg/kg b.w was selected for further study. The antihyperglycemic effect of extract (15.6%) was found to be better than that of glibenclamide (600 μg/kg b.w), the reference drug used which produced 11.47% fall in blood glucose levels.

Subsequently, daily administration of 200 mg/kg b.w of 12 h germinated aqueous extract for the period of 3 weeks exhibits remarkable glycemic control in diabetic rats. The results are summarized in Tables 3 and 4 which shows substantial decrease in levels of FBG (48.6%) and GHb (30.2%) in diabetic rats after period of 21 days. Glucose tolerance was also found to be significantly improved after period of 21 days which make it effective in controlling post-prandial hyperglycemia.

In current study, residue (200 mg/kg b.w) left at the end of extraction (containing fiber) was also administered orally to rats and it was found to decrease blood glucose levels to 5.97% only. However in comparison to this, aqueous extract of 12 h germinated soybean seeds (200 mg/kg b.w) when administered orally produced 15.6% fall in blood glucose levels. These results reveal that fibre is not the major component of Glycine max seeds that produce antihyperglycemic effect.

In addition, the effect of extract was evaluated on body weight and it was found that extract treated rats undergo immediate weight loss in first seven days and after that it was increased and maintained to nearly normal in a 21 day treatment (Table 5).

Effect of seed extract on lipid profile
The effect of aqueous extract on serum lipid profile is recapitulated in Table 6. A significant increase in serum lipids, except HDL-c, was observed in diabetic rats when compared with healthy rats (p<0.001). Treatment with extract for 3 weeks shows a marked reduction (p<0.001) in levels of TC, TG and LDL. This lipid lowering effect produced by extract was found to be better than that of glibenclamide.

Table 1. Preliminary screening of aqueous and organic extracts of germinated Glycine max seeds for antihyperglycemic activity in STZ+NAD induced diabetic rats

<table>
<thead>
<tr>
<th>Glycine max extracts</th>
<th>Germination</th>
<th>12 h</th>
<th>24 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Chloroform</td>
<td>--</td>
<td>--</td>
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<td>--</td>
</tr>
<tr>
<td>Acetone</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Ethanol</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Aqueous</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

++, significantly effective; +, effective; --, non effective
Gupta & Sharma: Antihyperglycemic effect of germinated Glycine max seeds

Table 2. Effect of different doses of aqueous extract of 12 h germinated Glycine max seeds on FBG in STZ+NAD induced diabetic rats (one day treatment)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment (0 h)</th>
<th>90 min (% fall)</th>
<th>3 h (% fall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>85.6±6.9</td>
<td>86.6±6.9 (-)</td>
<td>87±6.7 (-)</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>260.2±15.8</td>
<td>261.8±15.1 (-)</td>
<td>262.2±16.4 (-)</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>265±8.3</td>
<td>249.8±8.1 (%5.74)</td>
<td>234.6±10* (%11.47)</td>
</tr>
<tr>
<td>Diabetic + 100 mg/kg extract</td>
<td>257.2±20.6</td>
<td>249±21.7 (%3.13)</td>
<td>242.8±21.4 (%5.59)</td>
</tr>
<tr>
<td>Diabetic + 200 mg/kg extract</td>
<td>266.6±11.3</td>
<td>241±12.1* (%9.6)</td>
<td>224.9±10.5* (%15.6)</td>
</tr>
<tr>
<td>Diabetic + 400 mg/kg extract</td>
<td>268±8.8</td>
<td>249.5±9 (%6.9)</td>
<td>229±8.5* (%14.5)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5). *p<0.05 compared to 0 h.

Table 3. Antihyperglycemic effect of aqueous extract of Glycine max seeds on FBG in STZ+NAD induced diabetic rats at weekly intervals (3 week treatment)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 15</th>
<th>Day 21 (% fall)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>85.6±6.9</td>
<td>86.6±6.96</td>
<td>87±6.78</td>
<td>86.8±6.94 (-)</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>221.8±17.1</td>
<td>267±17.3</td>
<td>292±16.5</td>
<td>304.6±17.6 (-)</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>218.6±15.9</td>
<td>181.2±16.1*</td>
<td>146±16.5*</td>
<td>121.8±16.7* (%44.2)</td>
</tr>
<tr>
<td>Diabetic + extract</td>
<td>216±16.3</td>
<td>178.2±16.9*</td>
<td>137.4±17.5*</td>
<td>110.9±17.3* (48.6)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5). *p<0.05 compared to day 0 (the day when extract was administered to the rats and blood glucose levels were checked before administration of extract gr glibenclamide.

Table 4. Effect of aqueous extract (200 mg/kg b.w) of 12 h germinated Glycine max seed on GHb and OGTT in STZ+NAD induced diabetic rats after 21 day treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 21</th>
<th>Day 0</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.42±0.13</td>
<td>3.42±0.13</td>
<td>89.6±3.2</td>
<td>90±2.9</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>6.14±0.3</td>
<td>7.26±0.29</td>
<td>235±5.7</td>
<td>278.4±5.4*</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>6.26±0.38</td>
<td>4.84±0.21*</td>
<td>238.3±3.6</td>
<td>225±4.9*</td>
</tr>
<tr>
<td>Diabetic + extract</td>
<td>6.14±0.28</td>
<td>4.28±0.19*</td>
<td>237±6.3</td>
<td>130.6±5.6*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5). *p<0.05 compared to day 0.

Discussion

Diabetes mellitus is one of the major chronic diseases which affect millions of people worldwide. It is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Research in past few years confirmed that medicinal plants play an important role in diabetes management. Furthermore, certain bioactive compounds have also been isolated from certain medicinal plants showing more efficacy and less side effects than that of standard drugs.

