

Effect of *Citrullus Colocynthis* Pulp on Renal Function in Streptozotocin-Induced Diabetic Rats

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Original Article

Abstract

Introduction: Diabetic nephropathy is one of the most serious microvascular complications of diabetes and the main cause of end-stage renal disease. Various herbs have been used to control diabetes complications. This study aimed at investigating the effect of *Citrullus colocynthis* pulp on diabetes-induced renal damage in male rats.

Methods: A total of 36 rats were divided into 6 groups: 1- normal (N), 2- normal + 10 mg/kg colocynth (N+C10), 3- normal + 30 mg/kg colocynth (N+C30), 4- diabetic (D), 5- diabetic+10 mg/kg colocynth (D+C10), and 6- diabetic+30 mg/kg colocynth (D+C30). The rats received colocynth pulp through gavage for two weeks. The obtained data were analyzed through ANOVA and Tukey test using SPSS 16.

Results: The diabetic rats showed a sharp rise in the volume of urine, clearance of albumin, clearance of creatinine, and serum urea. Administration of colocynth in diabetic rats improved the changes to normal.

Conclusion: The results of this study show that colocynth pulp has the potential to treat diabetes and to prevent the relevant kidney damages.

Key words: Diabetes Mellitus - *Citrullus Colocynthis* – Rat.

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Introduction:

Diabetes is a syndrome characterized by impaired metabolism of carbohydrate, fat, and protein. It develops due to inheritance, aging, poor diet, high cholesterol, pancreas infection, reduced glucose consumption, and other factors. Diabetes is one of the most important disorders and if left undiagnosed, it may lead to rapid weakness and eventually to death (1). It is estimated that the number of diabetics in the world will increase to 300 million by 2025. United States of America and

developing countries such as India and China are now the countries with a significant number of patients. Diabetes is the third leading cause of death after cancer and cardiovascular disease (2).

Many studies have shown an increase in lipid peroxidation or oxidative stress in patients with diabetes (3).

It seems that oxidative stress occur in diabetes through increased production of reactive oxygen species (ROS), reduced anti-oxidative defense, and change in cellular redox status. Patients with diabetes

may be especially susceptible to acute and chronic oxidative stress which enhances further development of diabetic complications (4).

The kidney damage in diabetes will increase substantially. For example, one of the first characteristics of diabetic renal damage is increased glomerular filtration rate (GFR) and serum urea. Finally, diabetic nephropathy decreases renal function and changes its structure (5).

Diabetic nephropathy, discovered in 1936 by Paul Kimmelstiel and Clifford Wilson, is known as Kimmelstiel Wilson syndrome. Its morphological characteristics include renal hypertrophy, thickening of glomerular basement membrane, extending mesenchyme, tubular atrophy, interstitial fibrosis, and arterial thickening. Diabetic nephropathy is associated with increased mortality of patients with diabetes. It seems that diabetic nephropathy occurs as a result of interaction between metabolic and hemodynamic factors (6).

Herbal medicines have been used as a major treatment for diseases over the years. Since active ingredients in herbal remedies are associated with other compounds, they are always in a biological equilibrium, and hence they are not accumulated in the body and not cause side effects, giving them considerable advantages compared to chemical drugs. Before the discovery of insulin and conventional diabetes drugs, patients were treated with medicinal plants and traditional treatments. More than 1200 medicinal plants are shown to have positive impact on reducing blood sugar levels or its complications (7).

As a member of the Cucurbitaceae family, colocynthis is traditionally used as an anti-diabetic drug and as a laxative in several Asian countries (8). It is growing widely in Asia, especially in East Asian countries. Colocynthis is administered for diabetes by groceries in several cities of Iran as dried fruit in different doses of 300-800 mg/kg (9), but the patients are not monitored after treatment in terms of its efficacy or toxicity; although, empirical studies have confirmed its hypoglycaemic properties (10,11).

It has been shown that various extracts of colocynthis fruit has anti-diabetic activity. Nimla *et al.* (2000) reported that its seed's amino acids are able to stimulate the release of insulin from the pancreas of rats. It was also reported that aqueous extract of colocynthis seeds improves the toxic effects of STZ (11). Its fruit contains saponins of cucurbitacin E, G glycoside, alkaloids, and derivatives of caffeic acid

such as chlorogenic acid (12-14). In addition, colocynthis can lead to diarrhea due to increased peristalsis of the small intestine. It seems that this plant can lead to impairment of absorption of other foods through increased disposal of glucose.

