Effect of proteinous compounds from Portulaca oleracea L. plant on some biochemical parameters in mice

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Abstract
Two proteinous compounds (A and B) were isolated by gel filtration chromatography of the acetone precipitate of the aqueous extract of *Portulaca oleracea* L. plant. Comparative apparent molecular weights determination by gel filtration using standard proteins gave a value of 28184 and 2291 dalton respectively. The effect of the isolated proteinous compounds A, B and the hormone of insulin on some biochemical parameters such as blood glucose, cholesterol, triglycerides, total lipids levels and glycogen content in liver tissues was studied in fasting mice. A single intraperitoneal injection of the isolated compounds (A, B) at a dose of 77.5 mg/kg B.W. was given to normal mice which showed a significant decrease of serum glucose, cholesterol, triglycerides, total lipids level and glycogen content in liver tissues when compared to the controlled group, whereas insulin and compound B showed a significant increase in glycogen content of liver tissues.

Introduction
Managing the diet and/or pharmacological therapies are used in the treatment of diabetes mellitus to normalize the metabolic activity of glucose level [1]. A great deal of attention has been recently focused on the therapeutic use of traditional medicinal plants. In folk medicine, many plants provide a vast resource of novel compounds with potential for the development of new antidiabetic drugs [2]. *Portulaca oleracea* L. (portulacaceae) is a medicinal plant recommended by Ayurvedic for cooling, alterative, diuretic, antispasmodic, useful as an article of diet in scurvey, liver diseases and urinary affections. It has also been used for sore nipples and mouth ulcers [3,4] , and antidiabetic sufuf called (A).
QURS ZIABITUS [5]. This plant is commonly known as Baglatul humga, kurfa and purslane [3]. However the plant grows as a weed in the fields, vegetable plots, on roadside verges and by the sea [6]. P. oleracea extracts have an effect on alloxan diabetes [7]. The plant is recommended as a cholesterol lowering agent [8].

This study was carried out to investigate the isolation of proteinous compounds from the aqueous extract of the whole Portulaca oleracea L. plant above the ground and the hypoglycemic activity and other parameters on mice and, therefore, to determine their molecular weights.

Molecular weight determination and hypoglycemic activity may give some idea about structural similarity and the mode of action compared to insulin (insulin-like substance).

Materials and Methods

Plant material: Portulaca oleracea L. plant was purchased from the local market. It was classified according to plant taxonomy and plant classification [9, 10].

Preparation of crude extract:

The aqueous crude extract was prepared by freezing and thawing the plant (1000g) with liquid nitrogen several times after being homogenized for five minutes using a blender to rupture the cell membrane.

A ratio of 3:1 v/w of distilled water was added and the crude homogenate was stirred for additional two hours in ice bath, then kept in a refrigerator overnight. The mixture was filtered through several layers of moselin (cheese-cloth) to remove all the residual materials. Finally the filtrate was then centrifuged using a refrigerated centrifuge for 15 minutes at 12000xg. The supernatant (crude extract), after reducing its volume to about 1/3 by lyophilization, was kept for further investigation. Total protein concentration was determined by a modified lowry method. [11].

Precipitation of the protein:

The proteinous substance was separated from the crude extract by cold acetone precipitation technique[12]. Cold acetone was added to the crude extract (720 ml) at a ratio of 40:60 V/V with slow stirring at 0°C. The mixture was left for 24 hours at 0°C and the precipitated protein was isolated by cold centrifugation for 15 minutes at 12000xg. The proteinous precipitate was dried by lyophilization to obtain a solid substance.

Fractionation of the total protein:

The isolated proteinous precipitate was fractionated by gel filtration chromatography using Sephadex G75 on a 4.5x92 cm column with distilled water as eluent. Each peak component (A and B) was pooled, frozen then dried by lyophilization in air-tight sample tube in a freezer (-30°C) for further investigation.

Intraperitoneal injection:

Groups of healthy male adult mice weighing 30–35g were obtained from the animal house of the College of Veterinary Medicine, University of Mosul. The mice’s fasting period was 16 hr and divided randomly into four groups each containing five mice. Group one was kept as a control group while the other three groups were injected intraperitoneally (77.5 mg/kg B.W.) with the fractionated proteinous compounds A and B (Groups 3 and 4) and (6.64 iu/kg B.W.) with insulin, (Actrapid 40 iu/ml Novo Nordisk A/S, Denmark) as group 2. After two hours of injection, blood samples were collected for analysis by the orbital sinus puncture under ether anaesthesia, using non-heparinized microhematocrit capillary tubes [13].

