Evaluating the Effect of *Silybum marianum* Extract on Blood Glucose, Liver and Kidney Functions in Diabetic Rats

Ahmed Saber Abu-zaiton

Al-albayt University, Department of Biological Sciences

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Abstract

The aims of this study was to evaluate the effects of *Silybum marianum* extract on blood glucose level, cholesterol, liver enzymes and kidney functions in diabetic rats. Alloxan-induced diabetic rats were injected intraperitoneally separately with low dose (200 mg/kg), high dose (400mg/kg) of extract for 14 days. Treatment with *S.Marianum* for diabetic rats at dose 400 mg/kg led to a major reduction in fasting serum glucose about 38.42% and 15% in amylase and 37% in cholesterol. The concentrations of the liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) were significantly (p<0.05) reduced by the injected extract. At dose 400 mg/kg of extract treated animals, creatinine concentration was decreased. The administration of extract at dose 400 mg/kg to diabetic rats was able to decrease glucose levels significantly (p<0.05) compared with dose 200 mg/kg as well as liver enzymes. It may be concluded that *S.Marianum* extract demonstrated remarkable anti-diabetic activity in alloxan induced-diabetic rats. It also could have protective effect against hepatic and renal disorders.

Keywords: Alloxan, diabetic rats, *Silybum Marianum*, Hepatic enzymes
1. Introduction

Diabetes mellitus is one of the chronic diseases resulting either from insulin secretion due to an autoimmune disorder leading to deficiency of pancreatic $\beta$-cells to produce type I diabetes or insulin resistance which may be caused by defects in signal transduction leading to abnormal insulin receptors which produce type II diabetes resulting in hyperglycemia[1]. Internationally, around 346 million people had diabetes according to World Health Organization reports, in which 1% of them in the Western countries and 10% of them from world population [2].

Epidemiological studies in Jordan have suggested that the number of diabetics is high and continuous increasing. With comparison to 1996, 15% of Jordanian adults had diabetes during the year 2004 with an increase of 7%, also children aged between 0 to14 years considers the lowest but rising [3]. Many studies indicate that about half of the patients with type II diabetes are still undetected [4].

In most countries, diabetes is considered one of the main reasons for hospitalization. Diabetes is viewed as one of the main causes for diseases and death, mainly through the increased danger of vascular complications [5].

Although the endless risk of diabetes on the body, many scientists indicated that diabetes mellitus is under control of diet, exercise and chemotherapy, but the problem with the chemotherapy, the high cost of pharmaceutical drugs, toxicity and undesirable side effects or contraindications, and clinical limitation are some of the risks in case of modern medicine [6].

Nowadays, because the high cost of pharmaceutical drugs and the side effect of it on the body, many researchers are interested in the use of herbal drugs. It has been estimated that about 800 plants are involved in alternative medicine to treat diabetes [7, 8]. The anti-diabetic therapeutic potential of herbs has been assessed experimental models [9].

One of these herbs is *Silybum marianum*, it is an annual or biennial herb belongs to the family Asteraceae. Its common name is Milk thistle. This plant has been used as extract for liver diseases [10]. The main active ingredients are alkaloids, flavonoids, saponin, tannin, phenolic compounds and many more collectively known as Silymarin available commercially as standardized extract .there are various diseases treated by this plant including many hepatic diseases which are characterized by necrosis and functional loss [11]. *S.marianum* has various effects including antioxidant, against inflammation, immunomodulatory, antiviral and hepatoprotective influences. It reduces incidence of certain forms of cancer and other treatment properties [10]. Recently, it has been demonstrated that the protective effect of *S.marianum* is most likely because it's free- radical scavenging activity [12]. Silybum extracts did not cause any significant side effect. [13, 14].
The current study however attempts to explore the biochemical effect of *S. marianum* on blood sugar, cholesterol, liver and kidney functions. So, the present study is performed to study the proposed mechanism beyond antihyperglycemic influences of *S. marianum* on alloxan induced diabetic rats.

2. Materials and Methods

*Plant extraction:* The aerial parts of plants were prepared by thorough washing with water and then dried by air in shade at room temperature. Then, it mechanically powdered and sieved. 40g of powdered leaves were prepared for 12 hours in 200 mL of ethanol. The extracts were then filtered by Whitman filter paper. The collected extract was evaporated by special apparatus (rotary evaporator) to remove the solvent. Pure and dried ethanol extracts of the samples were refrigerated until used. The extractive yield were found 8.25% ethanol extract of *Silybum marianum*.

