Effect of *Abelmoschus esculentus* L. Bioactive Components on Blood Parameters and Histopathological Changes in the Kidney of Diabetic Albino Rats

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**Abstract:** Diabetes is a health problem spread all over the world and it is one of the main causes of death, resulting from high levels of glucose in the blood as a result of the inability of the beta cells in the pancreas to secrete insulin or the inability of the cells of the body to receive insulin properly. The biologically active compounds present in plant extracts may be a promising treatment and the best economical alternative for diabetic kidney patients.

The aim of the study was to prove the effect of the active compounds isolated from the okra (*Abelmoschus esculentus*) plant and its role in reducing blood glucose, creatinine and urea levels, as well as its role in alleviating histological changes in diabetic kidney patients. Twenty-four adult male white rats were used in this study. The rats were divided into four equal groups- (i) the control group (group I), (ii) the affected control group treated with alloxan (group II), (iii) the group treated with alloxan and phenol extract separated from okra pods (group III), and (iv) the group treated with alloxan and polysaccharides separated from okra pods (group IV). Blood samples were taken to determine the levels of glucose, urea and creatinine. The histological changes in the kidneys were also examined. It was found that treatment with phenol extract and polysaccharides separated from okra pods at a dose of 200 mg/kg for a month had a positive effect in reducing blood glucose levels, improving urea and creatinine levels, and reducing kidney tissue damage. The active compounds present in okra (phenols, polysaccharides) play a major role in improving kidney function, creatine and urea, so great consideration should be given to using okra as a complementary treatment alongside regular anti-diabetic medication.

**Keywords:** Okra, Diabetic, Kidney, Diabetes mellitus, Phenols, Polysaccharides


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**Introduction**

Diabetes is a metabolic disorder that results from a failure to secrete insulin from the pancreas or a defect in the body's cells receiving insulin. This defect causes high blood sugar and thus results in
various physiological imbalances such as retinopathy, nephropathy, and neuropathy, which affects the quality of life of affected individuals. Diabetes mellitus turns into insulin-dependent diabetes (type 1), which accounts for 5-10% of the cases (Dabelea et al., 2014). The cause of its occurrence is the destruction of β-cells that secrete insulin in the islets of Langerhans in the pancreas, which leads to diabetes. As for non-diabetes Insulin-dependent (type 2). This type of diabetes is more prevalent than type 1, accounting for 90% of cases (Bhatti et al., 2017). This type of diabetes is characterized by insulin resistance, a condition in which cells fail to respond to insulin properly (van Herpt et al., 2020). Diabetic kidney (kidney failure) is one of the greenest complications of diabetes, especially if the patient suffers from high blood pressure or symptoms of coronary arteries, as the kidneys of diabetes affect about 15-25% of patients with type 1 diabetes, as it occurs by 30-40%. Among patients with type 2 diabetes (Wu et al., 2022), symptoms of kidney failure include weakness, chronic fatigue, swelling around the eyes, swelling of the hands and feet, which limits the basic work of the kidneys, which is the elimination of toxins from the body. It leads to burning sensation when urinating with the repetition of the number of times (Asadzadeh et al., 2022).

The okra plant *Abelmoschus esculentus*, is one of the most important economic crops cultivated in Iraq and the other countries of the world, as it spreads in large geographical areas from Africa to Asia and from southern Europe to America in all temperate and medium tropical regions such as India, Japan, Iran, Turkey, Bangladesh, Pakistan, Malaysia, Thailand, Brazil, and Ethiopia (Qureshi, 2007; Gemede et al., 2014; Dumanoğlu, 2022). It belongs to the Malvaceae family (Boras et al., 2011; Jwar et al., 2021), and it is an annual dicotyledonous herb. The entire okra plant is edible, including the pods, leaves, and flowers. Its name varies according to the country in which it is located. The name of okra is of West African origin. But it is often used in the United States and the Philippines, it is known in many English-speaking countries as lady’s fingers or quiabo in Portugal, in French gumbo, bhindi in India, as molokhia in the Maghreb, or in the Middle East it is known as okra. The name varies according to the region and dialect (Adekanmi, 2020). It occupies an important medical position because it contains biologically active compounds, and the colloids present in the plant consist mainly of sugars, flavonoids, alkaloids, and phenols (Hu et al., 2014). Phenolic compounds are considered from the group of secondary metabolites, and they are one of the main biologically active components in the Okra fruits (Xia et al., 2015), which has multiple medical benefits. The polyphenols produced from a natural source are characterized by anti-cancer, anti-inflammatory, anti-heart disease and anti-hypertensive effects (Bhuyan and Basu, 2017; Shahid and Yeo, 2018; Gulcin, 2020). Polysaccharides are natural biopolymers found in abundance in the biosphere (Hamidi et al., 2022). 114 types of sugars were detected, 78 of which are anti-diabetic. Sugars have very large and wide applications in food, medicine, and other fields. Finally, their use also depends on the unique physical and chemical properties (Sun et al., 2021).

