



Renoprotective Effects of *Trigonella foenum* and *Cinnamon* on Type 2 Diabetic Rats

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Abstract

Background: Herbal medicine is used in all parts of the world mainly for prevention and treatment of various disorders due to better cultural suitability, lower cost and less side effects.

Objectives: The aim of this study was to determine the hypoglycemic and kidney-protective effects of the aqueous extract of *Trigonella foenum* and *Cinnamon* on diabetic rats.

Methods: In this experimental study, rats were randomly divided into 6 groups as follows: Group 1: control group in which animals received chow diet, group 2: diabetic rats, group 3: diabetic rat + 2% *T. foenum* extract (w/w), group 4: diabetic rat + 8% of *Trigonella foenum* extract (w/w), group 5: diabetic rat + 2% *Cinnamon* extract (w/w) and group 6: diabetic rat + 8% of *Cinnamon* extract (w/w). Aqueous extract of *T. foenum* leaves and *Cinnamon* were administered to diabetic rats for 4 weeks. The malondialdehyde (MDA) level and total antioxidant capacity were also measured in kidney of the animals. In addition, morphological changes of the kidney were also analyzed by the light microscope.

Results: *Trigonella foenum* and *Cinnamon* extract in diabetic animals significantly reduced MDA levels and restored antioxidant capacity ($P < .05$). *T. foenum* and *Cinnamon* also normalized plasma urea and creatinine concentration in diabetic rats ($P < .05$). Administration of *T. foenum* and *Cinnamon* extract especially at the dose of 8 mg/kg normalized histopathological changes of kidney in diabetic animal.

Conclusions: The findings of this experiment showed that *T. foenum* extract and *Cinnamon* restored antioxidant capacity and structural changes of kidney.

Keywords: Diabetes, Cinnamon, Herbal medicine, Rat, *Trigonella foenum*

Received: 18 January 2017
Accepted: 14 April 2017
ePublished: 27 April 2017



Background

Type 2 diabetes is accepted as one of the metabolic disorders, which is known by disturbance in glucose, lipid and protein metabolism. In 2013, International Diabetes Federation reported that 382 million people suffer from diabetes, and it is projected to reach 592 million people by 2035 (1). Hyperglycemia and oxidative stress are known as main factors in diabetic complications (2). In this respect, natural products which are capable of reducing blood glucose and scavenging of free radicals play major role in treatment of diabetes (3-6).

The glucose lowering effects of some medicinal plants has been determined and well recognized in different studies (7). In fact, during previous decade there has been increasing attention to the application of medicinal plants in the control and management of metabolic disorders. Various extracts of certain herbal medicine and its derivatives have been reported to contain high amount of phenolic components which are known as a hypoglycemic and antioxidant agents (8). In this regard,

Trigonella foenum and *Cinnamon* are traditional famous plants which displayed glucose lowering and antioxidant effects in different studies (9,10). The useful effects of *Cinnamon* on diabetes have been reported in various studies (7,11-13).

Mishkinsky et al (14) reported that *T. foenum* seeds have hypoglycemic effects when administrated orally to animal models. They proposed that glucose lowering effect of these seeds is attributed to high levels of alkaloids. The seeds of *T. foenum* are broadly suggested for treatment of type 2 diabetic patients (15). Ajabnoor et al (16) showed the hypoglycemic effect of this plant in diabetic models. *T. foenum* has shown beneficial effect in different types of diabetic models. However, some experiments stated that this plant has no effect on the blood glucose levels of normal rats, but are able to reduce glucose in diabetic animals (15). Faruque et al reported that its hypoglycemic effects are related to inhibition of intestinal glucose absorption by suppression of disaccharidases in the small intestine (17). While, *T. foenum* displayed lipid and glucose

lowering affects, kidney protective effect of leaves is less studied (18). On the other hand, few studies have been established to assess the comparative properties of water extract of *Cinnamon* with *T. foenum*. Consequently, current experiment was designed to determine the kidney-protective effect of aqueous *Cinnamon* and *T. foenum* extract in type 2 diabetic animals.

Objectives

This study was designed to determine the effect of *T. foenum* and *Cinnamon* on kidney biochemical markers, antioxidant and histological alterations in type 2 diabetic rats.