*Experimental Animals:* male Sprague Dawley rats 190–220g were used throughout the studies. They were kept in standard environment condition, supplied standard pellets and water ad libitum. All the rats were kept in cages and retained with natural 12 hour light and dark cycle. The research was conducted at Jordan University of Science and Technology, animal house unit.

*Induction of alloxanic diabetes:* alloxan (Sigma Chemicals, USA) was dissolved in normal saline at a dose of 140 mg/kg. Rats were injected intraperitoneally [15]. Following injecting rats with alloxan by one hour, rats were allowed to be fed standard pellets and water ad libitum. After that, the experimental rats were administered 20% glucose solution for 24 hours to prevent hypoglycemia. After 72hr of Injection, fasting blood glucose level (estimated by glucometer) was measured. Animals with blood glucose concentration over 200 mg/dl were selected for the study.

*Design of the experiment:* In this experiment study, rats were assigned randomly into four groups (N=6): Group I: control rats. Group II: diabetic rats. Group III: Diabetic rats treated with alcoholic extract (200mg/kg) .Group IV: Diabetic rats treated with alcoholic extract (400mg/kg). Changes in body weight were observed once a week. After 14 days of experiment, weights of body measured and the rats were sacrificed.
**Sample collection:** At the end of experiment, the rats were anesthetized and blood sample was collected by heart puncture in fresh vials containing anticoagulant agent. The blood sample centrifuged at 3000 rpm for 15 min, and was analyzed for cholesterol, creatinine, urea, AST, ALT, Albumin and blood glucose level.

**Statistical analysis:** Data were represented Mean ± SD. Variations between groups were tested for significance using one way ANOVA followed by Tukey test. Significance was considered at $P \leq 0.05$.

2. Results

Table 1 shows the range of blood sugar and pancreatic amylase in study groups. The results showed that there was a significant ($P < 0.05$) increase in blood glucose and amylase concentration in diabetic groups. The administration of extract (200mg/kg, 400 mg/kg) for rats with diabetes was able to restore blood glucose and decrease the concentration of amylase enzyme significantly. The administration of extract at dose 400 mg/kg reveals more reduction in blood glucose more than extract dose 200 mg/kg.

Rats with induced diabetes had significant ($P < 0.05$) increase levels of total cholesterol. The administration of extract at dose 400 mg/kg decreases the level of cholesterol significantly ($P < 0.05$). It was also shown that administration of extract in both doses led to restoring body weight changes.

Table 1. Blood glucose, pancreatic amylase, cholesterol and changes in body weight among study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Glucose (mg/dl)</th>
<th>Amylase (U/L)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial</td>
<td>final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>203.2 ±22.4</td>
<td>229.5 ±16.3</td>
<td>88.2±12.8</td>
<td>69.8±11.5</td>
</tr>
<tr>
<td>Alloxan</td>
<td>218.4 ±17.2</td>
<td>198.2 ±21.9*</td>
<td>386.5±24.9*</td>
<td>143.2±12.6*</td>
</tr>
<tr>
<td>Allox+Ex 200</td>
<td>213.1 ±15.9</td>
<td>208.6±13.6</td>
<td>255.6±21.8</td>
<td>125.4±10.8</td>
</tr>
<tr>
<td>Allox+Ex 400</td>
<td>220.4 ±17.1</td>
<td>211.8±13.9</td>
<td>238.5±19**</td>
<td>121.6±9.8**</td>
</tr>
</tbody>
</table>

* indicates significant difference ($P<0.05$). ** indicates significant difference ($P \leq 0.05$) from the alloxan group. Allox:Alloxan, Ex:Extract.

The changes in the activities of hepatic enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and kidney functions parameters: creatinine and urea of study groups are presented in Table 2. An obvious increase in the Albumin, AST and ALT level was noticed in diabetic animals. Both doses of extract lead to significant reversed changes in the activities of liver enzymes AST and ALT. The results showed that extract at dose 400 mg/kg to diabetic rats to have stronger effect on liver enzymes compared with dose 200mg/kg.
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The diabetic rats exhibited significant ($P < 0.05$) increase in the creatinine and urea concentration at end of experiment. In extract dose 400 mg/kg treated animals, creatinine concentration was decreased after 14 days of experiment to normal levels. On the other hand, $S.marianum$ (200 mg/kg) was shown to have more effects in retaining urea concentration.