The aim of this study was to evaluate the effect of *Citrullus colocynthis* pulp on renal function in diabetic rats.

Methods:

The present study was conducted in 2012 at Shahid Bahonar University on male Wistar rats weighing 230-270 g. The rats were obtained from the Experimental Animals Breeding Center of Shahid Bahonar, Kerman. They were fed with usual diet of laboratory animals, and were kept in standard metal cages under controlled conditions of 12 hours of darkness and 12 hours of light at 23-25°C, without food and water restrictions.

Induction of diabetes

Diabetes was induced in rats using 65 mg/kg streptozotocin (STZ) dissolved in 0.5 mL cold saline just before use. The levels of glucose were measured in blood sample collected from the rats' foot 3 days after induction of diabetes (15). To this end, the animals were anesthetized and blood sugar was determined using a glucometer (Glucocard 01, GT-1920). Rats with fasting blood glucose greater than 300 mg/dL were considered diabetic.

Preparation of the herb

Dried fruit of colocynthis was obtained from a grocery and was confirmed by a plant expert. The fruit pulp was separated from the peel and seeds and the pulp was then ground in a mill and stored in a cool dry place. The powder was dissolved in one milliliter normal saline and administered orally through gavage followed by gavage of one milliliter normal saline. In this study, colocynthis was administered at doses of 10 mg/kg and 30 mg/kg (16).

Test plan

A total of 36 rats were used in this study. The rats were divided into 6 six-member groups of 1- normal (N) that received normal saline for two weeks; 2- normal+10 mg/kg colocynthis (N+C10) that received colocynthis pulp powder (dissolved in 1 mL normal saline) in a dose of 10 mg/kg; 3- normal+30 mg/kg

colocynthis (N+C30) that received colocynthis pulp powder (dissolved in 1 mL normal saline) in a dose of 30 mg/kg; 4- diabetic (D) that were induced diabetic and received normal saline; 5- diabetic+10 mg/kg colocynthis (D+C10) that were induced diabetic and received colocynthis pulp powder in a dose of 10 mg/kg; and 6- diabetic+30 mg/kg colocynthis (D+C30) that were induced diabetic and received colocynthis pulp powder in a dose of 30 mg/kg.

The experiments were performed for two weeks (17) and the rats were placed in metabolic cages on the thirteenth day to measure the volume of 24 hours urine. At the end of the experiment, the rats were kept fasting for 12 hours with free access to water and then they were euthanized using a guillotine and their blood samples were collected in sterile plastic tubes. The blood was allowed to clot at room temperature for 20 minutes and then was centrifuged at 3000 for 15 minutes. The obtained serum was stored at -20 °C. Biochemical factors were measured using a spectrophotometer.

Measurement of glucose

Glucose was measured through the glucose oxidase method using the kit of Darman Kav Company.

Measurement of urea

Urea was measured through the urease method using the kit of Darman Kav Company and a spectrophotometer at 340 nm.

Measurement of Albumin

The concentration of albumin was measured through the method of Thomas (1998) with the kits of Darman Kav Company and the enzymatic, colorimetric method which is the basis of photometry at 546 nm.

Measurement of creatinine

The concentration of serum creatinine was measured through the Jaffe method using the kits of Darman Kav Company. The concentration of urine creatinine was also measured by the same method. In this method, urine was diluted (1:49) and then the urinary creatinine concentration was measured at 492 nm. This test is also based on photometry.

Evaluating kidney function by calculating clearance

One of the most useful ways to measure kidney function for disposal of various substances is to evaluate the clearance of plasma from these compounds. Therefore, clearance of any substance is defined as volume of plasma which is completely cleared of that substance per unit of time.

Creatinine clearance

$$C_s = \frac{U_{cr} \times V}{P_{cr}} \quad (1)$$

Where C_s is the creatinine clearance, P_{cr} plasma concentrations of creatinine, V urinary flow rate per time unit, and U_{cr} the urinary creatinine concentration.

Albumin clearance

$$C_s = \frac{U_{albu} \times V}{P_{albu}} \quad (2)$$

Where C_s is the clearance rate of albumin, P_{albu} plasma concentration of albumin, V urinary flow rate per time unit, and U_{albu} the urinary concentration of albumin.

Data analysis

The obtained data were analyzed with SPSS-16. The mean of data in different groups was compared using one-way ANOVA and Tukey test. The results of this study were expressed as mean±SEM and p value<0.05 was considered significant.

Results:

Changes in serum glucose levels

Serum glucose levels in group D were significantly higher than other studied groups (p value<0.001). No significant increase was observed in groups D+C10 and D+C30 compared to groups N, N+C10, and N+C30 (p value<0.001) in terms of serum glucose (Fig. 1)

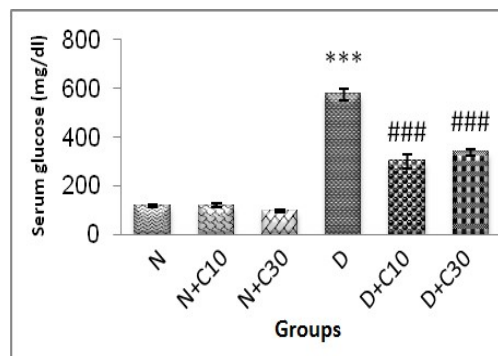


Figure 1- Comparison of the mean amount of glucose among all groups

Changes in urine volume

The mean volume of urine was significantly higher in group D than other studied groups

(p -value<0.001). A significant increase was observed in groups D+C10 and D+C30 compared to groups N, N+C10, and N+C30 (p -value <0.001) (Fig. 2).

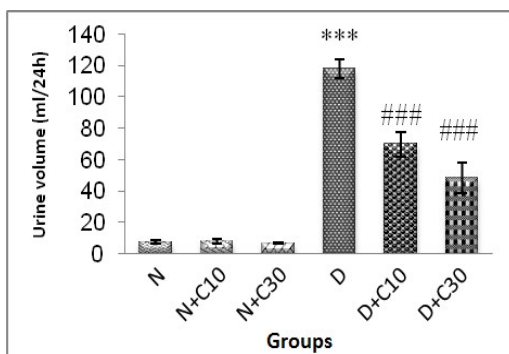


Figure 2- Comparison of urine volume among all groups

Changes in urea serum levels

The mean concentration of serum urea in group D was significantly higher than groups N, N+C10, and N+C30 (p -value<0.001). It was also significantly higher in group D+C10 compared to group N (p -value<0.001) and group N+C10 (p -value<0.01). The mean serum urea in group D+C30 was significantly higher than groups N and N+C10 (p -value<0.001). No significant difference was observed among other studied groups (Fig. 3).

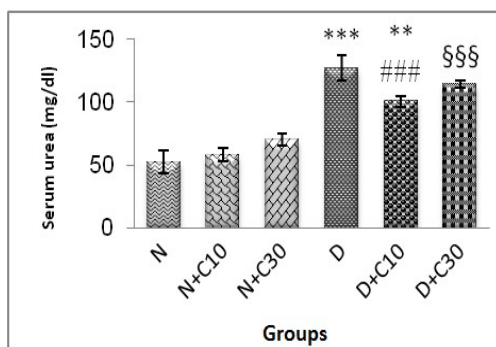


Figure 3- The mean level of serum urea among all groups

Changes in serum albumin

The mean level of serum albumin had no significant difference among all studied groups (Fig. 4).

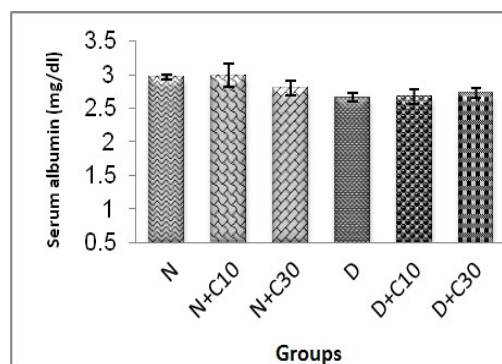


Figure 4- Comparison of serum albumin levels among all groups

Changes in serum creatinine levels

The mean level of serum creatinine in D group was significantly higher than groups N, N+C10, and N+C30 (p -value<0.05). There was no significant difference among other studied groups (Fig. 5).

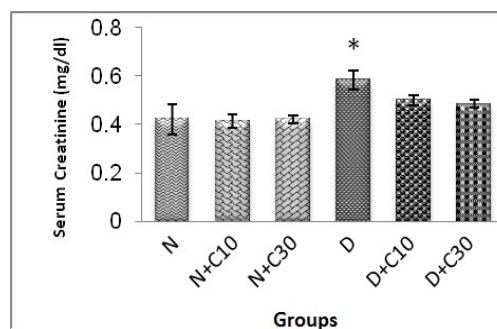


Figure 5- Comparison of mean levels of serum creatinine among all groups

Changes in creatinine clearance

The mean creatinine clearance was significantly higher in group D compared to groups N and N+C10 (p -value<0.001). No significant difference was observed among other studied groups (Fig. 6).

