The Effect of Protein Fractions of Avocado (Persea americana) on Biochemical Parameters in a Diabetic Rat Model

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Abstract

Introduction: The separation and inspection of the active protein constituents from the aqueous extract of avocado (Persea americana) by employing distinct biochemical ways is the primary target of this investigation. Material and Methods: Two compounds (A and B) were precipitated by ammonium sulfate and separated through gel filtration chromatography. Results: The glucose level decreased, compared to the oral administration because of the intraperitoneal administration of the protein fraction (B) and the concentrated aqueous extract. The molecular mass of the separated active protein fraction (peak B), was determined as 24,000 Daltons. Besides, certain blood components of alloxan-induced diabetic rats and normal rats were measured under the impact of the protein fraction B and the aqueous extract in doses of 50, 75, 100 and 125 mg per kilogram of body mass. The protein compound and the crude aqueous extract of the avocado with a dosage of 75 mg per kilogram of body weight significantly diminished the fasting blood sugar level in the normal rats compared to the normal set. Additionally, at the same dosage, a significant reduction in the serum total lipid and cholesterol levels was noticed. A noteworthy decline in the serum glucose, cholesterol, and total lipid levels in the diabetic rats, was beholden using the active protein in a dose of 75 mg/kg of body weight. Conclusion: This study showed that avocado (Persea americana) has the potential to be used for diabetes therapy due to active protein components.

Keywords: Avocado; Persea Americana; diabetes disease.

Introduction

Nearly 150 kinds of avocado can be found in tropical and subtropical regions, including Persea americana [1]. In ethnomedicine, the seed of the avocado was utilized in varied applications, including treatment for dysentery, diarrhea, intestinal parasites, toothache, as well as skincare and in the beauty manufacture. Several health advantages, including weight loss and controlling human weight, have been exploited from the seed oil, in particular when applied by obese for weight loss [1-3]. A recent review reports the impact of avocado (Persea americana) on distinct elements of the metabolic syndrome, such as a combination of hazard parameters, like elevated cholesterol, blood sugar, body mass index, and blood pressure. All these parameters may cause a rise in the hazard of cardiovascular diseases and type 2 diabetes.

Avocados possess the most favorable impact on lipid profiles, with alterations of HDL-cholesterol, LDL-cholesterol, triglycerides, phospholipids, and total cholesterol. However, the seed, peel, leaves, and flesh of avocados have varied impacts on elements of the metabolic syndrome [4].

The primary target of this research was to study the impact of the protein constituents separated from the aqueous extract of avocado (Persea americana) on biochemical specifications in an experimental animal model with the finding of an active constituent, harboring insulin-like structure or action.
Material and Methods

The crude aqueous extract preparation

Avocado (Persea americana) acquired from the local market in Iraq, weighing about 0.75 kg, cut in small sizes, combined with cold distilled water (DW) in a ratio of 1:3 w/v, and subsequently blended evenly for five minutes, using a mixer. The untreated, resulting material was mixed while on the ice, for another two hours, and subsequently kept in a refrigerator during the night.

Afterward, the blend was passing through some layers of filter to separate all remaining components. Ultimately, for isolating the supernatant, the mixture or the filtrate was then spun down at 8000 x g in a cold centrifuge for 15 minutes. Then, the supernatant was lyophilized in order to reduce the volume to about 1/3 for further use. The Lowry method was used to assess the total protein.

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Precipitation of the proteins

The ammonium sulfate precipitation was exploited to separate the active protein from the cold extract, and then, while stirring at 0°C, the addition of the cold crude aqueous extract was performed at a ratio of 75:100 w/v. The combination was kept in a fridge for 24 h, and then the mixture was spun down at 8000 x g for 15 minutes to separate the precipitated protein. Then, lyophilization was used to dry the precipitate. The sample was maintained at -20°C for further use.
**Fractionation of total protein**

Gel filtration chromatography was employed for the fractionation, using Sephadex G75, resulting in one considerable peak, by the elution volume of 411 ml (Figure 1).

The protein level per peak was quantitatively measured after gel filtration chromatography, resulting in 15.9% and 46.1% proteins in peak A and B, respectively.

A pre-calibrated column having known protein molecular weight (MW) was used after gel filtration chromatography to determine the MM of the protein, as demonstrated in Table 1. The MW of the protein in peak B and A was estimated at 23000 and 42000 D, as shown in Figure 2.

