Eman M.S. Shaheen

Protective effect of Fragaria ananassa against streptozotocin-induced diabetes in rats

ABSTRACT:
The present study aimed to clarify the effect of strawberry (Fragaria ananassa) leaves extract on some physiological parameters of streptozotocin-induced diabetes in rats. Diabetic rats were orally treated daily with three doses (50, 100, and 200 mg/kg) of strawberry leaves extract for 30 days. Diabetic rats exhibited significant increases in plasma glucose, triglycerides, total cholesterol, LDL-cholesterol, malondialdehyde levels in addition to the activities of catalase, alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) as compared to the control. On the other side, significant decreases in insulin, total protein, albumin, globulin, A/G ratio, HDL-cholesterol and superoxide dismutase levels were recorded. Treatment of diabetic rats with strawberry leaves extract significantly decreased plasma glucose, ALT, AST, triglycerides, total cholesterol, LDL-cholesterol, malondialdehyde and significantly increased insulin, total protein, albumin, globulin, A/G ratio, HDL-cholesterol and superoxide dismutase levels as compared to the diabetic rats. Therefore, the present data declared the efficiency of strawberry leaves extract for relieving the metabolic hazard of diabetics. Furthermore, the treatment with 200 mg/kg B.W. was more effective than treatment with 50 mg or 100 mg/kg B.W. the extract.

KEY WORDS:
Strawberry Leaves Extract, Diabetic Rats, Streptozotocin and Hyperglycaemia.

INTRODUCTION:
Diabetes mellitus (DM) is one of the most common chronic disease that may cause death. It is a metabolic disorder characterized by hyperglycaemia resulting from defective insulin secretion or resistance to insulin action or both. DM involves a high level of blood glucose, which contributes to an increase in free radical production (Perry, 2001; Saravanan and Ponnurugan, 2011; Demir et al., 2013). Diabetes leads to serious complications (Fiorentino et al., 2013) The treatment of diabetes is based on insulin and/or oral hypoglycaemic drugs (Daisy et al., 2009). These drugs act by various mechanisms to control blood glucose level, but many side effects have been reported (Patel et al., 2012).

Nowadays, there is a considerable interest in the field of medicinal plants due to their natural origin and fewer side effects (Jarlald et al., 2008). Many natural plants and their parts were recently used as traditional drugs for many human diseases where these plants are natural sources for antioxidants. Strawberry (Fragaria ananassa) is a hybrid species of genus Fragaria contains numerous important dietary components and, it is a rich source of vitamin C which neutralizes harmful effects occurred by reactive oxygen species (ROS). It also contains significant levels of ellagic acid, which is thought to be an anticaarcinogenic (Ashrafuzzaman et al., 2013). Strawberry leaves contain many bioactive compounds including; tannins, flavonoids, ascorbic acid and essential oil (Wang and Jiao, 2000). Strawberry leaves are used as anti-hypercholesterolemic and blood pressure lowering agent. Also, it is used for treatments of gastrointestinal disorders, respiratory tract inflammation, as well as a diuretic and expels kidney stones and intestinal worms. It may improve anaemia, hepatitis, nervous and immune systems (Duru, 2012). Strawberry fruits also have anticaarcinogenic activities (Wedge et al., 2001) and anti-thrombotic effects (Naemura et al., 2005). Till now, there is no attempt to test the efficiency of strawberry leaves extract to alleviate the harm consequences induced in diabetic rats. Therefore, the present study is proposed to explore this hypothesis.

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MATERIAL AND METHODS:

Preparation of strawberry leaves extract:
Strawberry (*Fragaria ananassa*) leaves were collected in January 2014 from Benha plant farms, Egypt. Mature fresh and healthy leaves were collected in the morning and immediately taken to the laboratory. The leaves were air-dried for one week at 20°C in the dark. 100g of the dried leaves powder was extracted with 1000 ml distilled water for 60 min and then filtered. Water extracted leaves were evaporated under vacuum using rotary evaporator (Buchi R-110) to give a crude residue.

Experimental animals:
White male albino rats (*Rattus norvegicus*) weighing 200 ± 10 g were purchased from the Centre of Laboratory Animals, Venoms & crude antisera production, Helwan, Cairo, Egypt. Animals were maintained under the laboratory conditions. Animals were exposed to 12 hours light and dark cycle at Temperature (20 ± 2°C) Rats were fed a laboratory rodent chow (18 - 24% protein, 4 - 7% fat and 60 - 75% carbohydrate) and water *ad libitum*. Rats were acclimated to laboratory conditions for 1 week before the onset of the experiment.

