ANTI-HYPERLIPIDEMIC ACTIVITY OF HYPERFORIN: AN IN VIVO STUDY IN RATS

Srikanth Ineedi, Vikas Kumar*

Pharmacology Research Laboratory, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-221 005, India

(*Corresponding author: vikas.phe@itbhu.ac.in)

Summary

The objective of the study was to evaluate the putative antihyperlipidemic activity of hyperforin in fructose fed hyperlipidemic rats and its hypolipidemic activity in normal rats. Hyperlipidemia was induced in rats by replacing drinking water with 10% fructose solution. Fructose feeding has elevated plasma levels of triglycerides, total cholesterol and LDL-cholesterol, whereas plasma HDL-cholesterol level has been decreased. Hyperforin treatment (10 mg/kg, i.p.) in fructose fed rats has shown to decrease the elevated levels of plasma triglycerides, total cholesterol and LDL-cholesterol and it has elevated the decreased levels of HDL-cholesterol. Treatment with a standard antihyperlipidemic drug, atorvastatin has also produced similar results. However, hyperforin treatment has not shown significant effect on lipid profile of normolipidemic rats. The results of the present study indicate that hyperforin has significant antihyperlipidemic activity, which is qualitatively comparable to that of atorvastatin.

Key words: Hyperforin, fructose feeding, hyperlipidemia, plasma lipid profile

Introduction

Hyperlipidemia, hyperlipoproteinemia or dyslipidemia is presence of elevated levels of lipids in the blood stream [1]. These lipids include fats, fatty acids, cholesterol, cholesterol esters, phospholipids and triglycerides [1]. Hyperlipidemia belongs to a group of disorders, collectively termed as metabolic syndrome or syndrome X [2], which is associated with increased risk for type 2 diabetes mellitus and coronary heart disease [3]. Today, in most of the developed countries, hyperlipidemia and its associated atherosclerosis is the leading cause of cardiac illness and deaths [4].

Hyperforin is a polyrenylated acylphloroglucinol derivative of *Hypericum perforatum*, also known as St. John’s wort (SJW) [5]. Ethanolic extract of SJW contains 1-5% hyperforin [6]. Hyperforin is one of the main constituents responsible for antidepressant activity of SJW [7, 8]. Apart from antidepressant activity, hyperforin has also been shown to produce antibacterial, anti-inflammatory and antitumoral activities [9-11].

Hyperforin has also been shown to have antioxidant activity. In an in vitro study, hyperforin has been shown to inhibit the generation of reactive oxygen species (ROS) in human isolated polymorphonuclear leukocytes (PMNL) [12]. Hyperforin has also shown strong inhibitory effect on oxidative burst of polymorphonuclear cells after stimulation with N-formylmethionyl-leucyl-phenylalanine [13].
The standardized extract of SJW containing hyperforin has shown to improve memory in scopolamine induced amnesic rats and this nootropic action is attributed to its antioxidant activity [14-17]. In another study, the standardized extract of SJW containing hyperforin has shown significant superoxide attenuation in human placental veins [18].

Insulin resistance is one of the main causes of disorders associated with metabolic syndrome [19] and plays an important role in development of hyperlipidemia [20]. Oxidative stress has been shown to be one of the man causes of insulin resistance, and several studies have demonstrated improvement of insulin sensitivity on treatment with antioxidants [3, 20, 21]. In one of our recent studies, hyperforin has normalized the altered lipid profile in streptozocin induced diabetic rats (unpublished data). Based on these findings, hyperforin was investigated for its putative hypolipidemic and antihyperlipidemic activities in normal and hyperlipidemic rats respectively.

Fructose administration induces hyperlipidemia by stimulating lipogenesis from liver [22]. Another cause of hyperlipidemia induction by fructose feeding is oxidative stress. Fructose has been shown to cause oxidative stress by elevating lipid peroxide, diene conjugates and reactive substances [22]. In the present study, effect of hyperforin on altered lipid profile of fructose fed rats was evaluated.

**Materials and Methods**

**Animals:** Charles foster albino rats of either sex, weighing 150 ± 20 g were obtained from Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi. Animals were allowed to access food and water ad libitum, unless mentioned otherwise. Temperature (25 ± 5°C) and humidity (55 ± 10%) were maintained constant and 12 hr light and 12 hr dark cycle was followed. Animals were habituated to the environment for 7 days before performing the experiment. The experimental procedures were in compliance with National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals (NIH Publication No. 80-23; revised 1978).

**Drugs and Chemicals:** Hyperforin was a gift sample from Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany. Fructose was purchased from Qualigens Fine Chemicals, Mumbai, India.