**The effects of the isolated protein component and the crude aqueous extract on glucose, total lipids, and cholesterol after intraperitoneal administration in normal rats**

The crude aqueous extract and associated protein impacts on total lipids, cholesterol, and glucose, in healthy rats are shown in Table 2.
Table I: Molecular weights and the associated elution volumes of separated proteins on a Sephadex G 75.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular weight (Dalton)</th>
<th>Elution volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue dextran</td>
<td>2000000</td>
<td>121</td>
</tr>
<tr>
<td>Bovine serum albumin (BSA)</td>
<td>67000</td>
<td>246</td>
</tr>
<tr>
<td>α- amylase</td>
<td>58000</td>
<td>324</td>
</tr>
<tr>
<td>Eggs albumin</td>
<td>45000</td>
<td>345</td>
</tr>
<tr>
<td>Pepsin</td>
<td>36000</td>
<td>375</td>
</tr>
<tr>
<td>Insulin hormone</td>
<td>5750</td>
<td>418</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>204</td>
<td>446</td>
</tr>
</tbody>
</table>

Table 2: Intraperitoneal administration of the crude aqueous extract and associated isolated protein components and the influences on serum total lipids, cholesterol and glucose, in normal rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mmol/L)</th>
<th>Cholesterol (mmol/L)</th>
<th>Total lipids (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.70 ± 0.435</td>
<td>2.90 ± 0.158</td>
<td>487.1 ± 11.762</td>
</tr>
<tr>
<td>Crude aqueous extract</td>
<td>3.50 ± 0.506*</td>
<td>2.44 ± 0.20</td>
<td>403.8 ± 99.97</td>
</tr>
<tr>
<td>peak A fraction at 125 mg/kg</td>
<td>3.94 ± 0.19</td>
<td>2.65 ± 0.10</td>
<td>413.5 ± 78.90</td>
</tr>
<tr>
<td>peak A fraction at 100 mg/kg</td>
<td>3.90 ± 0.25</td>
<td>2.50 ± 0.13</td>
<td>409.5 ± 78.78</td>
</tr>
<tr>
<td>peak A fraction at 75 mg/kg</td>
<td>3.76 ± 0.39</td>
<td>2.21 ± 0.21</td>
<td>393.9 ± 70.30</td>
</tr>
<tr>
<td>peak A fraction at 50 mg/kg</td>
<td>3.75 ± 0.32</td>
<td>2.16 ± 0.29</td>
<td>383.8 ± 73.53</td>
</tr>
<tr>
<td>peak B fraction at 125 mg/kg</td>
<td>3.76 ± 0.53</td>
<td>2.61 ± 0.14</td>
<td>377.5 ± 51.77*</td>
</tr>
<tr>
<td>peak B fraction at 100 mg/kg</td>
<td>3.71 ± 0.54</td>
<td>2.58 ± 0.17</td>
<td>371.5 ± 31.66**</td>
</tr>
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</tbody>
</table>

Note: * Significant difference at P<0.05; ** Significant difference at P<0.001.

Table 3: Effects of oral administration of the isolated protein compound and the crude aqueous extract on the lipid profile and serum glucose in normal rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mmol/L)</th>
<th>Cholesterol (mmol/L)</th>
<th>Total lipids (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.70 ± 0.435</td>
<td>2.90 ± 0.158</td>
<td>487.1 ± 11.76</td>
</tr>
<tr>
<td>Crude aqueous extract</td>
<td>4.25 ± 0.33*</td>
<td>2.53 ± 0.26</td>
<td>435 ± 51.5</td>
</tr>
<tr>
<td>Peak A fraction at 125 mg/kg</td>
<td>4.76 ± 0.07</td>
<td>2.84 ± 0.16</td>
<td>457 ± 63.10</td>
</tr>
<tr>
<td>Peak A fraction at 100 mg/kg</td>
<td>4.75 ± 0.21</td>
<td>2.82 ± 0.19</td>
<td>456 ± 68.00</td>
</tr>
<tr>
<td>Peak A fraction at 75 mg/kg</td>
<td>4.77 ± 0.74</td>
<td>2.78 ± 0.16</td>
<td>435 ± 78.12</td>
</tr>
<tr>
<td>Peak A fraction at 50 mg/kg</td>
<td>4.76 ± 0.77</td>
<td>2.71 ± 0.17</td>
<td>438 ± 79.17</td>
</tr>
<tr>
<td>Peak B fraction at 125 mg/kg</td>
<td>4.76 ± 0.90</td>
<td>2.71 ± 0.13</td>
<td>433.4 ± 76.10</td>
</tr>
<tr>
<td>Peak B fraction at 100 mg/kg</td>
<td>4.67 ± 0.86</td>
<td>2.78 ± 0.12</td>
<td>438.4 ± 71.33</td>
</tr>
</tbody>
</table>
After the administration of the protein fraction from peak A, no significant decline was beholden in the serum glucose in normal rats. 

