EFFECT OF ALCOHOLIC EXTRACT OF *Brassica oleracea* L. VAR. CAPITATA PLANT LEAVES ON GLUCOSE LEVEL AND ANTIOXIDANT ACTIVITY IN ALLOXAN-INDUCED DIABETIC RATS

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**ABSTRACT**

**Objective:** The aim of this study was to investigate the effect of ethanolic extract of *Brassica oleracea* var. Capitata (Cabbage) Plant Leaves of Glucose Level and Antioxidant Activity in Alloxan-Induced Diabetic Rats.

**Methods:** Thirty mature male rats were used in five groups, the first group kept as a normal group. The second group (Diabetic group) received a single dose of alloxan (150 mg/kg BW i.p.) to induction of Diabetes mellitus type 2 (T2DM) while the third, fourth, and fifth groups were diabetic rats received 150, 300, 600 mg/kg, respectively, of *Brassica oleracea* var. capitata ethanolic extracts orally for 60 days after induction of T2DM. Body weights were recorded after and before treatment. Animals were sacrificed after 24 h of the last dose and heart blood samples were collected for biochemical study.

**Results:** The presence of a significant decrease (\(P < 0.05\)) in the body weights and reduced Glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase1 (GPX1) levels and significant increase (\(P < 0.05\)) in the fasting plasma Glucose (FPG) and malondialdehyde (MDA) levels in the diabetic group compared with normal group. In contrast, it was noted the presence of significant increase (\(P < 0.05\)) in the body weights and GSH, SOD and GPX1 levels and significant decrease (\(P < 0.05\)) in the FPG and MDA levels in diabetic rats that treated with 300, 600 mg/kg BW of extract as compared with diabetic group rats.

**Conclusion:** The present study ascertains that the Ethanolic extracts of *Brassica oleracea* var. Capitata Plant Leaves possesses antihyperglycemic and antioxidant properties.

**INTRODUCTION**

Diabetes is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels\textsuperscript{5}. Diabetes Mellitus is associated with imbalance between generation of oxygen free radicals and endogenous antioxidant defense system leading to augmented oxidative stress where complete cure with insulin and oral hypoglycemic agents without side effect has been difficult\textsuperscript{6}. It has been reported that under diabetic conditions, persistent hyperglycemia may cause high ROS production via glucose auto-oxidation and/or protein glycation in various tissues\textsuperscript{7}. Also, there are reports on the altered antioxidative enzymes activities and increased lipid peroxidation in animal models and humans with diabetes\textsuperscript{5,6}.

The toxic side effects, contraindication and sometimes diminutions in response after prolonged use of antidiabetic drugs encouraged to search for therapeutic herbal remedies for safety, efficacy and economy. Many plants were known for their activity as antidiabetic herbal remedies and increased lipid peroxidation in animal models and humans with diabetes\textsuperscript{5,6}.
DM. Various nutritionally important vitamins, supplements and some vegetables, fruits including cappers, broccoli, grapes, tomatoes, carrots, spinach, nuts, etc. provides natural antioxidants which diminish the damage caused by oxidative stress in diabetes mellitus.

Brassica oleracea var. Capitata (Cabbage) belongs to the Brassicaceae family and is closely related to the broccoli, cauliflower and Brussels sprouts. It is a leafy vegetable crop that grows close to the ground. The leaves may be loose or tightly compacted, ranging in color from pale green to purple. The cabbage is one of the most important vegetable crops under cultivation. It is thought to have originated in the Mediterranean region. Cabbage is consumed either raw or processed in different ways, e.g., boiled or, fermented or, used in salads. Due to its antioxidant, anti-inflammatory and antibacterial properties, cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal disorders (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as in the treatment of minor cuts and wounds and mastitis. Fresh cabbage juice, prepared either separately or mixed with other vegetables such as carrot and celery, is often included in many commercial weight-loss diets.

Brassica oleracea var. Capitata and other Brassica vegetables have been found to have antioxidant, antihyperglycemic, anticancer, hypcholesterolemic and anticoagulant properties. It has high water content, is high in fiber, and has significant quantities of protein, calcium and iron. It is a rich source of vitamin A and vitamin C, in addition to containing some B vitamins. It also contains significant amounts of glutamine, an amino acid which has anti-inflammatory properties.