Materials and Methods

Preparation of Plant Extracts

Trigonella foenum leaves and *Cinnamon* were purchased from Hamadan market and then crushed. *T. foenum* and *Cinnamon* powders (40 g from each separately) were mixed with 400 mL of deionized water at 25°C for 48 hours. The solution was filtered, and the prepared filtrate was dried at 40°C in an incubator. The water extract was kept in dark condition at -20°C (19).

Animals

In this experimental study, male Wistar rats (200-220 g) were obtained from Hamadan University of Medical Sciences. During the experiment, the animals were fed with chow diet and kept at standard condition of 12/12 hours (light/dark) with the humidity of $55 \pm 5\%$. The animals were adapted for 1 week after being divided into different groups and prior to the beginning of the study (3).

Induction of Type 2 Diabetes

For induction of type 2 diabetes, 65 mg/kg of streptozotocin (STZ) was injected to animals after 10-12 hours of fasting. After 15 minutes, nicotinamide (110 mg/kg) was injected intraperitoneally (20). Seven days later blood glucose levels of rats were measured and glucose level more than 250 mg/dL were reflected type 2 diabetes.

Experimental Design

After acclimatization in animal house, the rats were randomly divided into 6 groups as follow: Group 1: control group in which animals received chow diet, group 2: diabetic rats, group 3: diabetic rat + 2% *T. foenum* extract (w/w), group 4: diabetic rat + 8% of *T. foenum* extract (w/w), groups 5: diabetic rat + 2% *Cinnamon* extract (w/w), and groups 6: diabetic rat + 8% of *Cinnamon* extract (w/w) (19). All procedures of this study were approved by the Ethic Committee of Hamadan Azad

University (Hamadan, Iran).

Determination of Oxidative Stress and Biochemical Markers

After 4 weeks, blood samples were taken from heart of all rats. After that, serum was prepared by centrifuge for 10 minutes at $1500 \times g$. The serum was used to measure glucose using a commercial kits (Pars Azmun Co. Iran) (4). Kidneys of the animals were removed and some parts were used for histopathological examination and the other parts for determination of oxidative stress markers. The malondialdehyde (MDA) level, as an indicator of lipid peroxidation, was determined using the thiobarbituric acid method (21). The results were stated as nmol of MDA/gram protein. Total antioxidant capacity (TAC) also was determined according to our previous papers (22). Some kidney markers such as urea and creatinine were measured enzymatically by using an automatic analyzer according to enzymatic kits (Pars Azmun, Tehran, Iran).

Histopathological Examination

The kidney of each rat was fixed quickly in 10% formalin solution and processed by conventional methods. Briefly, tissue was embedded in paraffin and 5 μm sections were arranged and stained with hematoxylin & eosin and evaluated under a light microscope (23).

Statistical Analysis

All statistical analyses were carried out using the SPSS statistical program package (SPSS; 16, Inc, USA). The data in this study were expressed as mean \pm standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey test was used for statistical comparison.

Results

Biochemical Test

Our previous report revealed that blood glucose significantly reduced and also showed significantly normalized liver enzyme ($P < .05$). Figure 1 shows that total antioxidant capacity in the kidney of diabetic rats markedly reduced in comparison with that of normal rats ($P < .05$). In animals treated with *T. foenum*, the levels of total antioxidant significantly increased compared with diabetic rats ($P < .05$).

MDA levels in the kidney of variously-treated animals are shown in Figure 2. This lipid peroxidation marker significantly increased in diabetic animals in comparison with normal group ($P < .05$). In *T. foenum* treated animals, this marker significantly normalized as compared with diabetic rats ($P < .05$).

Effects of *T. foenum* and *Cinnamon* on blood creatinine and urea levels are presented in Figures 3 and 4. In this

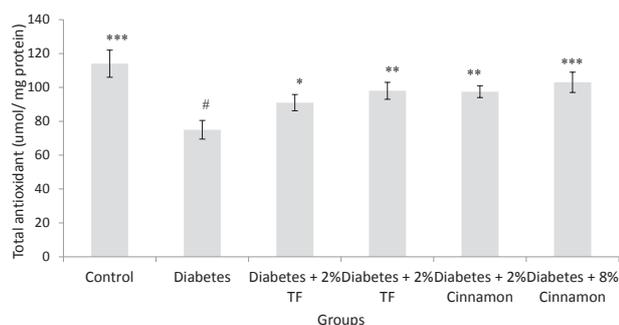


Figure 1. Total Antioxidant Capacity in Variously-Treated Animals. Data represent as mean \pm SEM. TF: *Trigonella foenum*. * $P < .05$, ** $P < .01$ and *** $P < .001$ compared with diabetic animals. # $P < .001$ compared with normal rats.