Oral administration of the crude aqueous extract, the isolated protein component and the effects on total lipids, cholesterol and glucose in normal rats

The data regarding the influence of protein components and the crude aqueous extract on total lipids, glucose, and cholesterol in normal rats are presented in Table 3. The data showed no significant decrease in fasting blood sugar (FBS) in normal rats due to the protein fraction (Peak A, peak B).

The intraperitoneal administration of the isolated protein compound and the crude aqueous extract, and the influences on lipid profile and glucose, in diabetic rats

The objective was to examine the impact of the protein compound of active peak and the crude aqueous extract of avocado (*Persea americana*) on serum cholesterol, total lipids, and glucose in alloxan-induced diabetic rats. Alloxan can induce diabetes by damaging the Langerhans cells, causing a reduction in the generation and secretion of insulin [4, 6]. The data regarding the impact of intraperitoneal administration on blood parameters in diabetic rats are presented in Table 4.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mmol/L)</th>
<th>Cholesterol (mmol/L)</th>
<th>Total lipids (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.6 ± 1.019</td>
<td>3.02 ± 0.680</td>
<td>657.15 ± 29.747</td>
</tr>
<tr>
<td>Crude aqueous</td>
<td>20.6 ± 3.65*</td>
<td>2.14 ± 0.43</td>
<td>478.51 ± 32.92*</td>
</tr>
<tr>
<td>Peak B fraction at 125 mg/kg</td>
<td>23.2 ± 4.13</td>
<td>2.37 ± 0.43</td>
<td>485.53 ± 15.62*</td>
</tr>
<tr>
<td>Peak B fraction at 100 mg/kg</td>
<td>20.5 ± 4.24</td>
<td>2.32 ± 0.72</td>
<td>487.34 ± 13.32*</td>
</tr>
<tr>
<td>Peak B fraction at 75 mg/kg</td>
<td>17.6 ± 8.10*</td>
<td>1.71 ± 0.37*</td>
<td>294.40 ± 32.38**</td>
</tr>
<tr>
<td>Peak B fraction at 50 mg/kg</td>
<td>25.6 ± 3.81</td>
<td>2.58 ± 0.81</td>
<td>473.40 ± 35.73*</td>
</tr>
</tbody>
</table>

Note: * Significant difference at P≤0.05; ** Significant difference at P<0.001.

Table 4: The influence of the isolated protein compound and the crude aqueous extract on serum total lipids, cholesterol, and glucose in diabetic rats.

Discussions

Intraperitoneal administration of the crude aqueous extract and associated isolated protein components and the influences on serum total lipids, cholesterol and glucose, in normal rats

A precious decline in the FBS level was attained after intraperitoneal administration of the crude aqueous extract of avocado (*Persea americana*), compared with the control group.

A regular avocado-containing diet results in less insulin requirement. Also, when body mass index (BMI, a scale for obesity) elevates, the favorable impacts on insulin requirements, improve even further [4]. In overweight people, the ratio of C-peptide/insulin is also lower, showing that the clearance speed of insulin from the liver is increased. This is happening in addition to the insulin-lowering characteristics of avocado-containing meals, which leads to a decrease in blood sugar levels [10].

The blood level of C-peptide is an indicator of the insulin produced by the pancreas. The pancreas makes proinsulin, which is split into insulin and C-peptide and then secreted into the circulation. Therefore, a low level of C-peptide signifies that a lower amount of insulin has been produced in the blood. As a result, the level of total lipids and cholesterol decreased in the blood, in comparison with the control group. The low cho-
Lesterol levels are a result of less cholesterol accumulation in the body, and high disintegration and discharge through the feces [4, 5].

