Research Article

BENEFICIAL EFFECTS OF GERANIOL ON PLASMA NON-ENZYMIC ANTIOXIDANTS IN HFD AND LOW DOSE OF STZ-INDUCED DIABETIC RATS

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Abstract

In the present study, we evaluated the beneficial effects of geraniol on altered plasma non-enzymatic antioxidants in high fat diet (HFD) and low dose streptozotocin (STZ) induced diabetic rats. Diabetes was induced in male albino Wistar rats by HFD in combination with a low dose of STZ (25 mg/kg body weight). Geraniol was administered orally at a dose of (25mg, 50mg, and 100mg/kg body weight) for 8 weeks. The effect of geraniol was studied on plasma non-enzymatic antioxidant. Diabetic rats also showed a significant (P < 0.05) decrease in plasma non-enzymatic antioxidant. Geraniol treatment near normalized in the biochemical parameters studied. Geraniol (100 mg/kg body weight) revealed the most significant effect than the other two doses. Thus, the present study exhibits antioxidant effects of geraniol in the circulation of HFD and low dose of STZ induced diabetic rats.

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Key words: Diabetes Mellitus, High fat diet, Streptozotocin, Geraniol, Non-enzymic antioxidant.

1. Introduction

Diabetes mellitus is a heterogeneous disorders having hyperglycemia, which is due to impaired glucose utilization resulting as of a defective or deficient insulin secretory reaction (Gupta et al., 1999). Several studies have testified that the rats fed with HFD develop insulin but not frank hyperglycemia or diabetes (Tanaka et al., 2007; Zhao et al., 2008; Flanagan et al., 2008). At the same time, STZ is broadly used to reproducibly encourage both insulin-dependent and non-insulin dependent diabetes mellitus presently by inducing cell injuries and death. (Szkudelski, 2001). Low dose STZ have to make a mild damage of insulin secretion, which is related to the future stage of type 2 diabetes (Reed et al., 2000; Srinivasan et al., 2005). Therefore, the present investigation have started to develop a rat model by feed with high fat diet combination of low-dose STZ that would future stage of type 2 diabetes (Reed et al., 2000; Srinivasan et al., 2005; Sahin et al., 2007). Overall, the world Diabetes mellitus (DM) is a most important and developing public health problem, with an estimated worldwide frequency of 415 million people in 2015, which is estimated to growth to 642 million people by 2040 (International Diabetes Federation, 2015).

Diabetes Mellitus is often controlled by synthetic agents such as sulfonylureas and α-glucosidase inhibitors, but they have several side effects.
effects (Posuwan, 2013). Geraniol is one of the monoterpenes compounds and it’s established in essential oils of fruits and herbs as like Curcuma longa (turmeric), Pelargonium graveolens, Sphaeranthus indicus and Cymbopogon martini. Also it is suggested to represent a new class of agents for biological actions such as anti-cancer, anti-microbial, anti-oxidant and anti-inflammatory, hypotension, hypocholesterolemic activity (Madankumar et al., 2013). Since, altered on non-enzymatic antioxidants plays a vital role in the pathogenesis and complications of DM, we considered the effect of geraniol on altered plasma of non-enzymatic antioxidants in HFD and low dose STZ induced diabetic rats.

2. Materials and Methods

Chemicals

Streptozotocin and geraniol were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade and purchased from E. Merck and Hi-media (Mumbai, India) and S.D-Fine Chemicals (Mumbai, India).

Experimental Animals

Male albino Wistar rats, weighting about 180 - 220 g were procured from central animal house, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. They were housed in clean, sterile, polypropylene cages under standard vivarium conditions (12 hrs light/dark cycles) with fixed amount of feed (Hindustan Lever Ltd., Bangalore, India) and water. The experimental protocol was approved by the Institutional Animal Ethical Committee, Annamalai University (Reg No. 1119, 2015).

