The Effect of Epicatechin on the Level of Lipid Peroxides and Antioxidant Enzymes in Some Tissues of Diabetic Rats

HODA W. EL-GAWLY, M.D.*; CHERINE MAURICE, M.D.*; EMAN ZAKRIA MAHMOUD, M.D.* and TAHER IBRAHIM EL-SERAFY, M.D.**

The Departments of Pharmacology* and Biochemistry**, Faculty of Medicine, Suez Canal University.

Abstract

Diabetes mellitus is a disease that is accompanied with reduction of endogenous antioxidants and an increase in oxidative stress in the human body. Antioxidants have been shown to reduce the risk of diabetes, improve glucose disposal and improve some of the associated complications.

In earlier reports, certain insulin-like effects of epicatechin was shown in rats. However, other researchers couldn't confirm these effects in their studies.

So, the present study was conducted on 50 adult male albino rats weighting from 180-200g. Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozocin (45mg/kg). After 48h of streptozocin administration, blood glucose levels were estimated in rats fasted overnight using blood glucose kits. Rats with blood glucose greater than 250mg/dl were considered diabetic and were divided randomly into 5 groups (10 animals/group). The antioxidant, antiperoxidative and hypoglycaemic effects of epicatechin alone and in combination with glibenclamide were assessed.

It was found that both epicatechin and glibenclamide had a significant antioxidant and antiperoxidative effects. However as regarding the hypoglycaemic effect, it was found that glibenclamide was better than epicatechin. The combination between epicatechin and glibenclamide was better than the use of each drug alone in controlling hyperglycaemia and in reducing the oxidative stress.

Key Words: Diabetes – Epicatechin – Lipid peroxides – Antioxidant enzymes – Glibenclamide.

Introduction

DIABETES mellitus is a common disease affecting 124 million individuals worldwide [1].

Diabetes is associated with high risk of atherosclerosis, renal, nervous system and ocular damage. Uncontrolled hyperglycaemia appears to be the principal biochemical abnormality that underlies the increased oxidative load in diabetes mellitus [2].

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus [3].

Free radicals are formed in diabetes by glucose oxidation, nonenzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins. High levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus [3].

Studies have shown that certain flavonoids exhibit hypoglycaemic effect [4].

Epicatechin is a member of a group of polyphenolic compounds collectively known as catechins, belonging to flavonoid family [5].

So, this study was conducted to examine the antiperoxidative and antioxidant effects of epicatechin on streptozocin induced diabetic male albino rats as well as its hypoglycaemic effect. Also, the hypoglycaemic effect of epicatechin either alone or in combination with glibenclamide was assessed.

Material and Methods

Animals:

50 male albino rats weighting 150-200gm were used in this study. Animals were housed under controlled environmental conditions with free access to food and water in polyethylene cage and were allowed for acclimatization before the start of the study.
Drugs:
- Epicatechin, sigma, St. Louis M.O. USA.
- Streptozotocin, sigma, St. Louis M.O. USA.
- Glibenclamide, Hoechst Orient, S.A.E. Cairo.

Induction of diabetes: By single intraperitoneal injection of freshly prepared streptozocin (45mg/kg) in 0.1M citrate buffer (pH 4.5) in a volume of 1ml/kg [6].

The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia.

After 48h of streptozocin administration, 0.5ml of blood glucose was withdrawn from retro orbital plexus of rats fasted overnight and tested for blood glucose. Rats with blood glucose greater than 250mg/dl were considered diabetic and randomly divided into groups [7].

Study design:
Rats were randomly divided into 5 groups (10 animals/group):
- Group 1: Normal nondiabetic rats receiving aqueous solution.
- Group 2: Diabetic control receiving aqueous solution.
- Group 3: Diabetic rats given 30mg/kg of epicatechin in aqueous solution intraperitoneally daily for five weeks [6].
- Group 4: Diabetic rats given glibenclamide (5mg/kg) in aqueous solution intraperitoneally daily [8].
- Group 5: Diabetic rats given glibenclamide (600ug/kg orally) in combination with epicatechin (30mg/kg in aqueous solution intraperitoneally daily) for five weeks [6].