Our data have also indicated that the administration of the protein component of peak B of avocado \((Persea americana)\) with a dosage of 75 mg/kg, has resulted in the maximum decline (64%) in the FBS level compared to the control set, as shown in Table 2. There is a chance that the decline might be the outcome of the insulin-like activity of the protein component of avocado \((Persea americana)\) [4] or due to the insulin-like shape of the protein component that attaches to insulin receptors and declines the FBS level. The protein component of peak B with a dosage of 75 mg per kilogram of body weight, presented in the same table, demonstrated a precious drop in total lipids and cholesterol. This might be the consequence of the inhibition of cholesterol production or a rise in the percentage of cholesterol excretion from the body, and the insulin-like activity may aid in lowering the serum cholesterol level [5, 11].

Avocado is a good point of supply for minerals, carotenoids, phenols, fatty acids, and vitamins. The anti-hypertensive, lipid-lowering, anti-obesity, anti-diabetic, anti-atherosclerotic, anti-thrombotic, and cardioprotective effects of avocado have been shown in other studies [4, 12, 13].

**Effects of oral administration of the isolated protein compound and the crude aqueous extract on lipid profile and serum glucose in normal rats**

Table 3 reports that the administration of the protein component led to a high decrease in total lipids, glucose, and cholesterol in diabetic rats, as in the case of normal rats. Nonetheless, the peak B protein compound at a dosage of 75 mg/kg was more influential in decreasing the previously mentioned characteristics in diabetic rats, in comparison to the normal rats. This declined that the impact of this investigation could help reduce the risk of hyperinsulinemia (high insulin levels in the blood), a complication related to type 2 diabetes [14, 15]. Therefore, in the diet of patients with non-insulin-dependent diabetes mellitus, the complex digestible carbohydrates can be replaced by mono-unsaturated fatty acids like avocado, as the principal source, which leads to the significant improvement of the lipid profile, maintenance over a suitable glycemic control, and present a proper alternative in managing the disease [14].

Some studies reported the insulin-like action of protein compounds, simplifying the entrance of glucose inside the cells, and leading to an increase in the associated metabolism [16-18].

Following oral administration of the protein fractions (Peak A and B) and the crude aqueous extract of avocado \((Persea americana)\), an increase in the serum glucose was found. This might be due to the constituents such as proteins, polysaccharides, amino acid, and fats in the crude aqueous extract. As glycolysis, glycolysis, and gluconeogenesis become active, these compounds, after metabolic reactions, increase serum glucose. An increase in the serum glucose was also found after oral administration of the protein fraction, Peak A and peak B; the proteins may be inactivated by the proteolytic enzymes or broken down by the gastric juice.

Taken all together, the intraperitoneal administration of the protein fraction and the plant extract have a significant hypoglycemic effect compared to the oral administration, and it is the most recommended path.

**The influence of the isolated protein compound and the crude aqueous extract on serum total lipids, cholesterol, and glucose in diabetic rats**

As presented in Table 4, the administration of the protein component led to a high decrease in total lipids, glucose, and cholesterol in diabetic rats, as in the case of normal rats. Nonetheless, the peak B protein compound with a dosage of 75 mg/kg was more influential in decreasing the above-mentioned characteristics in diabetic rats, in comparison to the normal rats. This lowering impact of this investigation could contribute to the reduction of the risk of hyperinsulinemia (high insulin in the blood), a complication related to type 2 diabetes [14, 15]. Therefore, in the diet of patients with non-insulin-dependent diabetes mellitus, the complex digestible carbohydrates can be replaced by mono-unsaturated fatty acids like avocado, as the principal source, which leads to the significant improvement of the lipid profile, maintenance over a suitable glycemic control, and present a proper alternative in managing the disease [14]. Some studies reported the insulin-like action of protein compound, simplifying the entrance of glucose inside the cells, and leading to an increase in the associated metabolism [16-18].

**Conclusion**

In terms of novelty, this study showed that avocado \((Persea americana)\) has the potential to be used for di-
abetes therapy due to active protein components. Fur-
thermore, the glucose and lipid ratio was smaller after
having an avocado, demonstrating high liver potency
in insulin clearance. Avocado also has antioxidants,
such as carotenoids and vitamin C, which might con-
tribute to the insulin regulation, which is applicable in
reducing the blood sugar and lipids.

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Conflict of Interest

The author declares no conflict of interest.

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