Induction of Experimental

The experimental groups were received beef tallow based high fat diet mixed with the commercial diet to diabetic complications (25 % fat, 15 % protein, 51 % starch and 5 % fiber) (Jian et al., 1998). After 1 month on their respective diets, experimental rats were injected intraperitoneally with streptozotocin (25 mg/kg body weight) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) (Sakr, 2010). STZ Injected rats were allowed to drink 20 % glucose solution overnight to overcome the initial drug - induced hypoglycemic mortality. The induction of DM in rats was confirmed by estimating the elevated plasma glucose levels, 72 hrs after STZ Injection. Rats with fasting plasma glucose levels more than 250 mg/dl were considered diabetic and chosen for the study.

Experimental Design

A total of 36 adult male albino Wistar rats were used and they were divided into six groups of 6 rats in each group as below. Geraniol was dissolved in corn oil and administered orally at different doses using an intragastric tube for a period of 8 weeks.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Normal control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>Normal rats were administered geraniol (100 mg/kg b.w/day/p.o) dissolved in 1 ml corn oil</td>
</tr>
<tr>
<td>Group III</td>
<td>Diabetic control rats</td>
</tr>
<tr>
<td>Group IV</td>
<td>Diabetic rats were administered geraniol (25 mg/kg b.w/day/p.o) dissolved in 1ml of corn oil</td>
</tr>
<tr>
<td>Group V</td>
<td>Diabetic rats were administered geraniol (50 mg/kg b.w/day/p.o) dissolved in 1 ml of corn oil</td>
</tr>
<tr>
<td>Group VI</td>
<td>Diabetic rats were administered geraniol (100 mg/kg b.w/day/p.o) dissolved in 1 ml of corn oil</td>
</tr>
</tbody>
</table>

Sample collection

At the end of the experimental period, the rats were deprived of food overnight, anesthetized intramuscularly using ketamine (24 mg/ kg body weight) and sacrificed by cervical decapitation. The blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3:1) for the estimation of plasma non-enzymatic antioxidants. Vitamins C, Vitamins E and reduced glutathione (GSH) were estimated in the plasma by standard methods.
Biochemical analysis

Assessment of reduced glutathione in pancreas and liver

The activity of reduced glutathione (GSH) was estimated by the method of Ellman. A known weight of tissue was homogenized in phosphate buffer. A volume of 0.5 mL of homogenate or plasma was pipetted out and precipitated with 2.0 mL of 5 % TCA. 2.0 mL of supernatant was taken after centrifugation and 4.0 mL of 0.3 M disodium hydrogen phosphate were added. The yellow colour developed was spectrophotometrically read at 412 nm. A series of standards (20 - 100 µg) were treated in a similar manner along with a blank containing 1.0 mL of buffer. The amount of glutathione was expressed as mg/dL of plasma or µg/mg of protein for tissues (Ellman, 1959).

Assessment of ascorbic acid (Vitamin-C) in pancreas and liver

The activity of ascorbic acid was estimated by the method of Roe and Kuether. To 0.5 mL of sample, 1.5 mL of 6 % TCA was added and allowed to stand for 5 min and centrifuged. To the supernatant, 0.3 g of acid washed norit was added, shaken vigorously and filtered. This converts ascorbic acid to dehydroascorbic acid. A volume of 0.5 mL of the filtrate was taken and 0.5 mL of DNPH was added, stopped and placed in a water bath at 37 °C for exactly 3 hrs. Removed, placed in ice-cold water and added 2.5 mL of 85 % sulphuric acid drop by drop. The contents of the tubes were mixed well and allowed to stand at room temperature for 30 min. A set of standards containing 20 - 100 µg of ascorbic acid were taken and processed similarly along with a blank containing 2.0 mL of 4 % TCA. The color developed was spectrophotometrically read at 520 nm. The values were expressed as mg/dL for plasma or µg/mg protein for tissue (Roe, 1943).

Assessment of α-tocopherol (Vitamin-E) in pancreas and liver

The activity of Vitamin-E was estimated by the method of Baker. To 0.5 mL of sample, 1.5 mL of ethanol was added, mixed and centrifuged. The supernatant was evaporated and to the precipitate, 3.0 mL of petroleum ether, 0.2 mL of 2, 2’ dipyridyl solution and 0.2 mL of ferric chloride solution were added. Mixed well and kept in dark for 5 min. An intense red color was developed. 4.0 mL of n-butanol was added to all the tubes and mixed well. Standard tocopherol in the range of 10 - 100 µg was taken and treated similarly along with a blank containing only the reagent. The colour in the n-butanol layer was spectrophotometrically read at 520 nm. The values were expressed as mg/dL for plasma or µg/mg protein for tissue (Baker et al., 1980).