MATERIALS AND METHODS

Collection of Plant and Preparation of Extract

The fresh plant of Brassica oleracea var. Capitata (cabbage) were obtained from local market. The leaves of the plant were dried under shade and powdered in a mechanical grinder. The powdered material (250 gms) was extracted successively in 80% ethanol using a Soxhlet apparatus at 45°C for 24 h. The extract was concentrated in vacuo and kept in a vacuum desiccator for complete removal of solvent and weighed.

Animals

Males of adult albino rats weighting 230–250 g were used, bred in the Animal House Lab, Faculty of Education for Girl/university of Kufa, Iraq. These animals were maintained under standard laboratory conditions and provided a standard diet and water ad libitum.

Induction and Assessment of Diabetes

Alloxan (Afco, India) was prepared in fresh normal saline. The 18 hrs previously fasted rats were injected intraperitoneally (ip) with a freshly prepared solution of alloxan monohydrate (150 mg/kg bwt) in a volume of 1 ml. After 72 hrs, blood samples were collected from the tail vein and fasting glucose levels were estimated by using a glucometer (ACCU-CHEK, Roche Diagnostics). The animals with blood glucose level ≥ 200 mg/dl were considered diabetic and included in the experiment. Also, fasting blood glucose values were determined just prior to killing the animals at the end of the experiment.

Experimental Design

A total of 30 rats (24 diabetic rats and 6 normal rats) were divided into five groups of six animals each and given the following treatment orally using an intragastric tube for the period of 60 days.

Group I: Normal control was given distilled water only.

Group II: Diabetic control was given distilled water only.

Group III: Diabetic rats were given 150 mg/kg BW of Brassica oleracea var. Capitata Ethanolic extracts dissolved in 1ml of distilled water for 60 days.

Group IV: Diabetic rats were given 300 mg/kg BW of Brassica oleracea var. Capitata Ethanolic extracts dissolved in 1ml of distilled water for 60 days.

Group V: Diabetic rats were given 600 mg/kg BW of Brassica oleracea var. Capitata Ethanolic extracts dissolved in 1ml of distilled water for 60 days.

Sample Collection

Weights of animals recorded at the beginning and the end of the experiment. The animals were sacrificed after 24 hrs of last dose and heart blood samples were collected for biochemical tests.

Biochemical Analysis

Estimation of Blood Glucose

The blood sample was collected from the tip of the rat tail vein and the glucose level was estimated using a glucose-oxidase-peroxidase reactive strips using glucometer.

Estimation of Serum GSH

Serum GSH was measured based on method that described by Burits and Ashood.

Estimation of Serum MDA, SOD and GPX1

Serum MDA, SOD and GPX1 levels were measured by enzyme-linked immuno sorbent assay (ELISA) methods using Rat MDA, SOD and GPX1 Elisa Kit (Elabscience, China).
Effect of Plant Extracts on Body Weight

As shown in Table 1, the results of this study showed body weight changes in experimental animals and the change represented by a percentage between the final and initial body weight. There was a significant decrease ($P < 0.05$) in the body weight of the diabetic control group rats (−16.09%) compared with normal control group rats which had a 23.21% increase in their body weight. There was no significant difference observed in diabetic rats treated with 150 mg/kg of plant extract compared with the diabetic control rats, but the decrease in body weight of these rats (−14.59%) was significant compared with normal rats. In diabetic rats treated with 300 mg/kg of plant extracts there was a significant increase (+20.05%) compared with the diabetic control rats and in diabetic rats treated with 600 mg/kg was no significant compare with normal rats. In addition to the above, a significant differences were observed when comparing the treatments with each other.

