Original Article

Renal Tissue Damages and Its Antioxidant Status Improved by Crab Shell Extract in Streptozotocin-induced Diabetic Rat

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Abstract

Background: Diabetic nephropathy is a complex and multifactorial adverse effect of diabetes mellitus (DM). Crab shell as a natural product is supposed to have antioxidant effect which is one of the important mechanisms to improve DM. The aim of this study was to investigate the effect of crab shell extract (CSE) on the histopathology and antioxidant status of kidney in diabetic rats. **Materials and Methods:** Forty-two adult Wistar rats (210 ± 10 g) were divided into six groups (n = 7). Streptozotocin (50 mg/kg) was administered interaperitonealy (IP) for inducing diabetes. Rats were treated for 14 days by CSE with 100, 200, and 400 mg/kg doses IP. Fasting blood glucose, body, and renal weight were evaluated. The antioxidant status of kidney's tissue was evaluated by determining the level of ferric-reducing antioxidant power (FRAP). Furthermore, urine samples were used to determine nitric oxide (NO) levels. Microscopic slides were prepared to compare kidney histology between groups. Data were analyzed by one-way analysis of variance with *post hoc* Tukey's test, and $P < 0.05$ was considered statistically significant. **Results:** CSE induced a significant reduction in blood glucose (P = 0.01) and a significant increase in total antioxidant capacity (FRAP) (P = 0.004). Furthermore, urine NO was decreased significantly (P = 0.000). The extract improved renal tissue changes caused by diabetes. **Conclusion:** CSE improved antioxidant status and diabetic histological changes of rat kidney, and it could be an alternative complementary therapy in diabetic-associated disorders.

Keywords: *Antioxidant, crab shell, diabetes mellitus, kidney*

Introduction

Diabetes mellitus (DM) is a group of metabolic disorder with complex etiology that occurs when the pancreas does not produce enough insulin. It was divided to four clinical categories: type 1 diabetes (autoimmune destruction of the β-cells), type 2 diabetes (progressive insulin defect), gestational diabetes (during pregnancy), and other specific types of diabetes due to other causes such as drug- or chemical-induced alterations.[1,2]

Diabetes is a major threat to global public health through late complications such as microvascular diseases including nephropathy, retinopathy, and neuropathy.[2,3] Impairment of kidney function is a prominent feature of DM characterized by proteinuria, loss of renal function, and hypertension in the end stage of disease. DM can affect kidney histology including thickening of glomerular basement membrane, mesangial expansion, and global glomerulosclerosis at the end stage of disease.[4]

Increasing evidence suggest that oxidative stress (OS) plays an important role in hyperglycemia-induced tissue injury as well as in early events relevant for the development DM.[5] The formation of advanced glycation end-products (AGEs), which are a group of modified proteins and/or lipids with damaging potential, is one contributing factor.[6] On the other hand, it has been demonstrated that AGEs increase the formation of reactive oxygen species (ROS) and impair antioxidant systems.[7] In renal tissue, AGEs regulate glomerular filtration and provide structural support through induction of apoptosis and vascular endothelial growth factor expression in mesangial cells.[8]

One of the most challenging problems in current drugs that used for the treatment of diabetes is induction of numerous side effects such as hypoglycemia, weight gain,

How to cite this article: Ebrahimi Z, Khazaei MR, Ghanbari E, Khazaei M. Renal tissue damages and its antioxidant status improved by crab shell extract in streptozotocin-induced diabetic rat. Adv Biomed Res 2019;8:41.

Received: March, 2019. **Accepted:** May, 2019.

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Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr_65_19

Quick Response Code:

headache, and elevating liver enzymes.[9] It has been shown that several diabetes-induced severe biochemical and histochemical alterations are found in the kidney of rats in which selenium or other antioxidants have a protective effect and decrease the side effect of diabetes.[10,11] This protection can be owing to the free radical scavenging activity.[10]

Derived polysaccharide of crustacean shells contains essential macro- and microelements such as calcium, copper, manganese, zinc, and selenium.[12] Furthermore, crustacean shells contain chitin and chitooligosaccharide (COS). Chitin is one of the most abundant organic compounds. Chitin, chitosan, and their derivatives have important biological properties including antioxidant, anti-inflammatory, antitumor, antimicrobial, and antidiabetic effect, and their antioxidant effects play a vital role in nutrition and human health.[13-15]