Tissue sampling: Kidney and heart tissues were collected in ice-cold containers, washed with ice chilled physiological saline, blotted dry and weighed. A tissue homogenate was prepared.

Biochemical assay for determination of: Lipid peroxides, glutathione activity, catalase, superoxide dismutase in tissues was done.

Data were analyzed using SPSS statistical software. Student's t-test was used to test for statistical differences between two groups. The significance level was considered at $P$ value <0.05 [9].

Results
The results shown in the Tables (1-9) and Figs. (1-9).
Table (3): The mean of superoxide dismutase activity in kidney tissue.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>SOD activity in kidney (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.22±0.19</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>0.61±0.10*</td>
</tr>
<tr>
<td>Diabetic treated with epicatechin</td>
<td>1.16±0.42*</td>
</tr>
<tr>
<td>Diabetic treated with glibenclamide</td>
<td>1.09±0.28*</td>
</tr>
<tr>
<td>Diabetic treated with both drugs</td>
<td>1.24±0.29*</td>
</tr>
</tbody>
</table>

*a* Significantly different from normal control group at \( p < 0.05 \).

*b* Significantly different from diabetic untreated group at \( p < 0.05 \).

Table (4): The mean of superoxide dismutase activity in heart tissue.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>SOD activity in heart (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.22±0.20</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>0.59±0.07*</td>
</tr>
<tr>
<td>Diabetic treated with epicatechin</td>
<td>1.14±0.42*</td>
</tr>
<tr>
<td>Diabetic treated with glibenclamide</td>
<td>1.09±0.28*</td>
</tr>
<tr>
<td>Diabetic treated with both drugs</td>
<td>1.24±0.29*</td>
</tr>
</tbody>
</table>

*a* Significantly different from normal control group at \( p < 0.05 \).

*b* Significantly different from diabetic untreated group at \( p < 0.05 \).

Table (5): The mean of glutathione peroxidase activity in kidney tissue.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>GPx activity in kidney (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.55±0.17*</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>0.23±0.06*</td>
</tr>
<tr>
<td>Diabetic treated with epicatechin</td>
<td>0.54±0.12*</td>
</tr>
<tr>
<td>Diabetic treated with glibenclamide</td>
<td>0.44±0.12</td>
</tr>
<tr>
<td>Diabetic treated with both drugs</td>
<td>0.56±0.28*</td>
</tr>
</tbody>
</table>

*a* Significantly different from normal control group at \( p < 0.05 \).

*b* Significantly different from diabetic untreated group at \( p < 0.05 \).
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Fig. (5): The mean of glutathione peroxidase activity in kidney tissue.

Table (6): The mean of glutathione peroxidase activity in heart tissue.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>GPx activity in heart (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.54±0.14* b</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>0.23±0.08* a</td>
</tr>
<tr>
<td>Diabetic treated with epicatechin</td>
<td>0.55±0.12* b</td>
</tr>
<tr>
<td>Diabetic treated with glibenclamide</td>
<td>0.43±0.13</td>
</tr>
<tr>
<td>Diabetic treated with both drugs</td>
<td>0.53±0.28* b</td>
</tr>
</tbody>
</table>

*a* Significantly different from normal control group at \( p<0.05 \).

*b* Significantly different from diabetic untreated group at \( p<0.05 \).

Fig. (6): The mean of glutathione peroxidase activity in heart tissue.

Table (7): The mean of catalase volume activity in kidney tissue.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>CAT volume activity in kidney (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>52.79±9.19* b</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>33.18±6.13* a</td>
</tr>
<tr>
<td>Diabetic treated with epicatechin</td>
<td>50.93±5.35* b</td>
</tr>
<tr>
<td>Diabetic treated with glibenclamide</td>
<td>50.75±6.11* b</td>
</tr>
<tr>
<td>Diabetic treated with both drugs</td>
<td>52.49±5.10* b</td>
</tr>
</tbody>
</table>

*a* Significantly different from normal control group at \( p<0.05 \).

*b* Significantly different from diabetic untreated group at \( p<0.05 \).

Fig. (7): The mean of catalase volume activity in kidney tissue.