Toxicity test:
The study was carried out using rats weighing 200 ±10g. Five groups (Four rats each) were administered orally with graded doses (50, 100, 200, 400, and 800 mg/kg) of the extract. During the toxicity test, there was no mortality in animals at all the tested doses of the extracts up to 800 mg/kg over a period of 14 days.

Induction of diabetes mellitus:
This was done by intraperitoneal (i.p.) administration of 45mg/kg B.W. Streptozotocin (STZ), purchased from (Sigma Co., USA), dissolved in citrate buffer (pH 4.5) according to El-Seifi et al. (1993). STZ induces diabetes within 3 days by destroying the beta cells. In order to overcome the hypoglycaemic coma that occurs within the first 24 hours following STZ injection, animals were given 5% glucose solution instead of drinking water for 2 days until sustained hyperglycaemia was established (Abd El-Moneim et al., 2002). Three days after STZ injection, the blood samples were withdrawn from the lateral tail vein and glucose concentration was measured from overnight fasted animals (10-12 h). Rats having glucose ranging from 180 to 200 mg/dl were considered as mild diabetic and included in the experiment (Abd El-Moneim et al., 2002).

Experimental Groups:
The rats were randomly distributed into five groups, six rats each, all rats fed with a standard diet.

Group I: Control rats (non-diabetic)
Group II: Diabetic rats orally received 1 ml distilled water once a day for 30 days (positive control).
Group III, group IV and group V: Induced diabetic rats of these groups were treated with strawberry leaves extract (50, 100, and 200 mg/kg B.W., respectively) dissolved in 1ml distilled water once a day for 30 days. Orally administration was done by intragastric tube.

Blood sampling:
At the end of the experimental period, animals of each group were fasted about 12 h and then anesthetized slightly by diethyl ether inhalation (Sinet et al., 1984). Blood samples were collected from post-caval vein and directly transported to tubes containing ethylenediaminetetraacetic acid (EDTA) (EL-Gomhorya Co. Egypt). All the tubes were centrifuged at 3000 rpm for 15 min by Hitech centrifuge and plasma free of haemolysis was separated and frozen at -20°C until biochemical assays.

Biochemical assays:
Plasma glucose level was determined according to the method of Burtis et al. (2006) using a reagent kit purchased from Roche Diagnostics (USA). Plasma insulin level was determined using reagent kits purchased from Roche Diagnostics (USA). Plasma triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol levels were determined using kits purchased from Roche Diagnostics (USA). Plasma protein and albumin were estimated according to Koller (1984) and Webster et al. (1974), respectively, using Diamond kit (USA) Plasma triglycerides, cholesterol, LDL- cholesterol and HDL-cholesterol levels were determined using kits purchased from Roche Diagnostics (USA) according to the methods of Shephard and Whiting (1990), Trinder (1969), Wieland and Sceidel (1983), and Hatch and Less (1968), respectively. Plasma malondialdehyde, catalase, and superoxide dismutase levels were determined according to the methods of Satoh (1978), Aebi (1984), and Nishikimi et al. (1972), respectively, using reagent kits purchased from Biodiagnostic company (Egypt).

Statistical analysis:
Data were expressed as mean ± SD. Data were analysed using statistical package for social science (SPSS) computer program, version 20.00. The values were analysed by one- way analysis of variance (ANOVA) followed by Duncan’s multiple range test (Duncan, 1957). values were considered significant at P < 0.05.
RESULTS:

Effect of strawberry leaves extract on plasma glucose and insulin:

STZ-diabetic rats showed highly significant increase in plasma glucose of all groups of STZ-diabetic rats as compared to control rats. Plasma glucose levels were significantly low (P < 0.05) in the STZ-diabetic rats and treated with 50, 100, and 200 mg/kg B.W. strawberry leaves extract as compared to diabetic rats. The effect was dose-dependent as the lowest blood glucose was found in rats treated up 200 mg/kg B.W. of the strawberry leaves extract. However, plasma glucose levels were significantly higher in all groups of STZ-diabetic rats as compared to control one. This indicating the potency of strawberry leaves extract to relief the elevation in blood glucose level and not full treatment.