The most important effect is antioxidant activity which has been demonstrated in several studies. The previous study showed that COS has a significant antidiabetic activity and is able to improve damages in diabetic nephropathy (DN).[16] COS has an anti-inflammatory effect and its exposure has been shown to downregulate the expression of tumor necrosis factor-alpha and interleukin-6 cytokines at the transcription level. Yoon *et al*. have also investigated the protective effects of COS against glycerol-induced acute renal failure (a model of renal OS).[17]

Experimentally induced diabetes with streptozotocin (STZ, an antimicrobial agent) is well characterized. STZ is the first choice for diabetes induction in animals which destroyed pancreatic β-cells and its effect depends on the dose, method of administration, and the animal strain. In the rats, STZ (40–50 mg/kg body weight) led to severe diabetes (blood glucose >200/300 mg/dL); STZ diabetic animals are most widely used for screening the compounds including natural products for their hypoglycemic/antihyperglycemic activities.[18]

STZ is also highly cytotoxic to the pancreatic beta-cells, and its diabetogenic properties are mediated through diverse mechanisms including increased OS due to nitric oxide (NO) release and ROS production.[19]

There is an essential need for effective and better hypoglycemic agent. Recently, much interest attracted in biological active compounds which derived from natural resources was seen, especially compounds that efficiently act on molecular targets, contributed in various diseases. Various natural compounds with antidiabetic activity are used to control diabetes; our previous studies were shown inhibitory effects of crab shell extract (CSE) on breast cancer cell line (MCF7) and[20] improved pancreatic tissue changes caused by diabetes.[15] The aim of this study was to investigate the effect of CSE on structural changes and ferric-reducing antioxidant power (FRAP) level of renal tissue and urinary biochemical parameters in diabetic rat.

Materials and Methods

Animals

In this experimental study, 42 adult Wistar male rats (210 ± 10 g) were used. The rats were maintained under standard conditions with a 12-h light and 12-h dark cycle at room temperature (23°C) constantly and humidity (50%–60%) and given access to food and tap water *ad libitum*. This study was done in accordance with the guidelines provided by the Animal Laboratory and approved by the Animal Care and Use Committee of Kermanshah University of Medical Science.

Experimental design

Rats were randomly divided into six groups (n = 7/group) as follow: the control and diabetic rats received 0.5 cc distilled water (extract solvent) IP daily and the CSE group received CSE (100 mg/kg). Diabetic rats were divided into three subgroups which treated with one of 100, 200, and 400 mg/kg CSE doses (dissolved in 0.5 cc distilled water) IP for 14 days. To induced diabetes, single dose of freshly prepared STZ (50 mg/kg dissolved in citrate buffer, pH = 4.5) was injected to rats intraperitoneally. Three days later, fasting blood glucose (FGB) was measured. Animals with FGB levels >250 mg/dl were considered diabetic.

At the end of experiment, animals were weighted and urine samples were obtained for NO measurement, then they were scarified, and the right kidneys were removed and were weighted. The kidneys were homogenated at 5000 rpm/min for 12 min and the homogenate solution was centrifuged in 4°C temperatures, and its clear solution was used to measure total antioxidant capacity (TAC) by FRAP method. Furthermore, the kidney-to-body weight ratio was calculated. The left kidneys of rats were dissected and were fixed in 10% formalin for histological study.

Preparations of crab shell hydroalcoholic extract

Crab was confirmed in term of genus and species (Potamon *Persicum*) by zoologist of Razi University, CS was washed and coarsely powdered by an electrical mill, and the powder was dissolved in ethanol (70%) for 48 h in darkness. It was subsequently filtered and dried to evaporate the alcohol; this powder was weighted and stored at 4°C.[20]

Ferric-reducing ability of power

TAC of kidney homogenate was evaluated using FRAP assay. The FRAP reagent was prepared as a mixture of 5 mL of 10 mM of 2,4,6-tris (2-pyridyl)-s-triazine in 50 mL of 0.1 M acetate buffer (pH 3.6) and 5 mL of FeCl3 (20 mM) in HCl (40 mM). Then, prepared FRAP reagent was incubated at 37°C for 15 min, and 200 μL of a kidney homogenate was mixed with 1.5 mL of the FRAP reagent and incubated for 15 min. The mixture was centrifuged

at 12000 rpm/15 min. Absorbance of the supernatant and standard sample was measured at 593 nm against blank.[5]

