Ferulic acid, a natural polyphenol, alleviates insulin resistance and hypertension in fructose fed rats: Effect on endothelial-dependent relaxation

Hany El-Bassossy a, b, *, Dina Badawy b, Thikryat Neamataallah a, Ahmed Fahmy b

a Department of Pharmacology, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia
b Department of Pharmacology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

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ABSTRACT

Ferulic acid (FER) is a polyphenolic compound contained in various types of fruits. It has a substantial therapeutic effect inhibitory activity against aldose reductase (AR) inhibition. In this study, we examined the effect of FER on fructose-fed rats in comparison to a standard AR inhibitor, zopolrestat (ZOP). We determined the protective role of FER against metabolic syndrome by examining serum insulin/Glucose levels, triglycerides (TGs), cholesterol and advanced glycation end product (AGE) in rats supplied with 10% fructose drinking water. In addition, blood pressure, vascular reactivity of isolated thoracic aortas and acetylcholine-induced NO were all evaluated to estimate the cardiovascular complications of metabolic syndrome (MetS) associated with fructose feeding. Animals were randomly divided into four groups: control, (+10% fructose, Fru), zopolrestat-treated fructose fed (Fru-zop) and ferulic acid-treated fructose fed rats (Fru-Fer). After 12 weeks of FER treatment, we found significant reduction in both hyperinsulinemia and elevated diastolic blood pressure associated with fructose-fed to levels comparable to those achieved with ZOP. Both FER and ZOP significantly augmented the impaired relaxation associated with fructose-fed, whereas neither showed any significant effect on the developed vasoconstriction. Isolated aortas from fructose-fed rats incubated with either FER or ZOP, reinstated normal relaxation response to acetylcholine (ACh). Furthermore, isolated aortas showed attenuated nitric oxide (NO) production following the addition of (ACh), while both FER and ZOP restored normal induction of NO. Taken together, the current study shows that, FER alleviated insulin resistance and hypertension associated with metabolic syndrome compared to the standard AR inhibitor (ZOP). This potential protective effect is at least mediated by restoring endothelial relaxation.

1. Introduction

Insulin resistance (IR) is a status in which insulin is unable to achieve its required biologic effects at its circulating levels [1]. Insulin resistance, hypertension and hyperglycaemia are the main causative factors of metabolic syndrome (MetS), which enhances the risk of cardiovascular diseases [2]. In hyperglycaemia, a surplus of glucose is reduced to sorbitol in the polyl pathway and further to fructose via a key enzyme called aldose reductase [3]. The reduction of glucose depletes an important cofactor of aldose reductase (AR) activity, called the reduced nicotinamide adenine dinucleotide phosphate (NADPH). Activation of the polyl pathway triggers metabolic abnormalities in tissues accumulated with sorbitol, including alterations in vascular structure and function [4]. Endothelial dysfunction also has been strongly connected with IR and subsequently vascular disorders [5]. Endothelial dysfunction—particularly impaired endothelium relaxation—is characterised by attenuated vascular reactivity to acetylcholine, in which the vasoactive action of nitric oxide (NO) in inhibited causing endothelium contraction [6].

FER is a natural polyphenol existed in various fruits and vegetables such as tomatoes, sweet corn and rice bran. It has antioxidant...
Effect of daily oral administration of ferulic acid (20 mg/kg) or zopolrestat (25 mg/kg) on the body weight, triglycerides, total cholesterol, LDL-cholesterol and advanced glycation end products (AGEs) in rats with fructose-fed rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>LDL-cholesterol (mg/dl)</th>
<th>AGEs (fluorescent units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>129.7 ± 16.5</td>
<td>52.1 ± 8.5</td>
<td>63.9 ± 7.4</td>
<td>26.9 ± 4.9</td>
<td>102.5 ± 3.9</td>
</tr>
<tr>
<td>+ Fructose (10%)</td>
<td>181.5 ± 9.9</td>
<td>88.7 ± 6.1</td>
<td>83.8 ± 5.1</td>
<td>35.2 ± 5.3</td>
<td>135.0 ± 10.4</td>
</tr>
<tr>
<td>+ Zopolrestat (20%)</td>
<td>191.7 ± 8.4</td>
<td>80.9 ± 8.5</td>
<td>102.3 ± 5.8</td>
<td>33.0 ± 6.6</td>
<td>120.7 ± 7.5</td>
</tr>
<tr>
<td>+ Fructose (10%) + Zopolrestat</td>
<td>196.6 ± 6.1</td>
<td>88.0 ± 7.4</td>
<td>72.82 ± 8.4</td>
<td>26.9 ± 4.9</td>
<td>118.0 ± 6.2</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SEM; N = 8 animals; *P < 0.05, **P < 0.01, compared with the corresponding control group values using One Way ANOVA and Dunnett’s post hoc test.
cleaned of adherent fat and connective tissue and cut into 3 rings (−3 mm length). One aortic ring per rat was mounted in the organ bath to examine vascular reactivity, and the other 2 rings were used to test ACh-induced NO production. In order to examine the acute effect of AR inhibition, aorta isolated from (+10% fructose) rats were incubated with zopolrestat or ferulic acid (10 or 100 μM, respectively) for 1 h to examine endothelial-dependent relaxation. Incubation parameters and inhibitory concentrations of ZOP and FER were determined from previous literature [4,17].

2.3. Biochemical measurements

Serum glucose level was determined calorimetrically as characterised previously [18], using a Randox reagent kit (Antrim, UK). Serum insulin level was assayed by sandwich ELISA (Millipore, Cairo, Egypt). TGs, total cholesterol and LDL cholesterol were estimated with commercially available kits. Serum AGE was determined as described previously [19]. The serum was briefly diluted in saline (1:15 v/v) and fluorescence intensity was determined by an LS45 fluorescence spectrophotometer at λex = 370 and λem = 440 nm (PerkinElmer, Cairo, Egypt).

2.4. Blood pressure recording

Blood pressure (BP) was recorded indirectly in restrained conscious rats by the tail-cuff method, as described in a previous work [20]. Rats were settled in the warming chamber (35 °C) and restrained for 10–20 min/day for 3 d prior to BP recording. Measuring BP was performed between 8:00 a.m. and 11:00 p.m. by the same investigator. After a stabilisation period of 5–10 min in the warming chamber, ten readings of the automated inflation-deflation cycle were recorded. A mean of 5 readings (of 5–10 mmHg range) was assigned as the BP.

2.5. Vascular reactivity

Vascular reactivity of isolated thoracic aortas was performed using the isolated artery method, as fully reported previously [21–23]. Contraction of the aortic rings was assessed by the increase in tension from cumulative additions of phenylephrine (PE, 10−9 to 10−3 M) or KCl (10–100 mM); Vasodilation of the aortas was evaluated by previous incubation of the aortic rings with sub-maximal concentrations of PE to produce similar pre-contraction responses in all studied groups. Acetylcholine (ACh, 10−9 to 10−3 M) cumulative concentrations were then added to the organ bath and the response was recorded.

2.6. ACh-induced NO production

The intracellular formation of NO from isolated aortas following activation by ACh was investigated as described previously [19,21]. Briefly, isolated aortic rings were loaded by 4-amin-5-methyl-aminono-2′,7′-difluoro fluorescein diacetate (DAF-FM) through incubation in a buffer at 37 °C for 30 min. Then, the rings were attached longitudinally in a specific organ chamber in which endothelium were exposed upward for fluorescence measurements using a LS45 fluorescence spectrophotometer (PerkinElmer®, Cairo, Egypt) with remote fibre optics calibrated at 485 nm excitation and 515 nm emission.

2.7. Drugs and chemicals

The following chemicals were used: DAF-FM, DCF-DA and pluronic acid were obtained from Molecular Probes, Cairo, Egypt. Fructose was purchased from El-Nasr Chemical Co., Cairo, Egypt, and ACh and PE were purchased from Sigma-Aldrich, Munich, Germany. ZOP was a gift from Pfizer® (Groton, CT, USA). ACh and PE were dissolved in cold buffer. ZOP and FER were administered orally suspended in 0.5% CMC.