**Materials and Methods**

In this study, 24 male albino rats (8-10 weeks; b wt. 250-300 g) were used. After inducing diabetes, the animals were prevented from eating for a whole day, weighed and injected with Alloxan at a concentration of 100 mg/kg (Obasi et al., 2019). The plant used is the pods of okra *Abelmoschus esculentus* L. (Baghdad okra available in Mosul markets).

**Extraction of phenolic active compounds from the okra plant:**

The phenolic compounds were separated and purified from the pods of the okra plant by the process of acid hydrolysis. By taking 25 ml of 1N HCl acid, where the thermal sublimation is carried out at 100 °C for 1 h, then it is left to cool and then emptied with a separation funnel, and 50 ml of ethyl acetate is added to it and shaken well in two stages, where two layers are obtained. The upper
first layer is the ethyl acetate layer and the second lower layer. The upper layer was taken and 3 g of anhydrous magnesium sulfate was added to it, then it was filtered using filter paper and placed in dark glass bottles and kept in the refrigerator until diagnosis in the chromatographic apparatus and studying the extent of its antioxidant effectiveness, based on the source (Harborne, 1998; Hamdoun, 2020).

**Extraction of β-glucan polysaccharides.**

The polysaccharides were separated by treating 50 g of crushed okra plant and mixing it with 50 g of hot alkaloids KOH for 6 h with continuous stirring in order to get rid of the monoprotein, after which the sample was centrifuged at a speed of 10000 rpm at 4 °C to separate the cells and then we added an amount of ethanol to the sediment, left it to settle for a whole day, then treated with an amount of acetone. It was placed in the filter funnel, where it was filtered through filter papers, and then transferred to the rotary evaporator at a temperature of 70 °C and at low pressure in order to get rid of the fat and obtaining dry sugar (Natakankitkul et al., 2016; Al-Ta’i, 2021). Then the active compounds of phenols and sugars were identified by means of HPLC (high-performance liquid chromatography).

**Inducing diabetes mellitus:**

The animals were prevented from eating for a full day, weighed and injected with Alloxan obtained from the British BDH company. It was injected into animals subcutaneously at a concentration of 100 mg/ml, which was prepared before giving the injection (Bagheri et al., 2021).

**Experiment design:**

The initial weights of the animals were recorded, then the rats were distributed into four groups (six animals each group), and treated as follows:

- **Group I:** Healthy control group, a normal group that was dosed with distilled water daily
- **Group II:** Control (experimental diabetes) group treated with alloxan (100 mg/ml) and was dosed with distilled water daily
- **Group III:** This group was dosed with alloxan (100 mg/ml) and phenol extract from the okra plant at a dose of 200 mg/kg of body weight every day for a month (Obasi et al., 2019)
- **Group IV:** This group was dosed with with alloxan (100 mg/ml) and polysaccharides from the okra plant at a dose of 200 mg/kg of body weight every day for a month (Liao et al., 2019).

**Collection of blood samples:**

Blood samples were collected from animals of all groups in the middle of the experiment and at the end of the experiment before sacrificing them, after imposing starvation on the animals for a period of 12 h. Blood samples were obtained from the eye socket and kept in sterile tubes. Then it was placed in a centrifuge for 15 min at a speed of 3000 rpm to obtain serum, then the required tests were performed for creatinine and urea. Glucose was determined with blood.

**Results and Discussion**

**Qualitative and quantitative diagnosis of phenolic compounds by HPLC technique of okra pods:**

Seven phenolic compounds were identified in okra pods—Apigenin, Catechine, and Gallic acid, Hydrobenzoic acid, Kaempferol, Rutin, and Sinaptic acid (Figs. 1, 2). Table 1 illustrates the percentage of phenolic compounds of the hot water extract before and after acidolysis.

**Qualitative and quantitative diagnosis of β-glucan by high-performance liquid chromatography (HPLC) of okra pods:**

Analytical charts were obtained in which the retention time of β-glucan in the study sample was determined compared to the retention time of the standard sample (Fig. 3). Figure 4 also shows the standard curve of β-glucan.