Plasma insulin level in diabetic rats were significantly (p < 0.05) decreased as compared to the control group. It was found that plasma insulin level was significantly increased in all diabetic rat groups receiving the strawberry leaves extract (Table 1).

Table 1. Effect of strawberry leaves extract on plasma glucose and insulin levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>125.46 ± 6.8a</td>
<td>251.6 ± 12.28a</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>5.89 ± 0.25a</td>
<td>0.61 ± 0.04a</td>
</tr>
</tbody>
</table>

All data expressed as mean ± SD for 6 animals.
Means superscripted with different letters are significantly different (P < 0.05)

Effect of strawberry leaves extract on liver functions:

Plasma ALT level of group II was significantly (p < 0.05) increased as compared to the control group. Treatment with strawberry leaves extract, especially 200 mg/kg B.W. decreases ALT level Plasma AST level in diabetic animal group was significantly (p < 0.05) high as compared to the control group (Table 2). Plasma ALT and AST levels of diabetic treated rats with 100 and 200 mg/kg B.W. showed statistically non significance difference from the control, whereas rats given the lowest dose showed significant increased value from the control.

Table 2. Effect of strawberry leaves extract on plasma liver enzyme activities.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>29 ± 3.5a</td>
<td>64.2 ± 4.49a</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>70.8 ± 5.8abc</td>
<td>107 ± 8.6a</td>
</tr>
</tbody>
</table>

All data expressed as mean ± SD for 6 animals.
abc: Means superscripted with different letters are significantly different (P < 0.05)

Effect of strawberry leaves extract on plasma total proteins, albumin and globulins:

Plasma total proteins, albumin, globulins levels and albumin/globulin ratio (A/G) showed significantly decreased values (P < 0.05) in diabetic rats compared with the control group. Plasma albumin was elevated in groups III, IV, and V as compared with those in group II. The highest dose (200 mg/kg) of strawberry leaves extract elevated total plasma proteins, albumin, globulin and A/G ratio more than those in lower doses (50 and 100 mg/kg) of strawberry leaves extract (Table 3).

Table 3. Effect of strawberry leaves extract on plasma total protein, albumin and globulins levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Total proteins (g/dl)</td>
<td>4.88 ± 0.79a</td>
<td>3.86 ± 0.24a</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.83 ± 0.15abc</td>
<td>3.12 ± 0.04a</td>
</tr>
<tr>
<td>Globulins (g/dl)</td>
<td>1.28 ± 0.77a</td>
<td>1.231 ± 0.25a</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>2.89 ± 0.02a</td>
<td>2.41 ± 0.09a</td>
</tr>
</tbody>
</table>

All data expressed as mean ± SD for 6 animals.
Means superscripted with different letters are significantly different (P < 0.05)
Effect of strawberry leaves extract on plasma lipid profile:

Plasma cholesterol, triglyceride, LDL-cholesterol and VLDL levels decreased significantly in diabetic rats' treatment with 50, 100 and 200 mg/kg B.W. strawberry leaves extract as compared to diabetic group (Table 4). Treatment with the extract resulted in a return of the level of HDL- and VLDL – cholesterol close to the levels in the control non-diabetic rats.

Plasma MDA activity of groups III, IV and V were significantly low as compared to the control group. Plasma CAT activity of group V was significantly high compared to those of group III and group IV. Plasma CAT activity of group IV was significantly high compared to that of group III.

Plasma superoxide dismutase (SOD) activity of group II showed significantly decreased as compared to groups I, III, IV and V. Treatment with the extract resulted in increasing plasma SOD in group V as compared to the group III and group IV (Table 5).

Effect of strawberry leaves extract on antioxidant parameters:

Plasma malondialdehyde (MDA) activity in group I, III, IV, and V were significantly low as compared to that of the diabetic group. Plasma MDA activity of groups III, IV and V were significantly (P< 0.05) high as compared to the non-diabetic group. Plasma MDA activity of group V was significantly low as compared to those of groups III and IV. Plasma catalase activity (CAT) of group II was significantly low as compared to those of groups I, III, IV, and V. Also, plasma CAT activity of groups III, IV and V were significantly low as compared to the non-diabetic group (Table 5).