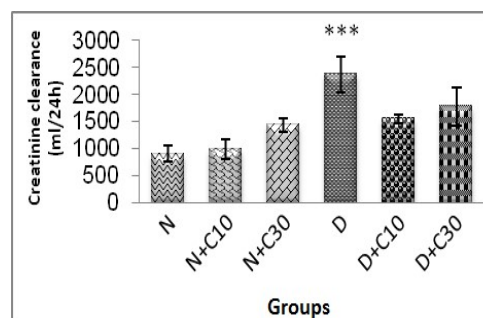


Figure 6- The mean creatinine clearance among all groups

Changes in albumin clearance

The mean clearance of albumin was significantly higher in groups D and D+C10 compared to groups N, N+C10, N+C30, and D+C30 (p value<0.001). A significant increase existed in group D compared to group D+C10 (p value<0.05). There was a significant increase in group D+C30 compared to groups N and N+C10 (p value<0.01) and N+C30 (p value<0.001). No significant difference was observed among other studied groups (Fig. 7).

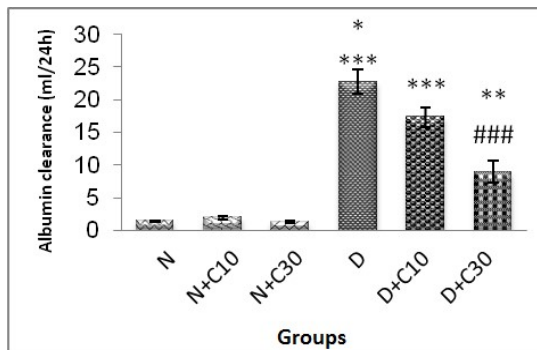


Figure 7- The mean albumin clearance among all groups

Conclusion:

This study showed that fasting blood glucose levels increased significantly 3 days after STZ injection and this increase continued. In addition, the rate of albumin and creatinine clearance as well as serum urea levels were increased in the diabetic group compared with the control group. This study aimed at evaluating the effect of *Citrullus colocynthis* pulp on diabetes-induced renal damage in male rats. Blood sugar levels were improved in animals treated with colocynth pulp, although blood glucose levels of the treated diabetic rats had a significant difference with that of the control group.

The anti-diabetic effect of colocynth pulp was shown in the present research; this was in agreement with previous studies which reported the hypoglycemic effects of saponoids extract derived from colocynth on normal and alloxan-induced diabetic rabbits in a short period of 0 to 24 hours (10), as well as *in vitro* stimulatory effect of a similar extract on insulin release from isolated pancreatic islets (11).

Polyuria is a symptom of diabetes developing due to increased levels of glucose. The risen glucose enters into the kidneys and increases osmotic

diuresis, leading to polyuria (18). Compared with the diabetic group, colocynth pulp had improving effects on urine volume in the colocynth pulp treated diabetic patients and these effects were better at a dose of 30 mg/dL and had a dose-dependent trend. Our results are in agreement with the findings of Garau *et al.* who showed that dried bitter melon (bitter family) supplements can prevent polyuria and polydipsia in diabetic rats, which can be due to its high fiber content. Fibers result in decreased glucose absorption during passage through the digestive tract (19). Administration of colocynth significantly reduces blood glucose levels in diabetic patients, and hence it may amend polyuria through hypoglycemic activity.

Renal damage increases substantially in diabetes. For example, increased GFR and serum urea levels are the first characteristic of diabetic nephropathy which ultimately results in decreased renal function and change in renal structure (5).

The following two reasons can lead to diabetic renal damage:

- Increased GFR which plays a crucial role in the pathophysiology of diabetic nephropathy (20).
- Increased blood sugar which impairs glomerular vessels and eventually increases the clearance rate of albumin (21).

Some researchers suggest that glomerular hyperfiltration in experimental diabetes is a result of increased reabsorption of glucose and sodium in proximal tubule which leads to secondary vasodilation through a tubular glomerular feedback (22). For example, as a result of increased proximal reabsorption, macula densa receives less salt, leading to activation of tubular glomerular feedback pathways which serves to increase the glomerular filtration. In fact, diabetes-related glomerular hyperfiltration may arise directly from increased proximal reabsorption of salt (23). This activity also increases the glomerular ultrafiltration.

Along with increased serum creatinine concentration, GFR, as a basic parameter of renal function, is also increased significantly in diabetes. GFR is usually evaluated through assessment of renal function and creatinine clearance (24).