2.8. Statistical analysis

Quantified values are expressed as mean ± standard error of the mean (SEM). The agonist maximum response (Emax) was calculated by a non-linear regression using a computer-based fitting program (Prism 5, Graphpad, CA, USA). Statistical significance was determined by a one-way analysis of variance (ANOVA) with Dunnett’s post hoc test.

3. Results

3.1. Metabolic syndrome parameters

The total caloric intake in the control and the (+10% fructose), groups was 532 and 642 kcal/kg/d, respectively, considering no significant difference in fluid consumption. Fructose (10%) delivered in drinking water for 12 weeks produced marked insulin resistance specified by the significant increasing in serum insulin (P < 0.001) and glucose (P < 0.01) levels to that found in the control group. Oral administration of ZOP and FER (25 and 20 mg/kg/d, respectively) significantly decreased the developed hyperinsulinemia (both at P < 0.001). However, Zopolrestat treatment caused distinctive, significant reduction in elevated glucose levels (P < 0.01) with no significant effect of FER on developed hyperglycaemia (Fig. 1). Fructose feeding was also associated with significant elevation in the body weight (P < 0.01), serum levels of triglycerides (P < 0.05), total cholesterol (P < 0.05) and advanced glycation end products (AGEs, P < 0.05). However, neither zopolrestat nor ferulic acid significantly affected these metabolic syndrome parameters (Table 1).

3.2. Blood pressure

Fructose feeding increased both systolic and diastolic blood pressure (both at P < 0.001) compared to the control group, whereas it caused no effect on the pulse rate (Fig. 2). Oral administration of ZOP and FER significantly reduced the elevated diastolic blood pressure (both at P < 0.001); however, neither significantly influenced the elevated systolic blood pressure (Fig. 2).

3.3. Vascular reactivity

Aortas isolated from rats with metabolic syndrome showed exaggerated vasoconstriction in response to phenylephrine (PE), as evidenced by a significant rise in apparent Emax (P < 0.001) when compared to the control group. However, neither ZOP nor FER treatment significantly affected aortic vasoconstriction in response to PE when compared to the (+10% fructose) group (Fig. 3a and Table 2). In response to ACh, aortas isolated from (+10% fructose) rats showed significant relaxation, which was reflected by a significant reduction in apparent Emax (p < 0.01) compared to the control. Interestingly, both zopolrestat and ferulic acid exhibited a significant increase in the apparent Emax (p < 0.01, in the case of ZOP) and in the apparent pD2 (p < 0.01, in the case of FER) when compared to the (+10% fructose) group (Fig. 3b and Table 2). This suggested the reverse action of zopolrestat and ferulic acid against MetS-induced ACh hypo-responsiveness.

In vitro incubation of isolated aortas from (+10% fructose) rats with zopolrestat or ferulic acid for 1 h retrieved normal responsiveness to ACh, indicated by the significant increase in apparent
pD₂ (P < 0.05, in the case of ZOP) and the significant increase in apparent Eₘₐₓ (P < 0.05, in case of FER) compared to the (+10% fructose) group (Fig. 4 and Table 3).

3.4. ACh-induced NO production

Ten percent fructose in drinking water significantly inhibited ACh-induced NO release when compared to the control group as indicated by a significant decrease in apparent Eₘₐₓ (P < 0.001). Surprisingly, both ZOP and FER treatment showed a significant increase in the production of NO when compared to the MetS group (both at P < 0.001, Fig. 5 and Table 2). Thus, both FER and ZOP prevent the reduction of NO release associated with MetS.