Discussion:

The present study showed a significant decrease in plasma glucose in diabetic rats treated with strawberry leaves extract (50, 100, or 200 mg/kg B.W.) as compared to that of untreated diabetic rats. The increased plasma glucose which was induced by destroying the β-cells of islets of Langerhans by streptozotocin obtained in the present study is similar to the findings of Perry (2001). The increase in blood glucose level may be due to low plasma insulin level after using STZ which destroy β-cells. These results were supported by those of Abd El-Moneim et al. (2002) and El-Shafey et al. (2013).

The obtained data in the present study evoked a reduction of blood glucose level in STZ induced diabetic rats treated with strawberry leaves extract as compared with STZ induced diabetic rats untreated with strawberry leaves extract. This is accompanied with a significant increase in insulin level in diabetic rats post treated with strawberry leaves extract. These results denote the ability of strawberry leaves extract to reduce the magnification of blood glucose in STZ-induced diabetic rats. The effects of strawberry leaves extract are regulating the activities of amylase and α-glycosidase that lowers the blood glucose content. This is associated with the existence of antioxidant activities of strawberry leaves extract are regulating the plasma antioxidant parameters:

Table 5. Effect of strawberry leaves extract on plasma MDA, CAT and SOD activities.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diabetic</th>
<th>Group III + 50 mg/kg extract</th>
<th>Group IV + 100 mg/kg extract</th>
<th>Group V + 200 mg/kg extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>81.2 ± 8.2b</td>
<td>147.2 ± 9.60c</td>
<td>76.4 ± 16.13ac</td>
<td>64.9 ± 7.89cde</td>
<td>52.4 ± 6.06fde</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>131.4 ± 4.77b</td>
<td>137.6 ± 6.4c</td>
<td>58.8 ± 5.8b</td>
<td>95.2 ± 16.7c</td>
<td>97.116.3b</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>18.8 ± 4.7b</td>
<td>8.76 ± 2.3a</td>
<td>17.0 ± 2.54a</td>
<td>17.80 ± 5.08b</td>
<td>17.4 ± 2.30c</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>31.68 ± 0.84d</td>
<td>40.13 ± 2.94e</td>
<td>18.08 ± 3.9b</td>
<td>16.82 ± 3.18b</td>
<td>14.40 ± 4.79h</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>30.80 ± 5.99e</td>
<td>50.44 ± 4.6a</td>
<td>31.58 ± 5.3b</td>
<td>25.7 ± 3.39b</td>
<td>25.053.9b</td>
</tr>
</tbody>
</table>

All data expressed as mean ± SD for 6 animals.

abcde: Means superscripted with different letters are significantly different (P < 0.05)

Table 4. Effect of strawberry leaves extract on plasma lipid profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diabetic</th>
<th>Group III + 50 mg/kg extract</th>
<th>Group IV + 100 mg/kg extract</th>
<th>Group V + 200 mg/kg extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml)</td>
<td>2.16 ± 0.25b</td>
<td>6.12 ± 0.15c</td>
<td>5.19 ± 0.12a</td>
<td>4.49 ± 0.35a</td>
<td>3.82 ± 0.16d</td>
</tr>
<tr>
<td>CAT (nmol/ml)</td>
<td>6.18 ± 0.30a</td>
<td>1.97 ± 0.36b</td>
<td>2.79 ± 0.38d</td>
<td>3.44 ± 0.30d</td>
<td>4.13 ± 0.16b</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>8.82 ± 0.41a</td>
<td>2.25 ± 0.23c</td>
<td>3.15 ± 0.18c</td>
<td>3.57 ± 0.35c</td>
<td>5.85 ± 0.44b</td>
</tr>
</tbody>
</table>

All data expressed as mean ± SD for 6 animals.

abcde: Means superscripted with different letters are significantly different (P < 0.05)
compounds as flavonoids, ellagic, and anthocyanin in strawberry leaves extract (Hannum, 2004). These results agreed with those of Mandave et al. (2013) and Mima (2013), as they reported that in vitro aqueous extract of strawberry fruits delayed carbohydrate absorption by inhibiting α-amylase and α-glucosidase enzyme activities which might control blood glucose.

Oxidative stress and lipid peroxidation are the characteristic features of chronic diabetes. Increased oxidative stress plays an important role in the development and progression of diabetes and its complications (Fiorentino et al., 2013). Diabetes is usually accompanied by increased production of free radicals, reactive oxygen species (ROS) and impaired antioxidant defenses (Maritim et al., 2003). The increment in free radical production may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn result in the elevated production of free radicals (Rajasekaran et al., 2005).