Table 2: The effect of $S.marianum$ extract and metformin on liver enzymes concentration and kidney function parameters of normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Albumin (g/dL)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>Creatinine (mmol/L)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.1±1.9</td>
<td>23.4±2.9</td>
<td>26.2±3.5</td>
<td>0.42±0.1</td>
<td>44.27±4.1</td>
</tr>
<tr>
<td>Alloxan</td>
<td>17.0±2.8*</td>
<td>48.6±5.8*</td>
<td>49.53±5.1*</td>
<td>1.97±0.2*</td>
<td>86.21±5.3*</td>
</tr>
<tr>
<td>Allox+Ex 200</td>
<td>22.8±3.1</td>
<td>41.7±4.5</td>
<td>42.4±4.9</td>
<td>1.55±0.2</td>
<td>53.24±4.7</td>
</tr>
<tr>
<td>Allox +E 400</td>
<td>25.4±3.8</td>
<td>39.4±3.9**</td>
<td>37.54±3.7</td>
<td>1.46±0.1**</td>
<td>73.53±5.1</td>
</tr>
</tbody>
</table>

* indicates significant difference ($P \leq 0.05$) from all other groups. ** indicates significant difference ($P \leq 0.05$) from the alloxan group. Allox: Alloxan, Ex: Extract, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

4. Discussion

Diabetes Mellitus is endocrine disorder which can be considered as the biggest impact on adults of working age in developing countries and major cause of high economic loss [16]. It has been pointed to the environment of numerous numbers of medicinal plants with long history in folk use in management of diabetic patient [17]. One such compound that has recently been the subject of active research is $Silybum marianum$.

In the present study, diabetes were induced diabetes in rats by injecting alloxan intraperitoneally. The cytotoxic effects of alloxan are due to its active damaging insulin secreting β-pancreatic cells, leading to reduce endogenous insulin release, and lower intake of glucose by the cells which leads to increases blood sugar level [18]. Administration of extract at dose 200 mg/kg and dose 400 mg/kg led to a major reduction in fasting serum glucose about 33.86%, 38.42% mg/kg respectively in blood glucose, at the end of experiment, reduction in amylase 12-15%, and total cholesterol 30-37% concentrations in each of the extract supplementation trials. The capacity of extract lower increased blood glucose concentration significantly ($P < 0.05$) within normal limits is considered crucial for the liver to restore its normal homeostasis in experimental diabetic rats. $S.marianum$ extract effect was potentiating the function of β-cells in sugar metabolism. It is may have an unidentified agent that improves the function of insulin in sugar metabolism. Such hypoglycemic effect of extract may increase the
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Effect of insulin in sugar metabolism in diabetic animals by making fat cells to respond more to insulin and it is highly possible to activate the enzyme that causes insulin to bind to cell receptors and to inhibit the enzyme that makes to block this process leading to increase insulin sensitivity.

Taking together, it is plausible to postulate that mechanism for the anti-diabetic potential of S.marianum is partly due to stimulate insulin secretion, release as well as regeneration of β-cells of Langerhans islets, activation of enzymes responsible for glucose utilization.

Administration of extract decreased the concentrations of liver enzymes AST, ALT and kidney functions parameters: creatinine and urea in diabetic rats. The concentration of plasma glucose was found to decrease significantly in diabetic rats treated with both extract doses, which is thought to be a result of the significant (P < 0.05) decrease in the level of liver enzymes, creatinine and urea [Table 2]. The decrease in the activities of gluconeogenesis leads to the decreased level of glucose in blood. It is hypothesized that a sequential metabolic association between increased glycolysis and decreased gluconeogenesis induced by S.marianum points to the possible biochemical mechanism through which sugar homeostasis and liver enzymes are maintained. [19]

Liver enzymes such as AST, ALT are released to circulation when liver cells are damaged by alloxan including muscle injury and viral hepatitis as well as cardiac problem. Increased levels of AST and ALT are indicative of cellular damage and make disorder in the functional activity of liver cell membrane. Albumin concentration on other hand is due to the function of liver cell. Increased albumin in serum is due to more synthesis, in the availability of increasing biliary pressure [20].

5. Conclusion

This investigation supports that Silybum marianum extract have anti-diabetic activities and effective in decreasing concentrations of blood glucose and cholesterol levels in diabetic rats. Results demonstrate positive effect on liver and kidney function. Administration of extract likely represents a safe and effective means to reduce the risk factors for the development of diabetic complications. More research on S.marianum should be undertaken because it has new potential management of diabetes and liver disorders.

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