4. Discussion

This study presented, for the first time, the potential protective effect of ferulic acid against insulin resistance and hypertension, the main components of the MetS compared to the standard AR inhibitor, zopolrestat. This is supported by the following findings: (i) FER improved ACh-induced relaxations caused by MetS, (ii)
impaired NO release following ACh stimulation in MetS was ameliorated by FER and (iii) FER reduced hyperinsulinemia and hypertension associated with MeS. Thus, it is suggested that the overall protective role of FER against MeS is mediated by the endothelial-dependent relaxation NO signaling.

In our study, we considered the most reliable model of MeS in rats by including fructose (10%) in drinking water [24]. This was sufficient to significantly develop hyperinsulinemia and insulin resistance in 6 weeks. Also, the (+10% fructose) animal group were characterised by increasing in both systolic and diastolic blood pressure, which is consistent with previous studies [20]. Elevated systolic (afterload) BP might be correlated to diminished myocardial contractility [25]. Whereas, enhanced diastolic (preload) BP is referred to the impaired vascular reactivity in the affected animals. Our results indicated that both FER and ZOP significantly reduced the elevations in diastolic BP associated with MetS. The effect of FER is explained by its AR inhibition, which is consistent with previous studies that reported that AR inhibition protects against development of hypertension in spontaneously hypertensive rat [26] and diabetic rat [27] models.

Our present study also highlighted the impairment of vascular reactivity because it is implicated in the development of hypertension [28] and particularly, MetS-triggered hypertension [29]. In (+10% fructose) animal group, we observed an increase in the contraction of isolated aortae in response PE and decreased in the relaxation in response ACh in (+10% fructose) animals. These findings are consistent with previous works on diabetes from our laboratory and others, which demonstrated that diabetes is associated with increased response to ZOP and FER [27,30] and impaired endothelium-dependent relaxation [22,31,32]. Comparable findings concerning impairment of endothelium-dependent dilatation in people with have been derived from several regional circulations [33]; also, hyperinsulinemia can trigger endothelial dysfunction and promote atherosclerosis by impairing endothelial-

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### Table 2

Effect of daily oral administration of ferulic acid (20 mg/kg) or zopolrestat (25 mg/kg) on the maximal response (Emax), Log EC50 and pD2 (−Log EC50) values of isolated aorta PE and ACh dose response curves and Emax and hill slope of NO generation curve in fructose-fed rats (10% in drinking water, for 12 weeks).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PE Emax</th>
<th>PE pD2</th>
<th>ACh Emax</th>
<th>ACh pD2</th>
<th>NO generation Emax</th>
<th>NO generation Hill slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>618.6 ± 18.2</td>
<td>7.3 ± 0.1</td>
<td>948 ± 3.6</td>
<td>7.1 ± 0.1</td>
<td>50.0 ± 1.8</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>Fructose (10%)</td>
<td>902.3 ± 31.8***</td>
<td>7.3 ± 0.1</td>
<td>693 ± 4.5*</td>
<td>6.6 ± 0.1</td>
<td>29.4 ± 1.9***</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>Fructose (10%)-zopolrestat</td>
<td>806.7 ± 46.8</td>
<td>6.7 ± 0.2</td>
<td>890 ± 4.5**</td>
<td>7.2 ± 0.1</td>
<td>61.9 ± 3.6***</td>
<td>0.18 ± 0.09</td>
</tr>
<tr>
<td>Fructose (10%)-ferulic acid</td>
<td>843.4 ± 45.3</td>
<td>6.7 ± 0.2</td>
<td>748 ± 4.3</td>
<td>7.4 ± 0.2**</td>
<td>59.6 ± 3.3***</td>
<td>0.10 ± 0.04</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± SEM; **P < 0.05, ***P < 0.001 compared with the corresponding control group values; *P < 0.05 compared with the corresponding (+10% fructose, Fru) group values; by One Way ANOVA and Newman-Keuls post hoc test.

