Effect of (+)-catechin hydrate on oxidative stress induced by high sucrose and high fat diet in male Wistar rats

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Increased lipid peroxidation and reduced glutathione levels in liver of rats fed high sucrose high fat (HSHF) diet were normalized by concomitant administration of (+)-catechin hydrate. Plasma non-enzymatic antioxidants viz. α-tocopherol, ascorbic acid and total thiols decrease were also significantly less in rats administered with (+)-catechin hydrate concomitantly with HSHF diet. Thus the present results indicate that (+)-catechin hydrate has antioxidant activity and is effective in reducing oxidative stress. The study is of clinical importance as oxidative stress is known to be the cause of many clinical manifestations viz. cancer, Parkinson’s disease, atherosclerosis, heart failure, myocardial infarction and many other diseases.

Keywords: Ascorbic acid, (+)-Catechin hydrate, Lipid peroxidation, Reduced glutathione, Thiols, α-Tocopherol

High sucrose high fat (HSHF) diet is very effective in the development of obese diabetic rat model1. In this model consistent hyperglycemia was observed. It was presumed that this diet may also be effective in causing oxidative stress as it is known that consistent hyperglycemia can result in the formation of reactive oxygen species (ROS) such as H2O22. Oxidative stress in the tissues may be due to alteration in lipid composition induced by high sucrose high fat diet. Studies have shown that sucrose and particularly fructose component may cause oxidative damage through increased glycation of proteins3. Sucrose feeding induces accumulation of advanced glycation end products and oxidative degradation of glucose or fructose adducts so formed can lead to the production of free radicals4. A short term consumption of sucrose rich diet is associated with high plasma lipid peroxidation, decrease in plasma α-tocopherol levels and greater susceptibility to peroxidation in various tissues5.

High level of fat particularly unsaturated fat in diet is also known to increase fat-mediated oxidative stress and decrease antioxidant enzyme activity6. Unsaturated fatty acid intake was found to adversely affect some indices of lipid peroxidation7.

Catechins (e.g., catechin, epicatechin, epigallocatechin and their gallates) are predominant form of flavonoids present in plants and have attracted particular attention due to their relatively high antioxidant capacity in biological systems8-10. Their maximum concentration is present in green tea (27%, w/w). Antioxidant capacity of catechins is largely related to the presence and positions of the hydroxyl groups11. Epigallocatechin gallate (EGCG) is more potent antioxidant than ascorbic acid and reduced glutathione in scavenging the alkyl peroxy radicals12. Catechins are known to upregulate antioxidant enzymes13 and scavenge ROS like superoxide (O2−), hydroxyl (OH·) and peroxyl radicals11.

There is virtually no information regarding the effects of pure catechin namely (+)-catechin hydrate. Thus the present study has been planned to explore in vivo effects of (+)-catechin hydrate on non-enzymatic antioxidants in plasma and liver in both control and high sucrose high fat diet fed rats.

Materials and Methods

Reagents — (+)-Catechin hydrate was purchased from Sigma–Aldrich Company, USA for in vivo experiments. 2,4,6-Tripyridyl-S-triazine (TPTZ), 2,4-dinitrophenyl hydrazine (DNPH) and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sisco Laboratories Pvt. Ltd, Mumbai, India. Sucrose, starch, casein, methionine, gelatin and all other analytical grade laboratory chemicals and reagents were
purchased from Merck (Germany) or SRL Chemicals (India). Ultra pure water prepared by labPURE-series Analytical & Ultraplusuf (BIO-AGE, Mohali, India) was used throughout the experiment. The preparations were made fresh every time before the commencement of the experiment. The preparations were purchased from chemist (Table 1).

Animals—Male Wistar rats weighing approximately 100-125 g were obtained from Central Animal House, Panjab University, Chandigarh. The animals were housed in polypropylene cages in hygienic conditions with rice husk bedding and had free access to tap water. Rats were provided with standard rodent chow (Ashirwad Industries) for a two week baseline period. This was followed by administration of experimental diet for 12 weeks. All the procedures and care of the animals were conducted in accordance with institutional guidelines and CPCSEA policies (Committee For The Purpose Of Control and Supervision of Experiments on Animals) throughout the experiment.

