Effect of Ginger on the activity of some antioxidant enzymes (Superoxide dismutase, and Catalase) of Alloxan Experimental Induced-Diabetic Rabbits

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ABSTRACT

Oxidative stress is considered as one of the important consequences of poor glycemic control. This study was designed to explore the possible clinical utility of ginger as an antioxidant enzymes. This study was performed on alloxan induced diabetic rabbits with age of (6,12) month's. The selected rabbits were allocated into four groups each include 10 rabbits. The first group was healthy rabbits as compared with second...
group. The second diabetic without treatment as control group with the last three groups. The third and forth group diabetic treated with extract 250mg/Kg, and 500mg/Kg of body weight respectively as a single daily dose for 6 weeks. The elevation procedure was base on the measurement of the level of (Superoxide dismutase (SOD), Catalase (CAT), and blood sugar. Analysis of data revealed significant, dose dependent effects of ginger on the antioxidant enzyme, SOD, and CAT were significantly elevated, while blood glucose were significantly reduced, associated with significant improvement in glycemic control.

INTRODUCTION

Diabetes mellitus is a disorder of carbohydrate metabolism, characterized by persistent hyperglycemia, glucosuria and polyuria (1-4). Diabetes mellitus is one of the pathological conditions that are always accompanied by oxidative stress, that is, with the preponderance of oxidative reactions over the anti-oxidative protection of tissues (5-6).

The enzymes responsible for detoxifying free radicals or regenerating antioxidant molecules can provide an indication of the level of stress experienced in a cell or tissue. These enzymes are usually measured by in vitro activity assays, although changes in transcription can also provide evidence of cell stress. In long-term diabetes, Catalase, GSH reductase, GSH peroxidase, and SOD decrease in complication-prone tissue. One study reports elevated CuZn-SOD activity in the blood, although the increased activity did not correct the deficiency of antioxidant capacity or hyperglycemia induced lipid peroxidation. The study suggested that treatment with oral antidiabetic drugs was responsible for decreases in GSH peroxidase and catalase below control levels(7,8).

Ginger (Zingiber officinale L., Family Zingiberaceae) roots are commonly used as culinary spice and medicinally used for its antioxidant, androgenic and hypoglycemic activities which were reported in animal models. The active ingredients of ginger roots and leaves such as zingerone, gingerdiol, zingibrene, gingerols and shogaols produced antioxidant activity(9-13).
MATERIALS AND METHODS

Diabetes was induced in rabbits by injection of alloxan tetrahydrate at a dose of 180 mg/kg body weight IV in marginal ear vein(14). soon the animal were injected with 10% of glucose solution S/C.

Forty rabbits 6-12 months old and their body weight ranged 1.5-2 kg were randomly assigned to four groups. Group I of rabbits(control group) were with healthy glucose level. Group II (diabetic group) received an IV injection of alloxan tetrahydrate. Groups III and IV of diabetic rabbits treated with extract of Ginger as a single daily dose 250, 500mg/kg body weight of extraction dissolve in 1cc DW orally for 6 weeks.

Ginger ethanolic Extraction:
The officinale (Ginger) was collected from local market of Baghdad, which dried and powdered, according to Bhandari method (15). Two kilograms of air-dried rhizomes of the herb was milled into fine powder mechanically and extracted in cold percolation with 95% ethanol for 24h. The extract was recovered and 95% ethanol was further added to the ginger powder and the extraction was continued. This process was repeated three times. The three extracts were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanolic extract was air-dried, finally give 80 grams of dark brown, gelatinous extract of ginger dried rhizomes. Without any further purification, the crude ethanolic extract was used for the experiments.

Preparation of blood samples
Before taking the blood samples animals were fasted for 12 hours, and the blood samples were obtained by the heart puncture (3-5ml). The blood was centrifuged in a test tube at 2500 rpm for 15 minutes, serum was separated from plasma using a Pasteur pipette supplied with rubber bulb, and transferred to another test tube.

Biochemical analysis:
Glucose determination was carried out according to the method ofTrinder(16)
Serum CAT was determined according to Aebi method manually(17). Superoxide dismutase activity in erythrocyte was determined by using a
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modified photochemical nitro-blue tetrazolium (NBT) method utilizing sodium cyanide as peroxidase inhibitors(17).

Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control group with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). P-value less than 0.05 were considered significant and are written in the parentheses.