In this concern, Rostamian et al. (2011) reported that administration of hydro-alcoholic strawberry leaves extract decreased partially glucose level. The strawberry leaves extract exerts beneficial effects by maintaining lipid metabolism, recovery of body antioxidant system, and controls blood pressure and blood sugar.

The increase in activities of plasma AST and ALT indicate that diabetes may induce hepatic dysfunction. Agreeing with our finding those found by Ohaeri (2001) that liver was necrotized in diabetic rats. Therefore, the increase in the activities of AST and ALT in serum may be mainly due to leakage of these enzymes from the liver cytosol into the bloodstream (Concepcion et al., 1993) which gives an indication on hepatotoxicity by CCl4—which leads to liver damage.

The present study showed a decline in total proteins, plasma albumin globulin and A/G ratio in diabetic rats. This agrees with hypoalbuminemia observed in diabetics (Porte and Halter, 1981). Hypoalbuminemia is a common problem in diabetic animals and is generally attributed in the presence of nephropathy. Also, diabetes is characterized by elevated levels of albuminuria (Yassin and Mwafy, 2007). An overall reduction in plasma total protein in diabetic animals and consequents albumin and A/G ratios were observed in the present study.

In the present study, rats treated with strawberry leaves extract post STZ-diabetic induction showed a significant decrease in triglyceride, cholesterol and LDL cholesterol and a significant increase in HDL- cholesterol as compared to those of untreated diabetic rats. With rats being affected with diabetes, LDL content would increase, and HDL content would decrease that agree with the results from Abou-Seif and Yussef (2004) and Winocour et al. (1992). The obtained results agreed with Rostamian et al. (2011) who reported that strawberry leaf extract could decrease triglyceride content and HDL will increase by decreased triglyceride. Treatment of strawberry extracts improved lipid profile and liver function and led to a significant increase in antioxidant status in diabetic rats (Mandave et al., 2017). Fotschki et al. (2018) compares the effects of two dietary strawberry extracts rich in monomeric or dimeric ellagitannins on liver triglyceride in Wister rats fed high-fructose diets they showed more effectively decrease in serum and liver triglyceride.

The recorded data showed a significant increase in malondialdehyde (MDA) level in diabetic rats compared to the control. This is similar with the observations of Maritim et al. (2003) and Ravi et al. (2004) as they reported that induction of diabetes in rats with STZ result in an increase in lipid peroxidation as MDA an indirect evidence of intensified free radical production. Treatment of diabetic rats with strawberry leaves extract lower the elevated MDA level in plasma compared with diabetic non-treated rats.

Plasma catalase is a haemoprotein, which is present virtually in all mammalian cells and is responsible for the reduction of H2O2 and protects tissues from highly reactive OH radicals (Sözmen et al., 2001). Diabetic rats exhibited a significant decrease in CAT and SOD activities as compared to the control group. The decreases in activities of SOD and CAT in diabetic rats may be due to increased production of reactive oxygen radicals that can themselves reduce the activity of these enzymes (Wohaib and Godian, 1987). The decrease in CAT activity could result from inactivation by glycation of the enzyme. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of oxidative damage. Therefore, removing O2 and OH is probably one of the most effective defences against diseases (Sankaranarayanan and Pari, 2011). SOD and CAT are important defence enzymes which convert superoxide to H2O2 (Abdel-Razek and Hassan, 2011). The reduction of these enzymes in diabetic rats may lead to several deleterious effects. Administration of strawberry leaves extract increase the activity of these enzymes as compared to the diabetic group and may help to avoid the deleterious effects of free radicals generated during diabetes. This effect is related to the flavonoids in strawberry leaves which are antioxidant compounds neutralizing the harmful effects of free radicals (Lobo et al., 2010).
Conclusion:
The current work showed the protective role of strawberry (Fragaria ananassa) leaves extracts on STZ – induced diabetic rats. Strawberry leaves extracts reduced the severity of hyperglycaemia by enhancing plasma insulin level and reducing plasma AST and ALT levels and elevating plasma albumin and reducing plasma lipid profile. It also enhancing antioxidant parameters as malondialdehyde, catalase and superoxide dismutase levels Therefore, it is recommended to use strawberry leaves extract as herbal medicine for diabetic patients.

REFERENCES:


