Evaluation of the diuretic potentials of naringenin in hypercholesterolemic rats

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Original Research Article

Abstract

Purpose: To investigate the diuretic potentials of naringenin (NGN) in obesity induced in rats by high fat diet (HFD).

Methods: To prepare HFD, normal pellet diet was crushed and thoroughly mixed with cholesterol powder (1 % w/w). The mixture was mixed with some water and made into pellets which were then oven-baked to dry. Four groups of male Wistar albino rats (n = 6) were used for the study. Normal control (group I) received normal pellet diet. Group 2 (HFD-only) was fed HFD for 28 days, while Groups 3 and 4 were co-administered HFD and NGN at doses of 50 and 100 mg/kg, respectively. All treatments were given orally, and lasted for 28 days. Twenty-four hours after the last dose of NGN, blood was collected from all rats and total cholesterol levels determined to confirm obesity. Thereafter, the rats were placed in metabolic cages and urine samples were collected at two time points (5 and 24 h) for measurement of urine volume, urinary pH, conductivity and electrolyte levels (Na, K and Cl).

Results: Treatment with HFD resulted in significantly (p < 0.05) increased serum cholesterol level (178.83 ± 5.43 mg/dL) when compared to normal control rats (88.17 ± 4.04 mg/dL). It also led to decrease in urinary volume (~50 %) at both time points (5 and 24 h) and in excretion of urinary electrolytes (sodium, potassium and chloride ions). However, the changes in these parameters were significantly reversed by NGN administration (p < 0.05).

Conclusion: These results demonstrate the diuretic activity of NGN in HFD-induced obese rats. Thus, NGN can be further explored for use in combination with hypolipidemic agents to tackle obesity.

Keywords: High-fat diet, Hypercholesterolemia, Naringenin, Obesity

INTRODUCTION

Epidemiologic data have shown that Western-style dietary patterns play a major role in the development of diabetes mellitus, hyperlipidemia and disorders of the cardiovascular system [1,2]. Studies have demonstrated a link between HFD and the etiology of hypertension, obesity and hyperlipidemia [3]. There are high chances of acute renal dysfunction from fluid shifts and hypovolemia in obese patients with BMI more than 60 kg/m². In addition, obesity is associated...
with significant decreases in urine output. Many types of drugs have been effectively used for the treatment of these clinical disorders, but the unwanted side effects of these agents limit their applications. The established lipid lowering drugs such as fibrates, statins and bile acid-binding resins have many side effects [4]. Therefore, there is a growing interest in the development of lipid-lowering drugs with diuretic activities from natural products for the treatment of obesity.

Daily intake of fruits and vegetables could be beneficial in the treatment of chronic diseases that make up metabolic syndrome [5,6]. Several studies have demonstrated the potential health benefits of flavonoids and anthocyanins against the various disorders associated with metabolic syndrome [7]. Citrus fruits are rich in flavonoids, the most predominant of which is naringenin (4',5,7-trihydroxyflavanone; NGN), a flavanone [8]. Studies have demonstrated that NGN possesses anticancer [9], lipid-lowering [10], anti-atherogenic [11] and antioxidant [12] properties.

The aim of the present study was to investigate the diuretic effect of NGN on HFD-induced obesity in Wistar albino rats.

EXPERIMENTAL

Reagents and chemicals

Naringenin powder and total cholesterol kit were products of Sigma–Aldrich Chemical Co., USA. The NGN doses used in this study were based on a previous investigation carried out [13].

Animals

Mature, male albino rats (Wistar strain) weighing 180-200g were supplied by the Animal Care Unit of College of Pharmacy, Prince Sattam bin Abdulaziz University, KSA. The rats were acclimatized for 7 days under standard laboratory conditions in an environment with 12h light/12h dark cycle. Standard pellet diet and water were made available to the rats ad libitum. The study was approved by the Ethical Review Committee, College of Pharmacy, Prince Sattam Bin Abdulaziz University, KSA (approval ref no. HAP-01-KJ-050). The experimental procedures were performed according to the guiding principles of the Institute for Laboratory Animal Research in Guide for the Care and Use of Laboratory Animals [14].

Preparation of cholesterol-supplemented diet

Normal pellet diet was ground and mixed thoroughly with cholesterol powder (1% w/w). The resultant mixture was mixed with water and made into pellets which were then oven-baked for proper drying to avoid fungal contamination.

Induction of obesity

Normal Wistar rats were fed with the mixed pellet diet containing 1% cholesterol (HFD) for 28 days.

Diuretic activity

Four groups of Wistar albino rats were used (6 rats per group). The assignment of rats to groups was done randomly. Rats in group 1 (normal control) received normal pellet diet only, while group 2 rats were fed HFD only. Groups 3 and 4 were fed HFD co-administered with NGN at doses of 50 and 100 mg/kg, respectively. All treatments lasted for 28 days. Twenty-four hours after the last NGN dose, blood was withdrawn from retro-orbital plexus for the estimation of serum cholesterol level. Subsequently, urine samples were collected at two time points (5 and 24 h) from the rats put in metabolism cages for 24h. The 24h urine samples were filtered at the time of urine collection and used for determination of urinary pH and conductivity. The remaining urine samples were stored at ~20 °C and used for analyses of electrolytes (Na+, K+ and Cl−).

Evaluation of urine parameters

Urinary conductivity was determined using Jenway conductivity meter (Jenway, UK), while urinary pH was measured using a pH meter (Hanna Instruments, Lynnfield, MA). The 24 h samples were then diluted (1:1000 in deionized water) and evaluated levels of K+, Na+ and Cl− using Professional Ion Chromatography (Metrohm, Switzerland).

Determination of saluretic activity, natriuretic activity and carbonic anhydrase inhibitory (CAI) activity

Saluretic activity was measured by simply summing up the concentrations of Na+ and K+ present in the urine samples of each group. Natriuretic activity was measured by calculating the ratio of Na+ and K+ ion concentrations in the urine samples, while CAI activity was estimated by calculating the ratio: Cl−/(Na+ + K+) [15].

Statistical analysis

Data are expressed as mean ± SEM, and were analyzed by one-way ANOVA, followed by Dunnett’s post hoc test for determination of inter-group variability using SPSS program (version 21.

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software package; SPSS Inc, USA). Values of $p < 0.05$ were taken as indicative of statistically significant differences.

**RESULTS**

**Effect of NGN on serum total cholesterol levels**

After feeding normal healthy rats with HFD, there were significant increases in serum total cholesterol levels in all the rats ($p < 0.05$). However, the HFD-induced increases in serum total cholesterol in the obese rats were significantly decreased ($p < 0.05$) by NGN treatment (Figure 1).

![Figure 1: Total cholesterol level (mg/dL) of rats with HFD-induced experimental obesity. Values represent mean ± SEM of six rats for each group; *$p < 0.05$, relative to control group (n = 6); #$p < 0.05$, relative to HFD-only group.](image)

**Effect of NGN on urine output and diuretic activity**

The results of urine volume analysis are presented in Table 1. Obesity induced by HFD resulted in significant decreases in urine output at both time points of sample collection i.e. 5h and 24h, relative to normal control ($p < 0.05$). Treatment with NGN at doses of 50 and 100 mg/kg resulted in significant increases in urine volume, when compared to the obese rats, showing the diuretic effect of NGN.

**Effect of NGN on urinary electrolyte excretion**

Table 3 shows the urinary electrolyte content of the HFD-only, NGN-treated (50 and 100 mg/kg) and control rats. Excretion of urinary electrolytes (Na+, K+ and Cl-) was significantly lowered in the obese rats after exposure to HFD, when compared to control rats. The two doses of NGN (50 and 100 mg/kg) resulted in significant increases in urinary electrolyte excretion, relative to the HFD-only group ($p < 0.05$).

**Effect of NGN on natriuretic activity, saluretic activity and CAI activity**

Oral administration of NGN to obese Wistar albino rats at 50mg/kg and 100mg/kg produced significant saluretic activity, but did not result in
inhibition of carbonic anhydrase activity (Table 4).

DISCUSSION

The World Health Organization (WHO) has defined obesity as an abnormal accumulation of adipose tissue which is detrimental to good health [16]. Increased lipid levels in blood are important risk factors for atherosclerosis, stroke and hypertension which are major health problems in developing countries. Studies have established an association between obesity and various co-morbidities such as hypertension, diabetes mellitus and dyslipidemia [17]. In the present study, the diuretic and anti-hypercholesterolemic effect of NGN in HFD-induced experimental obesity in Wistar albino rats was assessed. Obesity was induced by feeding high fat diet for 28 days, and confirmed by an increase in serum total cholesterol.

Studies have indicated that excess intake of high-fat diet is not accompanied by a relative increase in its oxidation, leading to accumulation of more lipids in adipose tissue and an increase in body weight [18,19]. This is in agreement with the results obtained in the present study, where feeding of HFD to normal rats led to significant increases in serum cholesterol levels. Furthermore, analyses of urine samples from the rats fed HFD for 28 days revealed significant decreases in urinary volume and significant decreases in excretion of Na⁺, K⁺ and Cl⁻. It has been reported that obesity in rats is associated with some degree of impairment of urinary bladder function through decreased urodynamic pressure without disturbance of glucose metabolism [20,21].

In the present study, oral administration of NGN to obese rats at 50mg/kg and 100mg/kg for 28 days produced anti-obesity and diuretic effects which were clearly evidenced by significant decreases in serum cholesterol levels and increased urinary volume.

In a previous study, NGN ameliorated increases in plasma lipid levels in HFD-fed rats [22]. Increases in plasma and tissue cholesterol levels of ethanol-fed rats have been attributed to up-regulation of HMG CoA reductase, the rate-limiting enzyme in the biosynthetic pathway of cholesterol [23]. Studies have demonstrated the inhibitory effect of NGM (50mg/kg) administered along with ethanol, on the activity of HMG CoA reductase [24].