Statistical Analysis

Data presented as means ± standard deviation (S.D.) and subjected to statistical significance were evaluated by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) software package version 17.0 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan’s Multiple Range Test (DMRT). Values are considered statistically significant when P<0.05.

3. Results

Table – 1 shows the levels of plasma on non-enzymatic antioxidants such as vitamin-C, vitamin-E and GSH in normal control and experimental rats. The levels of non-enzymatic antioxidants such as vitamin-C, vitamin-E and GSH were significantly (P<0.05) decreased in the plasma of HFD and low dose STZ-induced diabetic control rats compared to normal control rats. Treatment with geraniol to HFD and low dose STZ-induced diabetic rats restored the levels of the above mentioned non-enzymatic antioxidants in the plasma. For the biochemical parameters evaluated, geraniol treatment to normal rats (Group II) did not show any effect as compared to normal control group (Group I).
Table - 1: Changes in the levels of plasma on vitamin - C, vitamin – E and GSH

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin - C (mg/dl)</th>
<th>Vitamin – E (mg/dl)</th>
<th>GSH (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.01±0.22a</td>
<td>2.67±0.14a</td>
<td>25.68±2.19a</td>
</tr>
<tr>
<td>Normal+Geraniol(100mg/kg b.w.)</td>
<td>3.10±0.21a</td>
<td>2.83±0.16a</td>
<td>25.96±2.21a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.96±0.077c</td>
<td>0.69±0.05c</td>
<td>16.01±1.39c</td>
</tr>
<tr>
<td>Diabetic+Geraniol(100mg/kg b.w.)</td>
<td>2.77±0.177c</td>
<td>2.03±0.15c</td>
<td>18.06±1.50c</td>
</tr>
</tbody>
</table>

Values are represented as means±SD for six rats in each group. Values that are not sharing a common superscript letter (a–c) differ significantly at P<0.05 (DMRT).

4. Discussion

In our study geraniol improved the insulin secretion from remnant pancreatic cells, which in develop glucose consumption by peripheral tissues of HFD and low dose STZ - induced diabetic rats too by helping glucose uptake and breakdown. Therefore, geraniol scavenges extreme free radicals produced by HFD and low dose STZ and reduces oxidative stress, thereby protecting the tissues from the deleterious effects of non-enzymic antioxidant. Non-enzymic antioxidants show an excellent role in preventing the cells from oxidative damage. The non-enzymatic antioxidants such as vitamin-C, vitamin-E and GSH play a dynamic role in protecting the cells from hyperglycemia mediated oxidative stress. In this research, the HFD and low dose STZ-induced diabetic rats exposed a significant depletion of non-enzymatic antioxidants such as vitamin-C, vitamin-E and GSH in the plasma. This could be due to increased free radical production in DM (Umamaheswari and Stanely Mainzen Prince, 2007). Handling with geraniol increased the levels of plasma vitamin C, vitamin E, and GSH in HFD and low dose of STZ-induced diabetic rats. Treatment with geraniol to HFD and low dose STZ-induced diabetic rats restored the levels of the above mentioned non-enzymatic antioxidants in the plasma. For the biochemical parameters evaluated, geraniol treatment to normal rats did not show any effect as compared to normal control rats. This result shows the antioxidant potential of geraniol against hyperglycemia caused by excessive free radicals in HFD and low dose STZ-induced diabetic rats. In conclusion, geraniol treatment restored altered levels of plasma non-enzymatic antioxidants in HFD and low dose STZ-induced diabetic rats. Our investigation also revealed that geraniol improved altered non-enzymatic antioxidants in the HFD and low dose STZ-induced diabetic rats from oxidative stress related complications by benefit of its antihyperglycaemic and antioxidant effects.

5. References


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