In present study, the antihyperglycemic effect of germinated seeds of Glycine max was evaluated. Glycine max (soybean) is considered as a unique and complete food because of its rich nutrient content. These legumes contain complex carbohydrates, vegetable protein, dietary fibre, oligosaccharides, phytochemicals (isoflavones, saponins etc), and minerals. Its complex carbohydrates and dietary fibre contents contribute to its low glycemic indexes, which benefit diabetic individuals [26].

Table 5. Body weight changes (in grams) of rats after 21 day treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>200±0.24</td>
<td>186±1.24</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>200±0.31</td>
<td>150±2.31</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>199±0.27</td>
<td>194±0.99</td>
</tr>
<tr>
<td>Diabetic + extract</td>
<td>202±0.3</td>
<td>197±0.89</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5).

The diabetic rat models were developed by administration of NAD 15 min before STZ. Blood glucose levels were increased significantly after 48 h of STZ+NAD treatment. Destruction of pancreatic β-cells can be accounted as one of the reason for producing diabetes.

The preliminary assessment reveals that out of all the extracts prepared (organic and aqueous), the aqueous extract of 12 h germinated Glycine max seeds produced most significant antihyperglycemic activity. The antidiabetic activity of aqueous extract of soybean sprouts was also observed by McCue et al [16]. The data in present study indicates that
aqueous extracts of germinated *Glycine max* seeds produced antihyperglycemic activity, while maximum fall in blood glucose levels was found with aqueous extract of 12 h germinated seeds after 3 h of extract administration. The report published by McCue *et al* [16] also observed antidiabetic potential of water soluble extracts of soybean sprouts germinated for period of 1 to 10 days. They estimated the inhibitory activity of seed extract on porcine pancreatic α-amylase (PPA) and yeast α-glucosidase *in vitro*. This inhibition is correlated to decreased glucose release from starch and delayed carbohydrate absorption in the small intestine which may have potential in treatment of diabetes. On the other hand, some other published reports postulates that this decrease in blood glucose levels is associated with isoflavones that are proposed to reduce the glucose level by increasing the secretion of insulin [27-29].

Previous research also specify the role of dietary fibers for antihyperglycemic activity [14, 15], whereas in current study, residue (200 mg/kg b.w) left at the end of extraction (containing fiber), when administered orally to rats, found to decrease blood glucose levels to 5.97% only. However in comparison to this, aqueous extract of 12 h germinated soybean seeds (200 mg/kg b.w) when administered orally produced 15.6% fall in blood glucose levels. Therefore, our results indicate that fiber of soybean seeds is not the key component for producing antihyperglycemic activity and the key component is present in aqueous extract of 12 h germinated *Glycine max* seed extract which is yet to be identified.

The ethnopharmacological information about the doses of germinated *Glycine max* seeds is not previously indicated. So in this study, the effects of graded doses (100, 200 and 400 mg/kg b.w) of extract is summarized in Table 2 which show that the dose of 100 mg/kg b.w does not produce significant fall in FBG and doses of 200 and 400 mg/kg b.wt. produced at par reduction of 15.6% and 14.5%, respectively, in blood glucose levels. Therefore, we selected dose of 200 mg/kg b.w for further study. The antihyperglycemic effect of extract was found to be better than that of glibenclamide, the reference drug used.

Daily 200 mg/kg b.w dose of 12 h germinated aqueous extract when administered for the period of 3 weeks exhibits remarkable glycemic control in diabetic rats, as evident by substantial decrease in levels of FBG and GHb. Glucose tolerance, which is indicative of glucose utilization in the body, was also found to be significantly improved after period of 21 days which make it effective in controlling post-prandial hyperglycemia (Tables 3 and 4). After extensive literature search only one preliminary report [30] was found which suggest that soaked and germinated *Glycine max* seeds become blood sugar regulator.

The effect of aqueous extract on serum lipid profile is summarized in Table 5. A significant increase in serum lipids, except HDL-c was observed in diabetic rats as compared with healthy rats. Treatment with extract for 3 weeks shows a marked reduction in levels of TC, TG and LDL. Certain reports suggest that soybean dietary fibre plays a role in the reduction of cholesterol levels in hyperlipidemic individuals and has a protective effect on cardiovascular disease. However, the results of present study indicate that absence of fibre from soybean seed extract also leads to reduction in cholesterol levels and improvement of lipid profile. Therefore, in agreement of other reports [14, 31, 32], this study advocates the role of either soy protein or presence of other bioactive compound in 12 h germinated soybean seed extract which is responsible for improvement in lipid profile. This hypolipidemic effect of extract was found to be better than that of glibenclamide except in case of HDL.

In conclusion, our data indicates that aqueous extract of 12 hr germinated *Glycine max* seeds possess antihyperglycemic and antihyperlipidemic activities. It is also found to be effective in controlling post-prandial hyperglycemia and

Table 6. Hypolipidemic effect of 12 h germinated *Glycine max* seed extract (200 mg/kg b.w) in STZ+NAD induced diabetic rats after 3 weeks treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum lipid profile (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG</td>
</tr>
<tr>
<td>Normal control</td>
<td>72.8±6.91</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>211±9.3*</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>100.8±4.6**</td>
</tr>
<tr>
<td>Diabetic + extract</td>
<td>90±2.7**</td>
</tr>
</tbody>
</table>

*p<0.001 compared to normal control; **p<0.05 compared to diabetic control.
improving lipid profile specifically TG, TC and LDL. Thus may prevent the progression of diabetes and cardiovascular diseases. This extract is effective in controlling type 2 diabetes. However, the specific component of soybean extract responsible for these actions is yet to be discovered.

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