Determination of biochemical parameters:

Serum blood glucose, cholesterol and triglycerides levels were measured according to the enzymatic methods using Randox Kit for glucose, U.K.,[14] and bioMerieux for cholesterol and
triglycerides Kits, France [15, 16]. Total lipids levels were determined by the method of Chabrol and Chardonnet [17]. Glycogen content in the liver tissues was estimated by anthrone method [18].

**Statistical analysis:**

Results were expressed as mean ± S.E., and estimation of the significance of difference between control and proteinous compounds, insulin- treated groups was determined by student’s t – test [19].

The percentage of glycemic variation after two hours of injection for treated groups was calculated by applying the formula:

\[
\text{% Change of glycemia} = \frac{G_x - G_c}{G_c} \times 100
\]

where Gc and Gx are the values of control and glycemia after two hours [20]. This formula was applied for other parameters.

**Results and Discussion**

**Precipitation of the protein:**

Precipitation of total proteins from the crude extract was accomplished by cold acetone technique [12], but not by saturated ammonium sulphate technique [12], since the former can be easily removed by evaporation, besides the fact that the precipitating power of both reagents was similar. Moreover, dialysis of the proteinous fraction to get rid of ammonium sulphate may remove some of the low molecular weight proteins or peptides. The protein content of the precipitate was determined [11] and found to be 0.6841 % in the crude extract, then the efficiency of this method for the precipitation of the protein was 38.88 %, because the dried weight of the proteinous substance obtained after lyophilization of the isolated proteinous precipitate was 2.6551 g.

**Fractionation of the total protein:**

This was accomplished by gel filtration chromatography using Sephadex G75 to give two peaks (A and B) with an elution volumes of 815 ml and 1511 ml, respectively (Figure 1). Quantitative determination of total protein in each peak after gel filtration chromatography was performed by the modified lowry method and then the percentage of each component (peak) was obtained. The results of these components A and B were 46.15 % and 22.15 %, respectively in the proteinous substance. The comparative molecular weight of each component (A and B) was determined by gel chromatography on a pre – calibrated column using known molecular weight proteins, and was found to be 28184 and 2291 dalton, respectively.
Figure 1 Elution profile of total protein obtained by acetone precipitation on Sephadex G75 was used on a column of 4.5x 92 cm. Distilled water was used as eluent at flow rate 125 ml/hr. The arrows A and B represent the elution volumes of the top peaks A and B.

Effect of isolated compounds on some metabolic parameters:
Isolation and ability of the proteinous compounds of *Portulaca oleracea* L. Plant to reduce the level of serum glucose in normoglycemic mice and other parameters have never been studied before. The results of intraperitoneal injection with the isolated compounds A, B and insulin in mice are listed in Table 1.

Results depicted from Table 1 indicate that in comparison to the control group there was a remarkable decrease in the level of glucose in blood serum for two groups of fasting mice injected intraperitoneally (77.5 mg/kg B.W.) with each of the isolated compounds A and B. On the other hand, insulin was more effective in lowering serum glucose level for normal mice.

The result of treating normal mice with insulin showed a decrease in serum blood glucose level which was in agreement with many studies in normal individuals [21], in normal rats [22,23], and in broiler chicks [24]. The hypoglycemic effect of insulin may be due to the increase in the rate of entrance of various sugars and glucose into the cells through increasing the number of glucose transporters in the plasma membrane [25]. The results of compounds A and B showed a decrease in blood glucose level which is significant (P<0.001) for A but not for B compared to the control group, these results are in good agreement with the previous work on the hypoglycemic activity of the proteinous compounds isolated from the aqueous extract of other local plants. The results of ingestion of component A which was separated from *Salvia syriaca* plant showed a significant decrease of 36% in serum glucose level chicks [27]. Furthermore, the effect of the
crude, proteinous and non proteinous aqueous extracts of *Salvia syriaca* and *Brassica Campestris Linn* Var.rapa root showed a significant decrease in serum glucose level [26 - 28]. Thus, it may be concluded that these proteinous compounds potentiate insulin action on carbohydrate metabolism. It is suggested that the mechanism of action of the low molecular weight protein isolated from different local plants was similar to insulin in structure and action [13,26].

