EFFECT OF SPICES MIXTURE ON BLOOD GLUCOSE AND LIPIDS PROFILE IN EXPERIMENTALLY - INDUCED OXIDATIVE STRESS FEMALE RABBITS

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Abstract
The effect of spices mixture on blood glucose and lipids profile in oxidative stress rabbits was studied. Twenty female rabbits were randomly divided into four groups (five animals in each group). The first group was subjected to oxidative stress by the ad libitum supply of drinking water containing 0.5% H2O2. The second group was supplied with pellets containing 20% spices mixture. The spices mixture is composed of cinnamon, turmeric and cumin powder. The third group was subjected to H2O2 (0.5%) and supplied with pellets containing 20% spices mixture. The last group was considered as control and supplied with pellets and water. The period of experiment was lasted for 21 days.

The results showed that there was a significant (P<0.05) decrease in glucose, cholesterol, triglyceride, LDL-C and VLDL-C concentration, except HDL-C concentration which increased significantly (P<0.05) in the groups exposed with spices mixture and spices mixture + hydrogen peroxide compared with stress animals.

In conclusion, this study revealed that the supplementation of diets with moderate amounts of spices mixture might have some beneficial effects in oxidative stress conditions and reducing the risk of diabetes and atherosclerosis diseases through improving glucose and lipid metabolism.

Therefore, the active materials in these natural plants might extract and use in diabetes and atherosclerosis drug industry from natural resources.
Introduction

Plants used in traditional medicine to treat some diseases. The dietary components beneficial in the prevention and treatment of diseases have not been clearly defined, but it is postulated that spices may play a role [1]. For example, it has shown that naturally occurring phenolic compounds, isolated from common spice plants have protective effect against mutagenesis [2]. Cinnamon, cumin, turmeric and others are amongst such spices reported to have beneficial effects [3]. Cinnamon improves glucose and lipid metabolism, antioxidant status and capillary function [1]. While, cumin increased activity and excretion content of bile acids and also increased pancreas and small intestine digestive enzymes such as amylase, trypsin, chymotrypsin and lipase in rats [4]. In addition, turmeric has antioxidant, antihepatotoxic and anti-inflammatory properties [5]. Spices mixture (SM) is a food-seasoning item commonly used in Indian homes and in several oriental countries as flavouring agents. Most of the spices are reported to possess anti-inflammatory and anti-tumor properties [6]. In addition, many plant extracts and plant products have been shown to have significant antioxidant activity in protecting the human body against damage by reactive oxygen species [7].

In the light of previous findings of beneficial effects of dietary supplement containing mixture of raw spices on plasma glucose and lipids of normal animals [3]; this study is aimed to investigate the effects of spices mixture containing cinnamon, turmeric and cumin on blood glucose and lipid profile in oxidative stress-induced rabbits.

Material and Method

Experimental animals

Twenty female local rabbits with an average age of about 3-3.5 months and weight between 1200 – 1500 g were used. They were bred in special cages in Al-Nahrain University Research Center for Biotechnology, fed pellets (contain 20 % crude protein and 11% crude fibre, rich in protein and energy) and given tap water ad libitum during the experimental period. Concerning conditions of the laboratory, average temperature was about 22 - 25° C. and the light cycle was divided into 12 hours light: 12 hours dark [8, 9].

Design of the experiment

The animals were randomly divided into four groups (five animals in each group). The first group was subjected to experimentally-induced oxidative stress by the ad libitum supply of drinking water containing 0.5% H2O2 [9]. The second group was supplied with food (pellets) containing 20% spices mixture. The spices mixture is composed of cinnamon (Cinnamomum zeylonicum), turmeric (Curcuma longa) and cumin powders (Cuminum cyminum) that mixed at ratio 1:1:1. The third group was subjected to H2O2 (0.5%) and supplied with pellets containing 20% spices mixture. The last (fourth) group was considered as control and supplied with pellets and water. The period of experiment was lasted for 21 days.

Blood sample collection

After the period of the experiment was elapsed, blood was collected by heart puncture. The volume of blood was 8 ml and collected in glass tubes. The blood sample slowly expressed into the vial to reduce the risk of hemolysis after removing of the needles from syringes [10].