**Changes in glucose, creatinine and urea level:**

The blood glucose levels noticed at different intervals in various groups are depicted in Table 2. Tables 3 and 4 show the changes in the level of creatinine and urea in the serum.
Table 1: Percentages of phenolic compounds of the hot water extract before and after acidolysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Hot water extract %</th>
<th>Hot water extract after acidolysis %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Apigenin</td>
<td>41.5</td>
<td>52.6</td>
</tr>
<tr>
<td>2</td>
<td>Catechine</td>
<td>22.9</td>
<td>30.9</td>
</tr>
<tr>
<td>3</td>
<td>Gallic acid</td>
<td>81.6</td>
<td>98.9</td>
</tr>
<tr>
<td>4</td>
<td>Hydrobenzoic acid</td>
<td>16.9</td>
<td>24.5</td>
</tr>
<tr>
<td>5</td>
<td>Kaempferol</td>
<td>42.6</td>
<td>56.9</td>
</tr>
<tr>
<td>6</td>
<td>Rutin</td>
<td>55.9</td>
<td>64.7</td>
</tr>
<tr>
<td>7</td>
<td>Sinapic acid</td>
<td>12.6</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Fig. 1: Phenolic compounds separated from okra pods assayed by HPLC

**Histological changes:**

The details of histological changes in the kidney of control and treated rats are shown in Figures 5-11. Kidney of rats of group III and IV showed recovery of histological structure as compared to group II.

The results of the current study showed that the okra plant is rich in effective phenolic compounds which is in agreement with observations of Cosme et al. (2020), who have showed that phenols have multiple types with biological effects related to their antioxidant capacity, such as anti-inflammatory activity, prevention of kidney disease, and protection from chronic diseases. Santos-Buelga et al. (2019) reported that phenolic compounds have antioxidant activity, as they contribute to lowering glucose levels in patients with type 2 diabetes. In
Fig. 2: Phenolic compounds after acid hydrolysis of the extract of okra pods identified by HPLC.

Fig. 3: Isolated β-glucan from the extract of okra pods assayed by HPLC.
Table 2: Level of glucose in the blood of rats of various groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Interval</th>
<th>Two days after the injection of Alloxan</th>
<th>After two weeks of experiment</th>
<th>Four weeks later</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>at zero</td>
<td>86.50± 2.08 aA</td>
<td>76.48± 3.14 aA</td>
<td>83.67± 2.49 aA</td>
</tr>
<tr>
<td>Group II</td>
<td>82.83± 3.092 aA</td>
<td>400.50±4.49 bB</td>
<td>388.73± 3.29 dB</td>
<td>385.83± 4.94 bB</td>
</tr>
<tr>
<td>Group III</td>
<td>91.33± 4.03 aA</td>
<td>396.83±12.13 bB</td>
<td>316.00±17.27 cC</td>
<td>133.50± 6.13 dC</td>
</tr>
<tr>
<td>Group IV</td>
<td>88.50± 5.84 aA</td>
<td>401.50±4.90 bB</td>
<td>315.50±11.83 cC</td>
<td>171.00± 3.04 dD</td>
</tr>
</tbody>
</table>

The different capital letters indicate that there are significant differences, P<0.05. The different lowercase letters indicate that there are significant differences, P<0.05

Table 3: Levels of creatinine in the serum of rats of various groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Intervals</th>
<th>Two days after the injection of Alloxan</th>
<th>After two weeks of experiment</th>
<th>Four weeks later</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>at zero</td>
<td>0.61± 0.02 aA</td>
<td>0.62± 0.02 aA</td>
<td>0.61±0.02 aA</td>
</tr>
<tr>
<td>Group II</td>
<td>0.63± 0.02 aA</td>
<td>1.01±0.05 bB</td>
<td>1.10±0.06 dB</td>
<td>1.06±0.041 cB</td>
</tr>
<tr>
<td>Group III</td>
<td>0.63± 0.04 aA</td>
<td>1.04±0.05 bB</td>
<td>0.89±0.03 cB</td>
<td>0.77±0.02 dC</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.63±0.02 aA</td>
<td>0.97± 0.03 bB</td>
<td>0.80±0.05 cC</td>
<td>0.73±0.02 bC</td>
</tr>
</tbody>
</table>

The different capital letters indicate that there are significant differences, P<0.05. The different lowercase letters indicate that there are significant differences, P<0.05
Table 4: Percentage of urea in serum of rats of various groups

<table>
<thead>
<tr>
<th>Intervals at zero</th>
<th>Two days after the injection of Alloxan</th>
<th>After two weeks of experiment</th>
<th>Four weeks later</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>43.20 ± 1.74 aA</td>
<td>40.83 ± 1.35 aA</td>
<td>41.17 ± 1.49 aA</td>
</tr>
<tr>
<td>Group II</td>
<td>42.63 ± 2.22 aA</td>
<td>118.65 ± 2.58 bB</td>
<td>128.83 ± 4.41 bB</td>
</tr>
<tr>
<td>Group III</td>
<td>42.45 ± 1.67 aA</td>
<td>119.01 ± 3.37 bB</td>
<td>102.32 ± 2.09 bB</td>
</tr>
<tr>
<td>Group IV</td>
<td>41.33 ± 0.96 aA</td>
<td>121.68 ± 3.16 bB</td>
<td>102.30 ± 2.82 bB</td>
</tr>
</tbody>
</table>

The different capital letters indicate that there are significant differences, P<0.05. The different lowercase letters indicate that there are significant differences, P<0.05.

this study the polysaccharide separated from the okra plant has a good effect on reducing glucose in the blood which is in conformity with findings of Li et al. (2022) who have noticed that polysaccharides from orchids provide antidiabetic effects by lowering blood glucose levels.