**Experimental Methods:**

**Hypolipidemic activity in normal rats:** Animals were divided into three groups (six animals each) as Group I (control), Group II (hyperforin) and Group III (atorvastatin). Animals were weighed before and after the experiment. Each treatment was given for seven days. Group I was administered vehicle (0.3% CMC, p.o.), group II was administered with hyperforin (10 mg/kg, i.p.) and group III animals were administered with atorvastatin (80 mg/kg, p.o.). Hyperforin was suspended in 1% dimethylsulfoxide that contained 0.3% carboxymethylcellulose (CMC). On 7th day, after 1hr of administration of the last dose, blood samples were collected from overnight fasted rats by retro-orbital puncture. Plasma triglyceride and cholesterol levels were measured by spectrophotometric method using commercially available assay kits.

**Antihyperlipidemic activity in fructose fed hyperlipidemic rats:** Animals were divided into four groups (six animals each) as Group I (normal control), Group II (hyperlipidemic control), Group III (hyperforin) and Group IV (atorvastatin). Animals were weighed before the experiment, after fourteen days of fructose administration and after the drug treatment. Group I rats received normal drinking water and Group II-IV received 10% fructose solution throughout the 21 days study period.
Drug treatment (hyperforin and atorvastatin) was started from day 15 for seven days. During drug treatment, Groups I and II rats were administered vehicle (0.3% CMC, p.o.), group III rats were treated with hyperforin (10 mg/kg, i.p.) and group IV animals were treated with atorvastatin (80 mg/kg, p.o.). Hyperforin was suspended in 1% dimethylsulfoxide that contained 0.3% CMC. On 21st day, after 1hr of administration of the last dose, blood samples were collected from overnight fasted rats by retro-orbital puncture. Plasma triglyceride and cholesterol levels were measured by spectrophotometric method using commercially available assay kits.

**Statistical analysis:** The data were expressed as mean ± Standard Error of Mean (SEM). Statistical analyses were performed by one way analysis of variance (ANOVA) followed by Student-Neuman Keuls multiple comparison test (Graphpad prism version 5).

**Results**

**Hypolipidemic activity in normal rats:** There was no significant difference in the weight gain among rats of three groups throughout the study period. Rats treated with atorvastatin (80 mg/kg, p.o.) have shown significantly lower levels of plasma triglyceride, total cholesterol, LDL-cholesterol and significantly higher levels of HDL-cholesterol, compared to control rats (P<0.05). Hyperforin treated rats did not show significant changes in plasma levels of triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol compared to normal control rats (Table 1).

**Table 1: Effect of hyperforin on normolipidemic rats**

<table>
<thead>
<tr>
<th>Treatment (Dose)</th>
<th>Body weight (g)</th>
<th>Plasma triglycerides (mg/dL)</th>
<th>Plasma total cholesterol (mg/dL)</th>
<th>Plasma HDL cholesterol (mg/dL)</th>
<th>Plasma LDL cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (0.5 ml, p.o.)</td>
<td>148.33±5.10</td>
<td>152.00±5.10</td>
<td>107.54±5.14</td>
<td>88.80±3.49</td>
<td>41.36±2.36</td>
</tr>
<tr>
<td>Hyperforin (10 mg/kg, i.p.)</td>
<td>145.16±5.04</td>
<td>148.12±5.60</td>
<td>96.58±2.59</td>
<td>88.87±3.76</td>
<td>58.33±5.51</td>
</tr>
<tr>
<td>Atorvastatin (80 mg/kg, p.o.)</td>
<td>141.33±7.26</td>
<td>139.28±7.86</td>
<td>79.57±3.93***</td>
<td>68.93±3.65**</td>
<td>60.00±3.43*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). *, ** and *** indicate P<0.05, <0.01 and <0.001 respectively, compared to control.

**Antihyperlipidemic activity in fructose fed hyperlipidemic rats:** There was no significant difference in the weight gain among rats of four groups throughout the study period (Table 2). Fructose fed rats have shown significantly higher levels of plasma triglyceride, total cholesterol, LDL-cholesterol and significantly lower levels of HDL-cholesterol compared to normal control rats (P<0.05). In fructose fed rats, hyperforin (10 mg/kg, i.p.) and atorvastatin (80 mg/kg, p.o.) have decreased the elevated levels of plasma triglyceride, total cholesterol, LDL-cholesterol, whereas they have elevated the decreased level of HDL-cholesterol (P<0.05) (Table 3).
Table 2: Body weight changes in fructose fed rats