Serum was separated by putting the tubes in the centrifuge at 3000 rpm for 15 min at 37°C. Serum samples were stored at -4°C until biochemical tests were performed [11].
Biochemical parameters

Glucose, total cholesterol, HDL cholesterol and triacylglycerides were determined using assay kits (Biomaghreb Company, Tunis) for in vitro diagnosis use [11]. The kit for glucose assay depends on glucose oxidation by glucose-oxidase enzyme. While the kits for total cholesterol and triglycerides determination depend on enzymatic hydrolysis and oxidation. On the other hand, the HDL cholesterol kit depends on the precipitation reaction and supernatant formation.

Concerning the LDL-cholesterol concentration, Friedewald equation was used as below:

\[ [\text{LDL-Chol}] = [\text{Total-Chol}] - [\text{HDL-Chol}] - [\text{VLDL-Chol}] \]

While VLDL-Chol concentration was calculated as below:

\[ [\text{VLDL-Chol}] = \frac{\text{TG}}{5} \]

Statistical analysis

The results were analyzed statistically using analysis of variance (ANOVA) applicable to a completely randomized design. Then, the significance among means was tested depending on Duncan Multiple Range Test using SPSS program [13].

Results and discussion

(Table 1) illustrates the effect of spices mixture on glucose concentration in oxidative stress female rabbits. The results showed that there was a significant (P<0.05) decrease in glucose concentration in the group treated with spices mixture and spices mixture + hydrogen peroxide compared with stress animals. The glucose concentration means were 99.32, 104.91 and 245.23 mg/dl for spices, spices + stress and stress groups respectively. While there was no significant difference in glucose concentration in the group treated with spices mixture compared with control animals. The glucose concentration for control group was 98.23 mg/dl.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide (0.5%)</td>
<td>a 245.23 ± 1.81</td>
</tr>
<tr>
<td>Spices mixture (20%)</td>
<td>c 99.32 ± 0.88</td>
</tr>
<tr>
<td>Spices mixture + hydrogen peroxide</td>
<td>b 104.91 ± 1.84</td>
</tr>
<tr>
<td>Control</td>
<td>c 98.23 ± 1.66</td>
</tr>
</tbody>
</table>

* Similar letters indicate no significant differences and different letters indicate significant differences at p< 0.05.

Concerning the effect of spices mixture on cholesterol and triglycerides concentrations, the results demonstrated that there was a significant (P<0.05) decrease in cholesterol and triglyceride concentrations in the group treated with spices mixture and spices mixture + hydrogen peroxide compared with stress animals, while there was no significant difference in cholesterol and triglyceride concentrations in the group treated with spices mixture compared with control animals. The cholesterol concentration means were 110.65, 115.24, 106.62 and 162.50 mg/dl for spices, spices mixture + stress, control and stress groups, respectively. While the means of triglycerides concentration for these groups were 119.47, 122.66, 119.56 and 230.50 mg/dl, respectively. (Table 2).

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Cholesterol concentration (mg/dl)</th>
<th>Triglycerides concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide (0.5%)</td>
<td>a 162.50 ± 1.48</td>
<td>a 230.50 ± 1.52</td>
</tr>
<tr>
<td>Spices mixture (20%)</td>
<td>bc 110.65 ± 1.98</td>
<td>b 119.47 ± 1.84</td>
</tr>
<tr>
<td>Spices mixture + hydrogen peroxide</td>
<td>b 115.24 ± 1.94</td>
<td>b 122.66 ± 2.24</td>
</tr>
<tr>
<td>Control</td>
<td>c 106.62 ± 1.34</td>
<td>b 119.56 ± 1.56</td>
</tr>
</tbody>
</table>

* Similar letters indicate no significant differences and different letters indicate significant differences at p< 0.05.

On the other hand, the results in Table (3) revealed that there was a significant (P<0.05) increase in HDL-C concentration in the group treated with spices mixture and spices mixture +
hydrogen peroxide compared with stress animals. The HDL-C means were 44.95 and 21.27 mg/dl, respectively for spices mixture and stress animals. The results also showed that there was no significant difference in HDL-C means in the group treated with spices mixture and the group treated with spices mixture + hydrogen peroxide compared with control group. The means of HDL-C were 45.07 and 42.97 mg/dl, respectively for spices mixture + hydrogen peroxide and control animals. In addition, these results demonstrated that there was a significant (P<0.05) decrease in LDL-C and VLDL-C concentration in the group treated with spices mixture and spices mixture + hydrogen peroxide compared with stress animals, while there was no significant difference in these parameters in the group treated with spices mixture compared with control animals. The means of LDL-C in spices mixture, control and stress groups were 23.89, 23.11 and 46.10 mg/dl, respectively. For these groups mentioned, the means of VLDL-C were 37.68, 40.54 and 95.13 mg/dl, respectively. Furthermore, there was a significant (P<0.05) increase in LDL-C mean in the group treated with spices mixture + stress compared with control animals, while there was no significant difference in VLDL-C mean in the group treated with spices mixture + stress compared with control animals. The means of LDL-C and VLDL-C were 26.06 and 41.72 mg/dl, respectively for spices mixture + stress groups.