The study showed that alloxan (group II) negatively affects kidney functions, as it causes a rise in creatinine and urea levels (Table 3, 4). The results also showed that the diabetic group treated with phenols had a good positive change in the levels of creatinine and urea. These results are in agreement with the observations of Ganenan et al. (2020), who have reported that the rutin compound of phenols has a lowering effect on creatinine and urea levels in the blood, as phenols have a protective role for kidney functions.

Figure 5 shows normal histological features of the kidney of a male rat in the healthy control group (Group I). The rats of diabetic group (group II) showed many pathological changes as shown in Figures 6-9. The results of the present study is supported by the findings of Pourghasem et al. (2015) who have showed that diabetes causes damage to the kidneys. Present study also derives support from findings of Wang et al. (2017) who have also noticed damage to the kidney by observing the expansion of Bowman's capsule. Similar results were also obtained by Abdullah et al. (2019) in the kidney as a result of diabetes.

Rats of group III (induced Diabetes mellitus and treatment with phenols extracted from the okra plant) showed histological features represented by slight atrophy the renal glomeruli (Fig.10) which indicates preventive and reducing tissue lesions of the kidney. Huang et al. (2021) by using guava leaves with a high content of phenols, also noticed reduced tissue damage to the kidney with the reduction of diabetic nephropathy and the reduction of necrosis, congestion and swelling of tubules significantly with the improvement of renal tubular structures. The results of the current study also agreed with findings of Abd Elkader and Abdou (2022) as their results showed an improvement in kidney function and a reduction in inflammatory injury compared to a control group with diabetes. Phenols have a role in reducing oxidative stress either directly or indirectly. On the other hand, phenolic compounds have biological effects and may be used as antioxidants.

Figure 11 shows the results of histopathological changes in the kidneys of the diabetes group treated with β-glucan polysaccharide extracted from the pods of the okra plant. This is in conformity with the results of Zhou et al (2022), who used polysaccharides from the black onion plant on liver and kidney injuries and showed that polysaccharides improve the histological structure of the kidneys. The current
Fig. 5: Rat kidney of the control group showing normal architecture of the glomeruli (A), proximal renal tubules (B), and distal renal tubules (C). HE; 100X.

Fig. 6: Rat kidney of the diabetic group showing atrophy of glomeruli (A), dilatation of Bowman’s space (B), renal cyst (C), hyaline casts in the renal tubules (D) and vacuolar degeneration of epithelial cells lining renal tubules (E). HE; 100X.

Fig. 7: Rat kidney of the diabetic group showing atrophy of glomeruli (A), dilatation of Bowman’s space (B), renal cyst (C) and infiltration of inflammatory cells (D). HE; 100X.

Fig. 8: Rat kidney of the diabetic group showing atrophy of glomeruli (A), dilatation of Bowman’s space (B), infiltration of inflammatory cells (C) and vacuolar degeneration (D) and necrosis (E) of epithelial cells lining renal tubules. HE; 400X.
Fig. 9: Rat kidney of the diabetic group showing segmentation of glomeruli (A), dilatation of Bowman's space (B), and severe cell swelling (C) and necrosis (D) of epithelial cells lining renal tubules. HE; 400X.

Fig. 10: Rat kidney of the diabetic rats with phenols group showing slight atrophy of the glomeruli (A), dilatation of Bowman's capsule (B), and slight hemorrhage (C). HE; 100X.

Fig. 11: Kidney of the diabetic rats with OPS β-glucan group showing mild atrophy of glomeruli (A), vacuolar degeneration of epithelial cells lining renal tubules (B) and congestion of blood vessels (C). HE; 100X.

The study also derives support from the studies of Luo et al. (2019), who have reported that polysaccharides from guava leaves mitigated damage to the kidneys and pancreas.

**Conclusion**

It is concluded that the okra plant contains phenolic substances and polysaccharides, and that the development of diabetes mellitus in male white rats led to an increase in glucose levels and an increase in creatinine and urea levels. These levels decreased in rats treated with phenolic extract and polysaccharides separated from okra plant pods.

The results of the study also showed that the induction of diabetes mellitus in male rats led to changes in the kidney tissue compared with the healthy control group, while the treatment with phenols extract and polysaccharides showed a protective effect on the kidneys.

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References