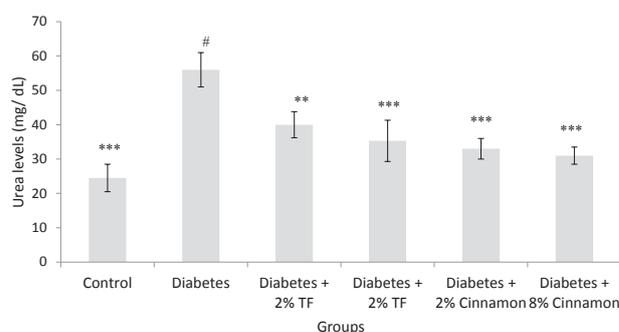


Figure 3. Urea Levels in Variously-Treated Animals. Data represent as mean \pm SEM. TF: *Trigonella foenum*. * $P < .05$, ** $P < .01$ and *** $P < .001$ compared with diabetic animals. # $P < .001$ compared with normal rats.

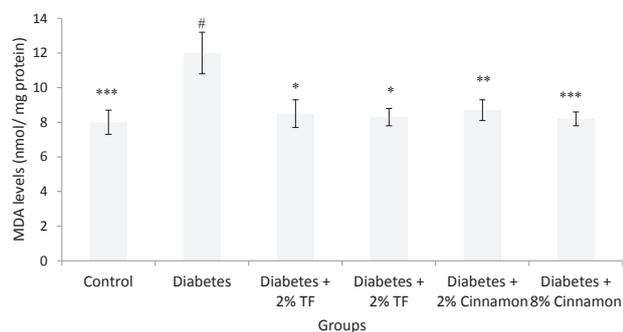


Figure 2. MDA Levels in Variously-Treated Animals. Data represent as mean \pm SEM. TF: *Trigonella foenum*. * $P < .05$, ** $P < .01$ and *** $P < .001$ compared with diabetic animals. # $P < .001$ compared with normal rats.

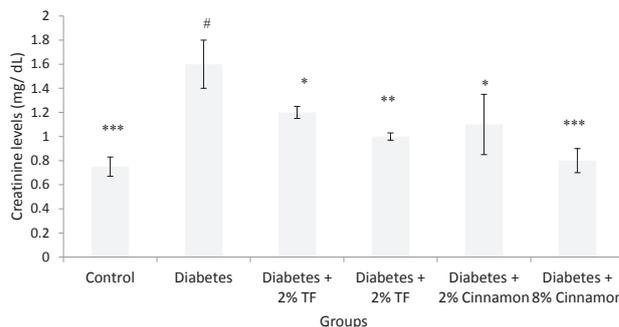


Figure 4. Creatinine Levels in Variously-Treated Animals. Data represent as mean \pm SEM. TF: *Trigonella foenum*. * $P < .05$, ** $P < .01$ and *** $P < .001$ compared with diabetic animals. # $P < .001$ compared with normal rats.

experiment, *T. foenum* and *Cinnamon* markedly normalized blood urea and creatinine levels especially at the dose of 8 g/kg ($P < .05$).

Histological Changes

In the control group, kidney cells showed regular proportions. The acinar cells were organized in lobules with noticeable nuclei. The islet cells were embedded in the acinar cells and surrounded by a fine capsule. Figure 3 illustrates the analysis of kidney with microscopic studies showing that in diabetic animals the islets size was reduced and necrosis with dense eosinophilic cytoplasm was observed as compared with control group. Treatment with *T. foenum* at the dose of 8 g/kg normalized islet structure and conserved kidney cells.

Discussion

A growing body of evidence proposes that natural antioxidants have a major role in the prevention and treatment of diabetic complications (24). Rise of reactive oxygen species (ROS) that produced by disturbance of free radicals hemostasis has been well-known in the diabetes (25). High blood glucose which leads to formation of Advanced glycation end products (AGEs)

along with ROS can motivate lipids peroxidation, different protein glycation, suppression of various enzymes and metabolism change known as the main reason of diabetic complications (2). It has been shown that insufficient free radical scavenging and overproduced ROS would cause different tissue damage especially in kidney cells (25). Also previous studies recognized that STZ diabetic models chiefly motivated ROS generation and consequently disrupted the kidney tissue. In fact, STZ causes cytotoxicity of kidney cells in diabetic animals (26).