Experimental design—Experimental diet was prepared in the laboratory (Table 1). Rats were randomly divided into following four groups of 6 each: Gr.I control diet (CD), Gr.II, control diet and (+)-catechin hydrate (CD + CH), Gr.III, high sucrose and high fat diet (HSHF), Gr.IV, high sucrose and high fat along with (+)-catechin hydrate (HFHS + CH). (+)-Catechin hydrate was solubilized in hot double distilled water (70 °C) and this solution at room temperature was orally administered to respective experimental groups with the help of canula at a dose of 110 mg/kg body weight. A little modified dose of catechin was given according to Kao et al. Rats were provided free access to experimental diet for 12 weeks period.

Plasma preparation and estimation of non enzymatic plasma antioxidants—At the start of the experiment and after every 4 weeks, animals were fasted overnight and blood samples were drawn by puncturing the orbital sinus of the animals under light anesthesia. Blood samples were drawn in vials containing anticoagulant potassium oxalate. Blood was centrifuged at 3000 g for 10 min. Plasma was aspirated and stored at 4 °C till further use for various non-enzymatic antioxidant estimations. Plasma α-tocopherol was estimated by the method of Martinek and plasma ascorbic acid by the method of Roe and Kuther. For the estimation of plasma total thiols, method of Ellman as modified by Sedlak and Lindsay was used.

Analysis of tissue lipid peroxidation and reduced glutathione levels—At the end of the stipulated period of 12 weeks animals were sacrificed and liver was carefully removed. Liver was adequately washed in ice cold normal saline (0.9% NaCl) followed by washing with homogenizing buffer. A 10% (w/v) tissue homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4) for determination of malondialdehyde (MDA) levels in liver by method of Wills. Reduced glutathione levels in liver were estimated as per Ellman. Proteins were estimated by using bovine serum albumin as standard.

Statistical analysis—Results were expressed as mean±SD. Statistical analysis was performed by using one-way ANOVA followed by Fischer’s least significance difference test. The statistical analysis was done using Jandel Sigma Stat Statistical Software version 2.0. Statistical significance of the results were calculated at least at P<0.05.

Results

MDA levels increased significantly (P<0.0001) in liver (46%) of HSHF group as compared to CD group rats (Fig. 1). Administration of (+)-catechin hydrate

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet (CD) (g)</th>
<th>High sucrose high fat diet (HSHF) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>658</td>
<td>Nil</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Nil</td>
<td>562</td>
</tr>
<tr>
<td>Casein</td>
<td>188</td>
<td>188</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Gelatin</td>
<td>14.1</td>
<td>14.1</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>41.4</td>
<td>82.4</td>
</tr>
<tr>
<td>Bran</td>
<td>37.6</td>
<td>37.6</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>9.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Mineral Mix</td>
<td>49.7</td>
<td>49.7</td>
</tr>
</tbody>
</table>

Supplied per kg of vitamin mix: 3 g thiamine mononitrate, 3 g riboflavin, 3.5 g pyridoxine hydrochloride, 15 g nicotinamide, 8 g D-calcium pantothenate, 1 g folic acid, 0.1 g d-biotin, 5 mg cyanocobalamin, 12.5 g cholecalciferol, 25 mg acetomenaphthone, 600 mg vitamin A acetate, 22 g d-α-tocopheryl acetate and 10 g choline chloride. To add these, following components purchased locally were added: 5.625 mL Abdec drops, 3.75 mL Avion drops, 17.5 mL Polybion and 30 mL Seacord.

Supplied per kg of mineral mix: 65.2 g NaCl, 105.7 g KCl, 200.2 g KH₂PO₄, 40.0 g FeCl₃·5H₂O, 512.4 g CaCO₃, 0.8 g KI, 0.9 g NaF, 1.4 g CuSO₄·5H₂O, 0.4 g MnSO₄, 0.05 g CoNO₃, and addition of MgSO₄·7H₂O to provide 507 mg of Mg. This mixture was prepared in the laboratory.
showed non-significant changes in liver MDA levels in CD + CH group as compared to CD group. However, (+)-catechin hydrate reduced liver lipid peroxidation in rats co administered with high sucrose high fat diet as HSHF + CH group rats had significantly lesser MDA levels in liver (31%) compared to rats of HSHF group (P<0.05).