Table (8): The mean of catalase volume activity in heart tissue.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>CAT volume activity in heart (U/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>52.48±8.23* b</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>34.27±5.06* a</td>
</tr>
<tr>
<td>Diabetic treated with epicatechin</td>
<td>50.20±4.38* b</td>
</tr>
<tr>
<td>Diabetic treated with glibenclamide</td>
<td>49.42±7.13* b</td>
</tr>
<tr>
<td>Diabetic treated with both drugs</td>
<td>53.36±7.05* b</td>
</tr>
</tbody>
</table>

*a* Significantly different from normal control group at \( p<0.05 \).

*b* Significantly different from diabetic untreated group at \( p<0.05 \).
Discussion

Diabetes is a chronic progressive disease that results in significant morbidity and mortality [10]. Moreover, it is predicted that between 2000 and 2025, the number of adults with diabetes in the world will increase from 150 million in 2000 to 300 million in 2025 [11].

In Egypt, the prevalence of diabetes in year 2000 was 2.623 and it is estimated to reach 6.726.000 in year 2030 [12].

The chronic hyperglycaemia produces multiple biochemical changes and diabetic oxidative stress could play a role in the progression and complications of the disease [6].

Letitia et al., [13], stated that diabetes is accompanied with a reduction of endogenous antioxidants and an increase in oxidative stress in the human body. Antioxidants have been shown to delay the onset of diabetes and improve some of the associated complications.

Although different types of oral hypoglycaemic drugs are available along with insulin for the treatment of diabetes, there is a growing interest in herbal remedies. Herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low costs [5].

So, more attention was directed towards the role and usage of natural antioxidants as a mean to prevent oxidative damage in diabetes with high oxidative stress [14].

Polyphenolic compounds are among the most commonly used dietary ingredients [15]. Epicatechin is a member of polyphenolic compounds collectively known as catechins, belonging to flavonoid family [5].

In India Ayurveda system of medicine, aqueous extract of the wood of Pterocarpus marsupium, commonly known as “bijasar” is used for treatment of diabetes. The active antidiabetic principle in its aqueous extract has been found to be epicatechin. In earlier reports, certain insulin like effects of epicatechin in rats and in erythrocytes of type 2 diabetic patients had been shown [16]. It is also known to regenerate B-cells in the alloxan and streptozocin induced diabetes in rats [6].

However, there are other researchers that couldn’t confirm these effects in their studies.
Sheehan et al., [17], found in their study on diabetic rats that epicatechin had no significant effect on blood glucose levels when compared to alloxan control animals.

So, this study was conducted on 50 male albino rats. Rats were randomly divided into 5 groups (10 animals/group). The first group was normal nondiabetic, the second was diabetic control receiving aqueous solution, the third one was diabetic group given epicatechin, the fourth was diabetic rats given glibenclamide, the fifth was diabetic rats given glibenclamide in combination with epicatechin.

The aim of the study was to assess the antiperoxidative, antioxidant and hypoglycaemic effects of epicatechin on streptozocin induced diabetic male albino rats. Also, we compared the effects of epicatechin with that of glibenclamide therapy on the level of blood glucose, lipid peroxides and antioxidant enzymes in order to compare their efficacy and to assess their efficacy as monotherapy or combined therapy.

From our study, it was found that diabetes mellitus was associated with increased oxidative stress. We found that the diabetic untreated rats showed significantly high levels of lipid peroxides. This result is similar to that of Caimi [18], who found that the level of hydroperoxides and thiobarbituric acid reactive substances were elevated in diabetic patients.

Also, Pon et al., [19], in their study on diabetic rats found that the level of lipid peroxides increased significantly in serum, heart and aorta of diabetic rats. They related the elevation in thiobarbituric acid reactive substances in diabetic rats to the increased levels of oxygen free radicals.

The exact cause of the increased levels of free radicals in diabetes is still not defined. However, several hypothesis have been suggested. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products and enhanced glucose flux through the polyol pathway. Moreover, it may be due to the activation of sorbitol system and functional limitation of the hexose monophosphate pathway [20].

So, the greater blood glucose concentrations are the main cause of the oxidative stress with experimental diabetes [21].