Administration of antioxidants to both human and animal models can be one of the beneficial methods to control blood glucose and ROS in diabetic patients (25). Pharmaceutical evidence reported that approximately 800 medicinal plants have been used as traditional medicines for treatment of metabolic disorders (27).

In this experiment, we showed that *T. foenum* has significant antioxidant capacity that would cause additional beneficial properties such as hypoglycemic and kidney regeneration effects. The relationship between definite complications in diabetic patients and kidney structural changes has been reported, but only limited results showed correlation of antioxidant effects of *T. foenum* and kidney structure changes (28).

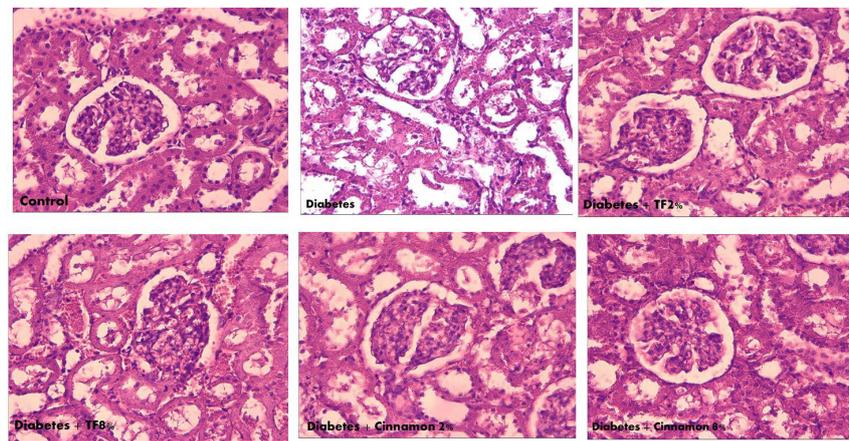


Figure 5. The Morphological Alteration of Kidney in Various-Treated Animals. *Trigonella foenum* and *Cinnamon* normalized kidney morphological changes in diabetic rats.

Sharma (29) stated that prescription of *T. foenum* in hyperlipidemic patients, markedly reduced cholesterol and lipoprotein levels. Our previous report proved that *T. foenum* markedly declined fructosamine and AGEs formation (19). Inhibition of AGEs by herbal medicine was suggested as one of the main targets of diabetic complications (6). On the other hand, increase in levels of MDA in the kidney is a sign of oxidative stress that has been stated as one of the basic reason of diabetic nephropathy (27). In this study, MDA levels were significantly less in *T. foenum* and *Cinnamon* groups compared with that of diabetic group.

The kidney injury was revealed by the rise of some waste materials like creatinine and urea in the blood. The results of this experiment showed evidence of kidney damage that is reflected by the change of these markers (30). Interestingly, our study demonstrated that *T. foenum* and *Cinnamon* significantly normalized creatinine and urea concentration in diabetic rats.

The current experiments showed changed kidney morphology in diabetic rats. Many epidemiological studies support the idea that control of hyperglycemia, increase of antioxidant activity, restore of insulin secretion and kidney structure are the major ways to manage type 2 diabetes (28). In diabetic animals, mild necrosis of tubular along with damage to brush border were observed. These changes were in line with the results of Renno et al (31) who reported the tubular necrosis, lining cells expansion and glycogen mass in the tubules. Ren et al (32) also stated noticeable increase in kidney weight and kidney fibrosis in diabetic animals.

Conclusions

This study illustrated that *T. foenum* and *Cinnamon* can improve antioxidant capacity and reduce lipid peroxidation. These medicinal plants also showed kidney protective effects. Therefore, *T. foenum* and *Cinnamon* are

suggested to use in diabetic patients.

Authors' Contribution

SMK prepared the draft of manuscript, did antioxidant tests and interpreted the data; MTG planned the study, prepared the manuscript and approved the final version of the manuscript; and EAO did animal handling and biochemical analysis and preparing the article draft.

Conflict of Interest Disclosures

No conflict of interest.

Funding/Support

We would like to thanks Hamadan Branch, Islamic Azad University for financial support of this study.

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