Significantly (P<0.001) decreased levels of reduced glutathione were observed in liver of HSHF rats compared to CD group rats (Fig. 1). Though the administration of (+)-catechin hydrate had non-significant effect in CD rats, the effect was significant (P<0.001) in HSHF + CH group rats. As compared to HSHF group, levels of reduced glutathione were found be significantly (P<0.001) higher in liver of HSHF + CH group.

Plasma α-Tocopherol, ascorbic acid and total thiols were respectively significantly lower in HSHF group during 4th, 8th and 12th week as compared to CD group (Table 2). At the end of 12 weeks, these levels were lower by 28.7% (P<0.0001), 35% (P<0.0001) and 37% (P<0.001) respectively compared to CD group.

Plasma α-Tocopherol and ascorbic acid remained unchanged in CD+CH group compared to CD throughout the study. Supplementation of (+)-catechin hydrate did not significantly affect plasma total thiols during 4th and 8th week but the change was significant (P<0.05) at the end of 12 weeks in CD + CH group compared to CD. In HSHF + CH group, plasma α-Tocopherol and ascorbic acid levels increased significantly (P<0.001 in each case) during 8th and 12th week compared to HSHF group. Compared to HSHF, plasma total thiols showed significant 28.3% (P<0.05) increase only at the end of the study in HSHF + CH group compared to HSHF group.

**Discussion**

The results clearly show that high sucrose and high fat diet produced oxidative stress showing depletion in non-enzymatic antioxidants in plasma as well as in the liver. Liver was affected in terms of lipid peroxidation and development of oxidative stress in

![Fig.1—Effect of (+)-catechin hydrate on liver lipid peroxidation and reduced glutathione levels in control diet fed and high sucrose high fat diet fed rats. [Values are mean ± SD from 6 animals/group. P values: ***<0.001 vs CD; **<0.0001 vs CD; *<0.05 vs HSHF; **<0.001 vs HSHF; NS1 non-significant change vs CD] (Table 2— Effect of (+)-catechin hydrate on (A) plasma α-Tocopherol, (B) ascorbic acid and (C) total thiols in control and high sucrose high fat diet fed rats [Values given as µmol/L are mean ± SD from 6 observations]

<table>
<thead>
<tr>
<th>Duration (Weeks)</th>
<th>CD</th>
<th>CD + CH</th>
<th>HSHF</th>
<th>HSHF + CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A 18.7 ± 0.98</td>
<td>19.2 ± 0.59</td>
<td>18.9 ± 2.39</td>
<td>19.0 ± 2.36</td>
</tr>
<tr>
<td></td>
<td>B 44.9 ± 1.83</td>
<td>44.4 ± 2.38</td>
<td>44.3 ± 1.60</td>
<td>45.0 ± 2.12</td>
</tr>
<tr>
<td></td>
<td>C 338 ± 8.22</td>
<td>334 ± 6.69</td>
<td>340 ± 2.16</td>
<td>334 ± 5.25</td>
</tr>
<tr>
<td>4</td>
<td>A 19.0 ± 1.25</td>
<td>20.0 ± 2.32 NS1</td>
<td>15.4 ± 1.33*</td>
<td>17.2 ± 2.83 NS2</td>
</tr>
<tr>
<td></td>
<td>B 45.0 ± 2.31</td>
<td>43.9 ± 3.37 NS1</td>
<td>30.2 ± 1.73***</td>
<td>34.7 ± 1.29 NS2</td>
</tr>
<tr>
<td></td>
<td>C 336 ± 7.14</td>
<td>338 ± 6.34 NS1</td>
<td>252 ± 7.88**</td>
<td>250 ± 4.65 NS2</td>
</tr>
<tr>
<td>8</td>
<td>A 19.5 ± 1.06</td>
<td>19.3 ± 2.87 NS1</td>
<td>14.0 ± 2.36*</td>
<td>20.2 ± 1.23 NS2</td>
</tr>
<tr>
<td></td>
<td>B 45.2 ± 1.82</td>
<td>43.6 ± 2.82 NS1</td>
<td>26.8 ± 1.79***</td>
<td>33.9 ± 1.76 NS4</td>
</tr>
<tr>
<td></td>
<td>C 339 ± 5.29</td>
<td>352 ± 9.98 NS1</td>
<td>230 ± 4.92***</td>
<td>240 ± 5.14 NS2</td>
</tr>
<tr>
<td>12</td>
<td>A 20.5 ± 2.28</td>
<td>20.9 ± 1.02 NS1</td>
<td>14.6 ± 1.66**</td>
<td>21.2 ± 1.54 NS2</td>
</tr>
<tr>
<td></td>
<td>B 45.2 ± 3.02</td>
<td>42.8 ± 3.46 NS1</td>
<td>29.4 ± 1.19***</td>
<td>34.9 ± 1.81 NS4</td>
</tr>
<tr>
<td></td>
<td>C 340 ± 4.56</td>
<td>371 ± 5.05*</td>
<td>214 ± 5.62*</td>
<td>275 ± 4.41 NS2</td>
</tr>
</tbody>
</table>