In fact, an increase in albuminuria in diabetic rats was observed in the second week of this study. This may arise from reduced reabsorption of albumin in proximal tubules, justifying the increased GFR. In accordance with this and compared with the control group, we found that GFR increases in very early

stages of diabetic nephropathy. This result is in agreement with the findings of Tajo *et al.* who proposed dysfunction of endocytosis in proximal tubule in a very early stage of diabetes (25).

We showed that treatment with colocynth reduced the increased GFR and improved renal function in STZ-induced diabetic rats. Colocynth significantly reduced urinary albumin excretion in the treated diabetic groups compared with diabetic rats.

Kidneys maintain optimal chemical composition of body fluids through urine acidification and removal of waste metabolites such as urea, uric acid, and creatinine. The levels of these metabolites in blood and urine increase in renal diseases. Our data suggest that the plasma levels of creatinine and urea increases in diabetic rats. This could be due to metabolic disorders in diabetes.

In this study, administration of colocynth pulp improved renal function and our data are in agreement with studies that showed orally-administered colocynth fruit extract results in decreased serum levels of urea, uric acid, and creatinine in diabetic rats. These findings are consistent with previous findings (26). As an appropriate treatment, *Citrullus colocynthis* showed beneficial effects on renal function indicating the anti-diabetic properties of this plant. The anti-diabetic properties of colocynth may be due to enhanced insulin secretion or increased peripheral utilization of glucose, or decreased gluconeogenesis (27). Despite the lack of current knowledge to explain the mechanisms of the effects of colocynth pulp on renal function in STZ-induced diabetes, the results of this study indicated that the colocynth can prevent diabetes-induced renal changes and it is able to prevent changes in renal clearance in diabetes.

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بررسی اثر پالپ هندوانه ابوجهل بر عملکرد کلیوی در موش‌های صحرایی نر دیابتی ناشی از استرپتوزوتوسین

طیبه باقری‌زاده^۱، علی گل^۲، حکیمه علمی^۳

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مجله پزشکی هرمزگان سال نوزدهم شماره چهارم ۹۴ صفحات ۲۴۴-۲۳۷.

چکیده

مقدمه: نفروپاتی دیابتی، یکی از جدی‌ترین عوارض میکروواسکولار دیابت و علت اصلی بیماری کلیوی مرحله آخر می‌باشد. گیاهان مختلفی برای کنترل عوارض دیابت استفاده شده‌اند. در مطالعه حاضر، اثر تجویز پالپ هندوانه ابوجهل بر روی آسیب‌های کلیوی ناشی از دیابت را در موش‌های صحرایی نر مورد بررسی قرار دادیم.

روش کار: ۳۶ سر موش صحرایی نر به ۶ گروه ۱- نرمال (N)، ۲- نرمال + هندوانه در دوز ۱۰ mg/kg b.w (N+C10)، ۳- نرمال + هندوانه در دوز ۳۰ mg/kg b.w (N+C30)، ۴- دیابتی (D)، ۵- دیابتی + هندوانه در دوز ۱۰ mg/kg b.w (D+C10) و ۶- دیابتی + هندوانه در دوز ۳۰ mg/kg b.w (D+C30) تقسیم شدند. موش‌های صحرایی پالپ هندوانه ابوجهل را از طریق گاواژ به مدت دو هفته دریافت نمودند. نتایج به دست آمده با نرم‌افزار SPSS نسخه ۱۶ و آزمون‌های ANOVA و Tukey تجزیه و تحلیل شدند.

نتایج: موش‌های صحرایی دیابتی افزایش شدیدی در میزان حجم ادرار، کلیرانس آلیومین، کلیرانس کراتینین و اوره سرم نشان دادند. تجویز هندوانه ابوجهل در موش‌های صحرایی دیابتی موجب بهبود این تغییرات به سمت طبیعی شد.

نتیجه‌گیری: نتایج به دست آمده از مطالعه حاضر نشان می‌دهد که پالپ هندوانه ابوجهل دارای پتانسیل برای درمان دیابت ملیتوس و جلوگیری از آسیب‌های کلیوی مرتبط می‌باشد.

کلیدواژه‌ها: دیابت شیرین - هندوانه ابوجهل - موش صحرایی.

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ارجاع: باقری‌زاده طیبه، گل علی، علمی حکیمه. بررسی اثر پالپ هندوانه ابوجهل بر عملکرد کلیوی در موش‌های صحرایی نر دیابتی ناشی از استرپتوزوتوسین. مجله پزشکی هرمزگان ۱۳۹۴؛

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