Table 1. Effect of Brassica oleracea var. capitata alcoholic extract of body weight (g)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Percentages of change %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>234.3 ± 7.66</td>
<td>288.7 ± 6.83</td>
<td>+23.21</td>
</tr>
<tr>
<td>Diabetic control group</td>
<td>231.8 ± 6.62</td>
<td>194.5 ± 5.99</td>
<td>−16.09</td>
</tr>
<tr>
<td>Diabetic + 150 mg extract</td>
<td>238.5 ± 7.12</td>
<td>203.7 ± 5.50</td>
<td>−14.59</td>
</tr>
<tr>
<td>Diabetic + 300 mg extract</td>
<td>236.2 ± 9.43</td>
<td>234.2 ± 8.13</td>
<td>−0.84</td>
</tr>
<tr>
<td>Diabetic + 600 mg extract</td>
<td>235.3 ± 10.76</td>
<td>282.5 ± 5.13</td>
<td>+20.05</td>
</tr>
<tr>
<td>H.S.D (P&lt;0.05)</td>
<td>11.842</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The numbers refer to mean of body weight ± standard deviation.
Various capital letters refer to present significant differences ($P<0.05$) between the treatments.
H.S.D: Honestly Significant Difference.

Effect of Plant Extracts on FPG and Plasma MDA Levels

The data presented in Table 2 show the presence of a significant increase ($P < 0.05$) in the means of FPG and plasma MDA levels (355.8 mg/dl and 226.35 ng/ml, respectively) in the rats of diabetic control group compared with normal rats (102 mg/dl and 64.36 ng/ml respectively). There was no significant difference observed in FPG and MDA levels of diabetic rats treated with 150 mg/kg of plant extract (358.5 mg/dl and 210.03 ng/ml respectively) compare with the diabetic control rats, but the increase in FPG and MDA levels of these rats was significant compare with normal rats. In contrast, it was noted the presence of significant decrease ($P < 0.05$) in FPG and MDA levels in the groups that treated with 300, 600 mg/kg of plant extracts compared with diabetic control rats, but the difference in FPG and MDA levels in the rats that treated with 600 mg/kg was no significant compare with normal rats. In addition to the above, a significant differences were observed when comparing the treatments with each other.

Table 2. Effect of Brassica oleracea var. Capitata alcoholic extract of FPG and plasma MDA levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FPG level (mg/dl)</th>
<th>MDA level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>102.0 ± 8.58</td>
<td>64.36 ± 8.57</td>
</tr>
<tr>
<td>Diabetic control group</td>
<td>355.8 ± 17.3</td>
<td>226.35 ± 18.65</td>
</tr>
<tr>
<td>Diabetic + 150 mg extract</td>
<td>358.5 ± 14.11</td>
<td>210.03 ± 13.47</td>
</tr>
<tr>
<td>Diabetic + 300 mg extract</td>
<td>159.2 ± 8.30</td>
<td>116.92 ± 9.64</td>
</tr>
<tr>
<td>Diabetic + 600 mg extract</td>
<td>116.8 ± 7.33</td>
<td>79.62 ± 10.35</td>
</tr>
<tr>
<td>H.S.D (P&lt;0.05)</td>
<td>19.8</td>
<td>20.89</td>
</tr>
</tbody>
</table>

The numbers refer to mean of FPG and plasma MDA levels ± standard deviation.
Various capital letters refer to present significant differences ($P<0.05$) between the treatments.
H.S.D: Honestly Significant Difference.

Effect of Plant Extracts of Plasma GSH, SOD and GPX1 Levels

The data presented in Table 3 show the presence of a significant decrease ($P < 0.05$) in means of plasma GSH, SOD and GPX1 levels in the rats of diabetic control group compared with normal rats. There was no significant difference observed in GSH, SOD and GPX1 levels of diabetic rats treated with 150 mg/kg of plant extract compared with the diabetic control rats, but the decrease in levels of GSH, SOD and GPX1 in these rats was significant compared with normal rats. In contrast, it was noted the presence of significant increase ($P < 0.05$) in means of plasma GSH, SOD and GPX1 levels in diabetic rats that treated with 300, 600 mg/kg BW of plant extracts compared with diabetic control group, but the difference in GSH, SOD and GPX1 levels in the rats that treated with 600 mg/kg was no significant compare with normal rats. In addition to the above, a significant
difference was observed when comparing the treatments with each other.