P values: *<0.05 vs CD; **<0.001 vs CD; ***<0.0001 vs CD; *<0.05 vs HSHF; **< 0.001 vs HSHF; NS1 non-significant change vs. CD; NS2 non-significant change vs. HSHF. All comparisons are made during same week.
rats administered high sucrose high fat diet. Increased oxidative stress in high sucrose high fat fed rats, leading to damage such as lipid peroxidation, could be due to oxygen free radical production or due to decreased protection by enzymatic and non-enzymatic antioxidants. Short term consumption of a high sucrose diet negatively affects the balance of free radical production and antioxidant defense in rats which leads to increased susceptibility of lipids to peroxidation. Administration of (+)-catechin hydrate reduced liver lipid peroxidation and hence oxidative stress in rats fed high sucrose high fat diet. Green tea extract reduces the levels of lipid peroxides in heart of streptozotocin diabetic rats. Yang et al. have shown that catechins prevent lipid peroxidation by scavenging lipid peroxyl radicals and by deactivation of oxidative stress sensitive transcription factor NF-κB. Drug induced oxidative stress is ameliorated by catechin administration. In the same study, catechins caused significant decrease in LPO levels, 

Glutathione deficiency in high sucrose high fat diet fed rats contributes to oxidative stress in these animals which may play a key role in pathogenesis of many diseases like diabetes. The observed significantly increased levels of GSH in liver may be due to antioxidant properties of bioactive (+)-catechin hydrate. Increase in tea consumption increases the index of total antioxidant status along with slight increases in serum GSH content. Polyphenols present in green tea may offer an indirect protection to prevent decrease of antioxidants such as vitamin E and C through glutathione and its related enzymes through antioxidants using an in vitro oxidation model for heart disease. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 rats administered high sucrose high fat diet. Increased oxidative stress in high sucrose high fat fed rats, leading to damage such as lipid peroxidation, could be due to oxygen free radical production or due to decreased protection by enzymatic and non-enzymatic antioxidants. Short term consumption of a high sucrose diet negatively affects the balance of free radical production and antioxidant defense in rats which leads to increased susceptibility of lipids to peroxidation. Administration of (+)-catechin hydrate reduced liver lipid peroxidation and hence oxidative stress in rats fed high sucrose high fat diet. Green tea extract reduces the levels of lipid peroxides in heart of streptozotocin diabetic rats. Yang et al. have shown that catechins prevent lipid peroxidation by scavenging lipid peroxyl radicals and by deactivation of oxidative stress sensitive transcription factor NF-κB. Drug induced oxidative stress is ameliorated by catechin administration. In the same study, catechins caused significant decrease in LPO levels, 

Conclusion

The present study clearly shows that high sucrose high fat diet produced a rat model of oxidative stress in which there is depletion of non-enzymatic antioxidants in both plasma and liver and (+)-catechin hydrate administration seems to be beneficial in restoring plasma antioxidants and reducing oxidative stress.

Acknowledgement

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