The expression of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, is known to be very low in islet cells as compared with other tissues and cells. Therefore, once B-cells face oxidative stress, they may be rather sensitive to it, suggesting that glycation and subsequent oxidative stress may in part mediate the toxic effect of hyperglycaemia. As direct support for this, glycation- mediated reactive oxygen species production reduces insulin gene transcription and also causes apoptosis of B-cells [22].

On the other hand, we found that the activity of all the measured antioxidant enzymes were decreased significantly in the diabetic untreated group as compared to their corresponding normal control group. This was supported by the results of Subbiah et al. [20], who detected in their studies that the level of antioxidant enzymes was decreased in diabetic rats.

Also, our findings were similar to the results of Pablo et al. [23], who found that hepatic glutathione levels were decreased in streptozocin-diabetic rats. Lipid peroxide levels were found increased in liver homogenates of streptozocin diabetic rats and the levels of the antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase are decreased in the liver of experimental diabetic rats.

So, we can conclude that the increased levels of lipid peroxides and the decreased activities of all the antioxidant enzymes may be explained as follows: The elevated generation of free radicals resulted in the consumption of antioxidant defense components which in turn may lead to oxidative damage to membranes and enhance susceptibility to lipid peroxidation.

On the other hand, in the present study, it was found that epicatechin had a significant antiperoxidative effect in all tissues of diabetic rats treated with epicatechin alone.

Our results were supported with the results of Pablo et al. [23], who suggested that catechins may provide a useful therapeutic option in the reversal of oxidative stress induced cardiac dysfunction in diabetes mellitus.

Xuczaj et al. [24], explained the antioxidative properties of catechins by their abilities to inhibit free radical generation, scavenge free radicals and chelate transition metal ions, mainly iron and copper, which are catalysts of free radical reactions.

Otherwise, glibenclamide had a significant antioxidant and antiperoxidative effects as it can increase the activity of antioxidant enzymes and
reduce the level of lipid peroxides. This was supported by the results of Roja et al. [25], who stated that glibenclamide besides having lowering effect, it seems to have antioxidant properties, as, it restored liver catalase and superoxide dismutase in streptozocin-induced diabetic rats.

Finally, we found that the antioxidant effects of epicatechin were better than that of glibenclamide. This could be explained by the fact that the antioxidant effect of glibenclamide could be due to improvement in glycaemic status which is intended to be the main cause of increased oxidative stress in diabetes. Thus the improvement in glycaemic status by glibenclamide suggests amelioration of the oxidative stress due to hyperglycaemia and as a consequence the antioxidant enzymes were not exhausted.

In our study, epicatechin alone had a significant hypoglycaemic effect. This finding was supported by the finding of Ting et al., 2007, who found that after 30 days treatment with catechins, plasma levels of glucose were significantly reduced as compared with that in diabetic control group.

In addition, several reports indicate certain insulin-like effects of epicatechin in rats and in erythrocytes of type 2 diabetic patients [16]. It is also known to regenerate B-cells in the alloxan and streptozocin-induced diabetes in rats [6].

Also, Myung et al. [26], found that epicatechin inhibited the deleterious effects of streptozocin on the pancreatic B-cells. They stated that epicatechin inhibited streptozocin induced hyperglycaemia and Beta-cells destruction in the rat pancreas and it blocked streptozocin induced nitric oxide production and inhibition of insulin release from the isolated islets.

Finally, we found that the use of glibenclamide alone had a better hypoglycaemic effect than the use of epicatechin alone. This is due to the synergistic inhibitory effect of glibenclamide and glucose on the ATP-sensitive potassium channel in the islet’s of langerhan’s, which may explain the rebound hypoglycaemic effects seen in some sulphophylorea overdose patients. This condition is treated with high concentration dextrose infusion [27]. So, we conclude that the use of epicatechin alone as a hypoglycaemic agent was not preferred as a substitution for glibenclamide.

From the above, we can conclude that the better antiperoxidative, antioxidant and hypoglycaemic effects of drug combination may be related to the effect of glibenclamide in controlling hyperglycaemia which may lead to the reduction of oxidative stress and also to the effect of epicatechin in increasing the activity of the endogenous antioxidant enzymes and also to its insulin like effect. Thus the combination of both drugs leads to potentiation of their effects leading to better control of glucose concentrations that cut the vicious circle of chronic hyperglycaemia and oxidative stress.

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