The decrease in serum glucose level of mice treated with high molecular weight protein compound (compound A) was in agreement with the results obtained by other investigators [13, 29, 30]. This suggests that the protein compounds with high molecular weight which were isolated from the aqueous extract of different plants (*Morus nigra L., Myrtus communis L., Erigeron bonariensis L., Peganum harmala L., Nigella sativa L., Pistacia atlantica & Cucurbita pepo Var condensa*) might contain sequence of amino acid similar to insulin which binds to specific insulin receptors located on the plasma membrane, this binding might mediate or facilitate the rate of uptake of glucose to the inside of the cell leading to hypoglycemic activity in mice. In addition, this hypoglycemic effect of the proteinous compounds is in agreement with the results obtained for a peptide, polypeptide isolated from fruit, seeds of *Momordica charantia* in patients with juvenile diabetes [31] and in normal and diabetic mice [32].

The decrease in cholesterol level for compounds A, B and insulin is found in Table 1. These results are in agreement with normoglycemic chicks ingested with the proteinous compounds isolated from *Salvia syriaca* aqueous extract [27]. This decrease in cholesterol level might be due to the inactivation of the regulatory enzyme β-hydroxy-β-methyl glutaryl – CoA reductase responsible for cholesterol biosynthesis [33]. The decrease is also in agreement with the hypocholesterolemic properties of fenugreek seeds on mice [34]. On the other hand, the decrease of cholesterol level when treated with insulin is in agreement with the results obtained from diabetic rats and rabbits [35, 36]. This might due to the inhibiting intestinal acyl CoA: cholesterol acyl transferase which is responsible for absorbing cholesterol from the intestine [37].

Triglycerides and total lipids levels also affected the isolated compounds A and B in the same table. The results show a significant (P<0.001) decrease in both triglycerides and total lipids levels. These results are in agreement with those of decreasing total lipids for the proteinous extract of *Olea Europaea* leaves [38], and decreasing cholesterol, triglyceride levels of the aqueous extract and seeds of *Trigonella foenum – graecum* for rats [39,40] and for rabbits [41]. This decrease might be due to the inhibition of lipase enzyme and preventing lipolysis of stored lipids [42, 43].

Finally, glycogen content of liver tissues was increased when the mice were injected intraperitoneally with the isolated compound B and insulin (Table 1). The increase in glycogen content is in agreement with the results obtained by other investigators for the insulin releasing effect of *Momordica charantia* seeds [44] and for insulin effect for increasing glycogen content in liver and muscle chicks and rats [43,45]. This increase might due to the activation of glycogen synthetase enzyme and inhibition or deactivation of phosphorylase kinase and glycogen phosphorylase enzymes [46], whereas the decrease for proteinous compound A is in agreement with the results obtained by other investigators for aqueous extract of garlic, onion, and *Aegle marmelose* plants [47, 48] and for the protein isolated from *Eminium spiculatum* leaves and *Brassica campestris Linn* Var. rapa root [49, 28]. This decrease might stimulate glycogenolysis due to the decrease in blood glucose which
activates glucagon hormone secretion [50, 51].

In conclusion, *Portulaca oleracea* L. plant contain active compounds with insulin - like action and / or structure, and having a significant depression of cholesterol, total lipid and triglycerides level and glycogen content in liver tissues.

Further experiments are now under current investigation in our laboratories to support the hypothesis that hypoglycemic plants may contain a compound having insulin like action and / or structure.

References

7. Stefanov, Zh., Ilarionov, I., Kolev D., Famatsiya ( Sofia), 1966, 16 (3), 27.
46. Fain J.N. Metabolism, 1984, 33, 7, 672.
**Table 1** Effect of proteinous compounds A, B separated from Portulaca oleracea L. plant and insulin hormone on serum glucose, cholesterol, triglycerides, total lipids levels and glycogen content of liver tissues in fasted mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample</th>
<th>Glucose level (mg/dL)</th>
<th>Cholesterol level (mg/dL)</th>
<th>Triglycerides level (mg/dL)</th>
<th>Total lipids level (mg/dL)</th>
<th>Glycogen content in liver tissues (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MEAN±SE</td>
<td>% Change</td>
<td>MEAN±SE</td>
<td>% Change</td>
<td>MEAN±SE</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>146±13.383</td>
<td>-</td>
<td>188±4.347</td>
<td>-</td>
<td>147±1.140</td>
</tr>
<tr>
<td>2</td>
<td>Insulin</td>
<td>14±1.414</td>
<td>90.410</td>
<td>170±2.07</td>
<td>3</td>
<td>139±1.224</td>
</tr>
<tr>
<td>3</td>
<td>Component A</td>
<td>63±2.949</td>
<td>-56.849</td>
<td>154±2.701</td>
<td>-18.085</td>
<td>90±1.516</td>
</tr>
</tbody>
</table>

- Significantly different from control to t-test at *P<0.05, **P<0.01, ***P<0.001.
- N = Number of mice in each group.