<table>
<thead>
<tr>
<th>Treatment (Dose)</th>
<th>Body weight (g)</th>
<th>Before fructose administration (day 0)</th>
<th>After fructose administration (day 14th)</th>
<th>After drug treatment (day 21st)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (0.5 ml, p.o.)</td>
<td>145.33±5.35</td>
<td>142.00±5.57</td>
<td>144.83±5.45</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemic control (0.5 ml, p.o.)</td>
<td>154.83±6.28</td>
<td>156.67±5.89</td>
<td>157.50±5.82</td>
<td></td>
</tr>
<tr>
<td>Hyperforin (10 mg/kg, i.p.)</td>
<td>138.67±4.54</td>
<td>140.17±3.94</td>
<td>143.18±5.04</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin (80 mg/kg, p.o.)</td>
<td>140.33±6.06</td>
<td>142.67±6.36</td>
<td>144.50±5.57</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Effect of hyperforin on fructose induced hyperlipidemic rats

<table>
<thead>
<tr>
<th>Treatment (Dose)</th>
<th>Plasma triglycerides (mg/dL)</th>
<th>Plasma total cholesterol (mg/dL)</th>
<th>Plasma HDL-cholesterol (mg/dL)</th>
<th>Plasma LDL-cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (0.5 ml, p.o.)</td>
<td>84.72±8.56</td>
<td>86.80±2.74</td>
<td>41.41±2.37</td>
<td>28.58±1.75</td>
</tr>
<tr>
<td>Hyperlipidemic control (0.5 ml, p.o.)</td>
<td>134.69±12.01*</td>
<td>121.01±6.03*</td>
<td>23.15±3.66*</td>
<td>72.15±8.47*</td>
</tr>
<tr>
<td>Hyperforin (10 mg/kg, i.p.)</td>
<td>105.16±6.21†</td>
<td>100.16±3.27††</td>
<td>43.26±3.58†</td>
<td>34.69±3.92†</td>
</tr>
<tr>
<td>Atorvastatin (80 mg/kg, p.o.)</td>
<td>97.75±4.11†</td>
<td>87.86±3.69†</td>
<td>42.76±2.77†</td>
<td>25.54±3.52†</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). * P<0.05, compared to normal control; † and †† P<0.05 and <0.01 respectively, compared to hyperlipidemic control.
Hyperforin treatment has not shown significant alteration of plasma lipid levels in normal rats, but normalized the altered plasma lipid levels in fructose fed rats. It is well established that fructose intake causes insulin resistance, hyperinsulinemia, hypertension and hyperlipidemia [3, 20, 22]. High consumption of fructose in food is considered one of the main causative factors for development of hyperlipidemia and obesity in sedentary life style [20]. Fructose feeding has been shown to induce hyperlipidemia in experimental animals also and three week administration of fructose in drinking water is a satisfactory model for induction of hyperlipidemia [3, 23, 24]. Fructose is a highly lipogenic nutrient. Fructose in diet enters liver and is metabolized to glyceraldehyde and dihydroxy acetone. These products enter glycolytic pathway providing both the triglyceride and acyl portion for the synthesis of triglyceride and free fatty acids [22, 25]. Elevated plasma free fatty acids promote fat oxidation and produce highly reactive oxygen species, thus causing oxidative stress [26].

Fructose itself can create oxidative stress by its metabolism [21]. Possible mechanism for direct induction of oxidative stress can be depletion of ATP due to its catabolism, down regulation of HMP-shunt by fructose, increased aldehyde formation and reduced generation of reducing equivalents [27]. Fructose also causes increased production of H$_2$O$_2$. Fructose has been shown to increase production of H$_2$O$_2$ and hydroxyl radicals in hamster pancreatic cells incubated with fructose in the presence of a metal ion catalyst [28]. Increase in production of reactive free radicals and oxidative stress causes insulin resistance and contributes to development of insulin resistance and hence hyperlipidemia. A number of studies on fructose fed rats have been reported suggesting antihyperlipidemic effect of drugs having inhibitory effects on oxidative stress and generation free radicals [3, 20, 21, 24]. In our study, fructose administration has elevated plasma levels of triglycerides, total cholesterol, LDL-cholesterol and decreased the level of plasma HDL-cholesterol. These findings are consonant with the lipogenic mechanism of fructose. Treating fructose fed rats with hyperforin at a dose of 10 mg/kg for seven days has decreased the elevated levels of triglycerides, total cholesterol, LDL-cholesterol and elevated the decreased levels of HDL-cholesterol. As hyperforin is a strong inhibitor of reactive oxygen molecules [12, 13, 14], the antihyperlipidemic activity of hyperforin could be attributed to its antioxidant activity. The antihyperlipidemic activity of hyperforin was qualitatively comparable to that of atorvastatin. However, hyperforin has not shown any effect on lipid profile of normolipidemic rats. In view of our novel findings, hyperforin may be potentially valuable in the treatment of hyperlipidemia.

Acknowledgements

Authors are thankful to Dr. Michael Noeldner, Senior Scientist, Pharmacology Division, Dr. Willmar Schwabe Gmbh & Co. KG, Karlsruhe, Germany for providing gift sample of hyperforin. Thanks are also due to University Grants Commission, New Delhi for financial assistance.

References