**Table 3: Effect of spices mixture on lipoprotein concentrations in oxidative stress female rabbits.**

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>HDL-C concentration (mg/dl)</th>
<th>LDL-C concentration (mg/dl)</th>
<th>VLDL-C concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
<td>21.27 ± 1.22</td>
<td>46.10 ± 0.30</td>
<td>95.13 ± 1.33</td>
</tr>
<tr>
<td>(0.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spices mixture</td>
<td>44.95 ± 1.04</td>
<td>23.89 ± 0.37</td>
<td>37.68 ± 2.17</td>
</tr>
<tr>
<td>(20%)</td>
<td></td>
<td>a</td>
<td>c</td>
</tr>
<tr>
<td>Spices mixture +</td>
<td>45.07 ± 1.72</td>
<td>26.06 ± 1.06</td>
<td>41.72 ± 2.29</td>
</tr>
<tr>
<td>hydrogen peroxide</td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Control</td>
<td>42.97 ± 0.99</td>
<td>23.11 ± 0.84</td>
<td>40.54 ± 2.08</td>
</tr>
</tbody>
</table>

* Similar letters indicate no significant differences and different letters indicate significant differences at p<0.05.

Recent studies have shown that the active constituents of these spices, in particular, cinnamon and turmeric have insulin-potentiating effects. Agents that improve insulin action could also have beneficial effects on lipid metabolism [6]. Furthermore, the aqueous extracts from theses spices have shown to increase in vitro glucose uptake and glycogen synthesis and to increase phosphorylation of the insulin receptor; in addition, these extracts are likely to aid in triggering the insulin cascade system. Because insulin also plays a key role in lipid metabolism, it postulated that consumption of these spices would lead to improved glucose and blood lipids in vivo [1]. Thus, botanical products that show antihyperglycemic effect have a positive influence on lipid metabolism. This is due to the inter-relationship between metabolism of glucose and lipid and to the regulatory influences of insulin on both the metabolic pathways [6].

Further, it has found that the active principle in cinnamon was identified to be methyl hydroxy chalcone polymers (MHCP). It proposed that MHCP is effective insulin mimetic which activates the pathways leading to glucose utilization in cells [6].

The hypocholesterolemic effect of spices mixture has been ascribed to inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme of the cholesterol synthetic pathway [4].

It has found that curcumin and cumin, (diferuloylmethane), the main coloring component of turmeric, have hypocholesterolemic, choleretic (enhanced bile secretion) and hydrocholagogic [enhanced biliary removal of cholesterol] effects.
Curcumin also has a beneficial effect on blood sugar in diabetics [4, 5].

The antioxidant effect of spices mixture in oxidative stress rabbits was in accordance with the result of [2] in which found that the administration of spices mixture (SM) reduces the levels of peroxidation markers in tissues and improves the antioxidant status. Therefore, it appears that superoxide radical scavenging action and inhibition of lipid peroxidation of spices mixture were responsible for the observed hypoglycemic action in stressed rabbits [7].

In addition, cinnamon extract (CE) administration to oxidative stress rabbits prevented the development of insulin resistance possibly by enhancing insulin-signaling pathway in skeletal muscle [6]. Furthermore, it has found that diabetic rats given curcumin showed a significant reduction in renal dysfunction and oxidative stress, which may indicate that curcumin has a protective role against diabetic nephropathy [5].

Thus food additives like spices, which have free radical scavenging activity, may be useful in controlling glucose and lipids levels in stressed patients [7].

The present study showed that the hypoglycemic and hypolipidemic was more in oxidative stress rabbits compared to non-oxidative stress rabbits after administration the same level of spices mixture to each groups.

In conclusion, this study revealed that the supplementation of diets with spices mixture might have some beneficial effects in oxidative stress conditions and reducing the risk of diabetes and atherosclerosis diseases through improving glucose and lipid metabolism. Therefore, the results of this research are considered useful in diabetic and atherosclerotic patients through small doses of this mixture is added to the diet or water. Furthermore, the pharmacists and chemists should put attention on the extraction the active materials in these natural plants and use them in diabetes and atherosclerosis drug industry from natural resources.

References