The findings of the present study indicate dose-dependent effect of NGN on urine excretion. Diuretic activity is considered to be good if diuretic index value is greater than 1.50, moderate for values between 1.00 and 1.50, mild if the values lie between 0.72 and 1.00, and absent if the value is less than 0.72 [25]. In the present study, the diuretic index values of the NGN-treated groups (50 and 100 mg/kg) were 1.83 and 2.19 (at 5h), and 1.71 and 2.39 (at

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**Table 3: Effect of NGN on urinary electrolyte excretion in normal and obese rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HFD-only</th>
<th>HFD + NGN 50 mg/kg</th>
<th>HFD + NGN 100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Na⁺ (mmol/L)</td>
<td>85.8±6.35</td>
<td>43.2±3.21*</td>
<td>99.2±5.72*</td>
<td>110.2±6.67*</td>
</tr>
<tr>
<td>Urinary K⁺ (mmol/L)</td>
<td>56.8±3.67</td>
<td>24.4±2.23*</td>
<td>67.4±4.35*</td>
<td>75.6±3.57*</td>
</tr>
<tr>
<td>Urinary Cl⁻ (mmol/L)</td>
<td>61.8±5.81</td>
<td>31.6±3.06*</td>
<td>67.8±4.33*</td>
<td>77.4±4.79*</td>
</tr>
<tr>
<td>Na⁺ index</td>
<td>1.00</td>
<td>0.50</td>
<td>1.16</td>
<td>1.28</td>
</tr>
<tr>
<td>K⁺ index</td>
<td>1.00</td>
<td>0.43</td>
<td>1.19</td>
<td>1.33</td>
</tr>
<tr>
<td>Cl⁻ index</td>
<td>1.00</td>
<td>0.51</td>
<td>1.10</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n = 6). Index = excretion in test group / excretion in control group.
*Significantly different, relative to control group (p < 0.05); #significantly different, relative to HFD-only group (p < 0.05).

**Table 4: Effect of NGN on natriuretic activity, saluretic activity and CAI activities in normal and obese rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HFD-only</th>
<th>HFD + NGN 50 mg/kg</th>
<th>HFD + NGN 100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saluretic activity</td>
<td>147.6±8.55</td>
<td>74.8±3.89*</td>
<td>167.0±8.15*</td>
<td>187.6±8.87*</td>
</tr>
<tr>
<td>Natriuretic activity</td>
<td>1.54±0.14</td>
<td>1.82±0.16</td>
<td>1.52±0.15</td>
<td>1.45±0.04</td>
</tr>
<tr>
<td>CAI activity</td>
<td>0.44±0.05</td>
<td>0.49±0.07</td>
<td>0.41±0.03</td>
<td>0.42±0.03</td>
</tr>
<tr>
<td>Saluretic Index</td>
<td>1.00</td>
<td>0.51</td>
<td>1.13</td>
<td>1.27</td>
</tr>
<tr>
<td>Natriuretic Index</td>
<td>1.00</td>
<td>1.18</td>
<td>0.99</td>
<td>0.94</td>
</tr>
<tr>
<td>CAI Index</td>
<td>1.00</td>
<td>1.11</td>
<td>0.93</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n = 6). Index = excretion in test group / excretion in control group.
*Significantly lower, relative to control group (p < 0.05); #significantly higher than corresponding value for HFD-only group (p < 0.05).
24h), respectively, indicating that NGN exhibited very good diuretic property especially at the higher dose of 100 mg/kg.

In addition, the excretion of Na\(^+\), K\(^+\) and Cl\(^-\) in urine of the obese rats treated with NGN was significantly increased, and there was slight alkalization of urine. Increased urinary Na\(^+\) excretion in the experimental animals after NGN administration is an indication of the potential of NGN as an antihypertensive agent [26].

Conductivity, an indirect index of urinary ion content, significantly decreased in the obese rats after administration of HFD for 28 days, but significantly increased by NGN treatment.

**CONCLUSION**

These results indicate that oral administration of NGN to hypercholesterolemic rats significantly mitigated increases in serum cholesterol, and produced significant diuretic effect. Thus, it can be reasonably suggested that NGN may be useful for the treatment of hypercholesterolemia associated with water and sodium retention. However, this is subject to toxicity and clinical studies.

**DECLARATIONS**

**Conflict of Interest**

Authors declare that they have no conflict of interest.

** Contribution of authors**

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Majid A. Ganaie, and Mohd N. Ansari designed the study. Abdul Samad, Tajdar H. Khan and Hassan A. Madkhali collected and analyzed the data. Mohd N. Ansari and Majid A. Ganaie wrote the manuscript with support of Syed Rizwan Ahamad. All authors read and approved the manuscript for publication.

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