RESULTS AND DISCUSSION

Table (1) showed that the glucose level is significantly increased in alloxan diabetic rabbits P<0.05 (9 ± 1.21) mmol/L compared with (3 ± 0.15) mmol/L in control rabbit. After treatment with 250 mg of ginger a significant decreased in glucose level (5 ± 0.43) mmol/L was observed compared with control group which indicated a positive correlation effect of ginger intake. The level of glucose was significantly reduced P<0.05 (4 ± 0.33) mmol/L in alloxan diabetic rabbits receiving 500mg of ginger compared with the level of control group.

Table (2) showed that the SOD level is significantly decreased in alloxan diabetic rabbits P<0.05 (29.78 ± 15.21 µ/ml compared with (150.96 ± 37.45) µ/ml in control rabbit. After treatment with 250 mg of ginger a significant decreased in SOD level (65.32 ± 17.6) µ/ml was observed compared with control group which indicated a positive correlation effect of ginger intake. The level of SOD was significantly reduced P<0.05 (71.3 ± 17.9) µ/ml in alloxan diabetic rabbits receiving 500mg of ginger compared with the level of control group.

Table (3) showed that the CAT level is significantly decreased in alloxan diabetic rabbits P<0.05 (10.14±0.23)µ/ml compared with (12.56±0.65) µ/ml in control rabbit. After treatment with 250 mg of ginger a significant decreased in CAT level (11.34±0.36) µ/ml was observed compared with control group which indicated a positive correlation effect of ginger intake. The level of CAT was significantly reduced P<0.05 (12±0.44) µ/ml in
alloxan diabetic rabbits receiving 500mg of ginger compared with the level of control group.

**Table-1: Effect of administering ginger extract on glucose level in healthy and diabetic rabbits**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mmol/L) Mean ±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3 ± 0.15</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>9 ± 1.21</td>
</tr>
<tr>
<td>Diabetic+ 250mg extract</td>
<td>5 ± 0.43</td>
</tr>
<tr>
<td>Diabetic+ 500mg extract</td>
<td>4 ± 0.33</td>
</tr>
</tbody>
</table>

P<0.05

**Table-2: Effect of administering ginger extract on SOD level in healthy and diabetic rabbits**

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (µ/ml) Mean ±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>150.96 ± 37.45</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>29.78 ± 15.21</td>
</tr>
<tr>
<td>Diabetic+ 250mg extract</td>
<td>65.32 ± 17.6</td>
</tr>
<tr>
<td>Diabetic+ 500mg extract</td>
<td>71.3 ± 17.9</td>
</tr>
</tbody>
</table>

P<0.05

**Table-3: Effect of administering ginger extract on CAT level in healthy and diabetic rabbits**

<table>
<thead>
<tr>
<th>Group</th>
<th>CAT (µ/ml) Mean ±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>12.56±0.65</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>10.14±0.23</td>
</tr>
<tr>
<td>Diabetic+ 250mg extract</td>
<td>11.34±0.36</td>
</tr>
<tr>
<td>Diabetic+ 500mg extract</td>
<td>12±0.44</td>
</tr>
</tbody>
</table>

P<0.05

Intravenous injection of alloxan rapidly damages the β cells of the islets of langerhans in pancreas. Destruction of pancreatic beta-cells by alloxan may results from reaction with glutathione or other sulphhydryl groups of proteins which would inactivate essential enzymes or coenzymes of the cell, alloxan injection may also results in generation of free radicals which cause breaking of DNA stands of beta-cells. Alloxan has also been
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shown to inactivate Ca^{2+}-and calmodulin-dependent protein kinase, the activity of this enzyme was related to insulin secretion (18,19). Flavanoids, terpenoids and a host of the secondary metabolites of many plants possess hypoglycemic effects in various experimental animal models (20,21). Many investigators reported that compounds of ginger such as 6-gingerol, tannins, polyphenolic compounds, and triterpenoids of possess hypoglycemic and other pharmacological properties (22,23).

Catalase and SOD are the two scavenging enzymes that remove toxic free radicals (24) are considered primary enzymes, since they are involved in direct elimination of reactive oxygen species (25). SOD is an important defense enzyme which catalyzes the dismutation of superoxide radicals (26) and CAT is a hemoprotein which catalyzes the reduction of H2O2 and protects tissue from highly reactive OH• radicals (27). The reduced activity of SOD and CAT in the liver and the kidney observed during diabetes may be due to deleterious effect of the accumulation of superoxide anion radicals and hydrogen peroxide. Studies conducted on the chemical compounds of ginger and onion shows that they contain antioxidants. Ginger contains vitamins, flavonoids which their antioxidant roles have been thoroughly been proved (28,29).

In conclusion, the results of the present study showed that the ginger have blood glucose lowering effects and it also had potent antioxidant properties, which may contribute towards preventing peroxidative damage.

REFERENCES


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