**Table 3. Effect of Brassica oleracea var. Capitata alcoholic extract of plasma GSH, SOD and GPX1 levels**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH (μmol/L)</th>
<th>SOD (ng/ml)</th>
<th>GPX1 (μmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>47.2 ±4.51</td>
<td>A</td>
<td>2.1 ± 0.19</td>
</tr>
<tr>
<td>Diabetic control group</td>
<td>27.2 ± 3.14</td>
<td>C</td>
<td>1.12 ± 0.24</td>
</tr>
<tr>
<td>Diabetic + 150mg extract</td>
<td>31.7 ± 2.80</td>
<td>C</td>
<td>1.66 ± 0.15</td>
</tr>
<tr>
<td>Diabetic + 300mg extract</td>
<td>38.1 ± 4.31</td>
<td>B</td>
<td>2.89 ± 0.21</td>
</tr>
<tr>
<td>Diabetic + 600mg extract</td>
<td>49.3 ± 5.30</td>
<td>A</td>
<td>3.99 ± 0.24</td>
</tr>
</tbody>
</table>

H.S.D = 11.842

The numbers refer to mean of FPG and plasma MDA levels ± standard deviation. Various capital letters refer to present significant differences ($P < 0.05$) between the treatments. H.S.D: Honestly Significant Difference.

**DISCUSSION**

Medicinal plants have a long history as a treatment for diabetes. With a disturbing rise in the prevalence of this metabolic disease and associated health care costs, interest in alternative or complementary therapies has grown. The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years. In this study, data in Table 1 show clearly significant increase in whole body weight of diabetic rats which received 300, 600 mg/kg respectively of plant extracts compared to the diabetic control group. This significant increase may be caused by the effect of plant extract which contain many of bioactive compounds such as flavonoids and alkaloids that act as anti-hyperglycemic and returned blood glucose level to normal range and enhanced glyco genesis activity by increasing the cellular uptake of glucose. This result is similar to the finding of Kataya & Hamza.

The results in Table 2 show a significant decrease of FPG and serum MDA levels in diabetic rats which received 300, 600 mg/kg respectively of plant extracts compared to diabetic control group rats. The significant effect of this extract on hyperglycemia is due to its effect in the improvement of insulin sensitivity, increase cellular uptake (decreasing the plasma level) and decreasing hepatic glucose production. This antidiabetic activity of extract may be related to the actions of ingredient active compound such as flavonoids and glycosides or may be provoke regenerated of β-cell in the pancreas, enhancement of insulin sensitivity and increase peripheral tissue uptake and utilization of glucose. These results are agreeable with Similar observations that were obtained by Mohammed & Al-Maliki. In other wise, the significant decrease of serum MDA level may be due to the antioxidant effectiveness of the plant because it contains multiple phenol compounds which play important role in the inhibition of lipid peroxidation.

The present investigation also indicates the effectiveness of Ethanolic extract of Brassica oleracea var. Capitata in increasing the serum GSH, SOD and GPX1 in diabetic rats which received 300, 600 mg/kg BW respectively of this extract compared to diabetic control group rats. Brassica oleracea extract may lead to stimulate insulin secretion and therefore increase the effectiveness of glucose-6-phosphate dehydrogenase, which leads to formation necessary reducing power (NADPH) for recycle glutathione from oxidized to reduced shape. This result is similar to the finding of Pourghassem-Gargari et al. The significant increase of antioxidant enzyme levels may be caused by the Cruciferous vegetables contain several chemical compounds, such as allyl isothiocyanate, iberverin, erucin, and sulforaphan, act as antioxidants or as inhibitors and/or inducers of phase I and phase II enzymes. Also, recent studies have shown that the cruciferous vegetables contain organic sulfur compounds (diallyl disulfide) and isothiocyanates which had the ability to modulate expression/activity of antioxidative enzymes and scavenging free radicals.

**CONCLUSION**

Concluded from the current study that the negative effects of Alloxan-induced T2DM on blood glucose level and antioxidant status of male rats can be reduced or prevented by treatment with Brassica oleracea var. Capitata leaf alcoholic extract which has anti-hyperglycemic and antioxidant activity.

**REFERENCES**