### Table 3

Effect of in vitro incubation with ferulic acid (100 µM) or zopolrestat (10 µM) for 1 h on the maximal response (Emax) and pD2 (−Log EC50) values of ACh dose response curves of the aorta isolated from fructose-fed rats (10% in drinking water, for 12 weeks).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ACh Emax</th>
<th>ACh pD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90.1 ± 4.7</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td>Fructose (10%)</td>
<td>67.2 ± 5.1*</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>Fructose (10%) + zopolrestat</td>
<td>83.0 ± 5.4</td>
<td>7.3 ± 0.2*</td>
</tr>
<tr>
<td>Fructose (10%) + ferulic acid</td>
<td>83.3 ± 3.9*</td>
<td>7.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E of mean; *P < 0.05, **P < 0.001 compared with the corresponding control group values; *P < 0.05, ***P < 0.001 compared with the corresponding Fructose (10%) group values; by One Way ANOVA and Newman-Keuls post hoc test.

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Fig. 4. The effect of ferulic acid or zopolrestat on aortic responsiveness to ACh. Isolated thoracic aortas were in vitro incubated with ferulic acid and zopolrestat (100 or 10 μM respectively) for 1 h. Aortic responsiveness to cumulative addition of acetylcholine (10⁻⁹ to 10⁻⁵ M) to the organ bath was recorded. *P < 0.05, compared with the corresponding control group values; **P < 0.05 compared with the corresponding (+10% fructose, Fru) group values; by One Way ANOVA and Newman-Keuls post hoc test.

Fig. 5. The effect of ferulic acid zopolrestat on the ACh-stimulated NO production. Ferulic acid and Zopolrestat (Fer, 20 and Zop, 25 mg/kg/d respectively) were administered daily for 6 weeks as suspension in 0.5% carboxy methyl cellulose (CMC) after 6 weeks of inducing MeS. Control group received CMC as a vehicle. Aortas were then isolated and NO release following ACh stimulation was assessed with the fluorescence probes 4-amino-5-methylamino-2′,7′-dichlorofluorescein diacetate (DAF-FM). **P < 0.001, compared with the corresponding control group values; ***P < 0.001 compared with the corresponding (+10% fructose, Fru) group values; by One Way ANOVA and Newman-Keuls post hoc test.
dependent vasodilatation [34,35]. Our data indicated that both FER and ZOP significantly prevented the diminished relaxation to ACh in aortas isolated from animals with MetS without affecting the response to PE. This coincides with previous reports in which zopolrestat recover the high glucose-induced reduction of flow-induced dilation [36]. On the other hand, short-term in vitro incubation of aortas isolated from (+10% fructose) animals with FER and ZOP restored normal endothelial dependent relaxation. This also indicates a direct involvement of the AR enzyme in the impairment of endothelial NO release associated with MetS. In mesangial cells, AR-regulated tumour necrosis factor alpha (TNF-alpha) induced nitric oxide synthase, which proposed a direct association between AR and NO generation [37]. In addition, the AR inhibition reduced the excessive basal NO generation induced by interleukin 1 beta in vascular tissue [38]. On the other hand, AR activity in lenses has been shown to be regulated by nitric oxide [39].

The mechanisms by which FER and ZOP prevent endothelium-dependent relaxation impairment is attributed to maintaining NO production. In the present study, both ZOP and FER prevented the impaired NO generation following ACh stimulation in MetS aortas. This is in accordance with the reported restoration of NO production by AR inhibition in human umbilical endothelial cells cultured in a medium with high glucose content [40]. These assumptions are also supported by previous work in which ZOP inhibited the reduction in flow-induced dilation induced by high glucose [36] and restored NO production in ischemic myocardial injury [41]. In addition, FER improved the bioavailability of stimulated NO in the aortas isolated from spontaneously hypertensive rats [42]. In conclusion, in comparison to the standard aldose-reductase inhibitor zopolrestat, ferulic acid alleviates the major factors of MetS, insulin resistance and hypertension. Ferulic acid is restoring endothelial relaxation, which explained its protective role against hypertension.

Conflict of interest

The funding agency, STDF, did not participate in designing the study, collection, and analysis or interpretation of data. Neither contributed in writing the report or in the decision to submit the paper for publication. There is no other funding source or conflict of interest.

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