Nitric oxide assay

Griess colorimetric method was used to measure NO levels. Briefly, 400 µL of urine specimens was deproteinized by adding 6 mg of zinc sulfate and was centrifuged at 12000 g for 12 min. Then, 100 µl of supernatant was added to 100 µL of vanadium chloride, 50 µL of sulfanilamide, and 50 µL of N-(1-naphthyl) ethylenediamine dihydrochloride in each well. After 30 min incubation at 37°C, the absorbance was measured at wavelengths of 540 and 630 nm. Values are expressed as µM.[21]

Histopathology assay

The kidney was fixed in 10% buffered formalin. Then, kidneys were processed and embedded in paraffin. Tissue sections (5 µm) were stained with hematoxylin and eosin. Histological alteration evaluated under the light microscope equipped with an image capture camera and a computer having image processing software (Motic 2000).[11] In this study, Jablonski scoring system (0–4) was used to report the histological damage in kidney injury.[22] The scoring system consists of histological injury in glomerular, endothelial, tubular, and interstitial components.

Statistical analysis

All data were showed as mean ± standard error. One-way analysis of variance, followed by *post hoc* Tukey's test, was used for comparison between different groups. $P < 0.05$ was considered statistically significant.

Results

FBG in the diabetic group showed a significant increase while the treatment with different doses of CSE significantly decreased FBG ($P = 0.000$) [Figure 1]. Body weight at the end of the experiment (day 14) significantly decreased in diabetic rats (163 ± 3.6 g) compared to control (225.5 ± 4 g) and increased in diabetic treated groups with 100, 200, and 400 mg/kg CSE (173.3 ± 14.3, 198.3 ± 1.2, and 178.7 ± 16.7 g, respectively) ($P = 0.011$). Furthermore, there was no significant difference between diabetic-treated groups [Figure 2a], and there was no significant difference in kidney weight and kidney/body weight ratio in different groups [Figure 2b].

Total antioxidant capacity

FRAP levels of kidney tissue in diabetic group (19.2 ± 2.3) were significantly ($P = 0.004$) lower than the control group (87.9 ± 2.7) and CSE (100 mg/kg) (79.8 ± 4.2). CSE significantly ($P = 0.004$) increased the TAC in the diabetic group treated with doses of 100 (64.9 ± 3.9), 200 (71.5 ± 0.3), and 400 mg/kg (58.14 ± 5.8) [Figure 3a].

Urine levels of nitric oxide

The mean level of urine NO increased in the diabetic group (402.25 ± 2.23 µM) compared to the control (124.5 ± 8 µM) and 100 mg/kg CSE (129.2 ± 9.5 µM), and it decreased significantly ($P = 0.000$) in diabetic treated with 100 (131.21 ± 4.9 µM), 200 (93.5 ± 24), and 400 (216.15 ± 7.4) mg/kg doses of CSE [Figure 3b].

Histological findings

Kidney of control [Figure 4a] and CSE (100 mg/kg)-treated rats [Figure 4b] showed the normal renal histological structure (score 0). Diabetic rats showed thickening of Bowman capsule and retraction of the glomerular tuft and necrosis in >25% of the proximal and distal tubules (score 4) [Figure 4c]. Diabetic rats treated with 100 [Figure 4d] and 200 mg/kg [Figure 4e] CSE showed

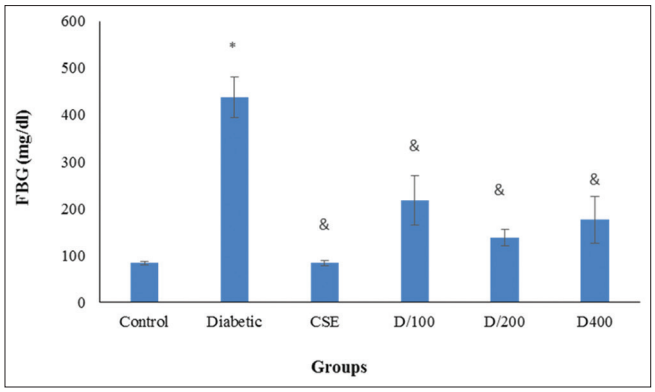

Figure 1: Crab shell extract effect on fasting blood glucose in control and experimental groups. Data are expressed as the mean ± standard error (SE). Significant difference: *compared to control group, and &compared to diabetic group ($P < 0.05$)

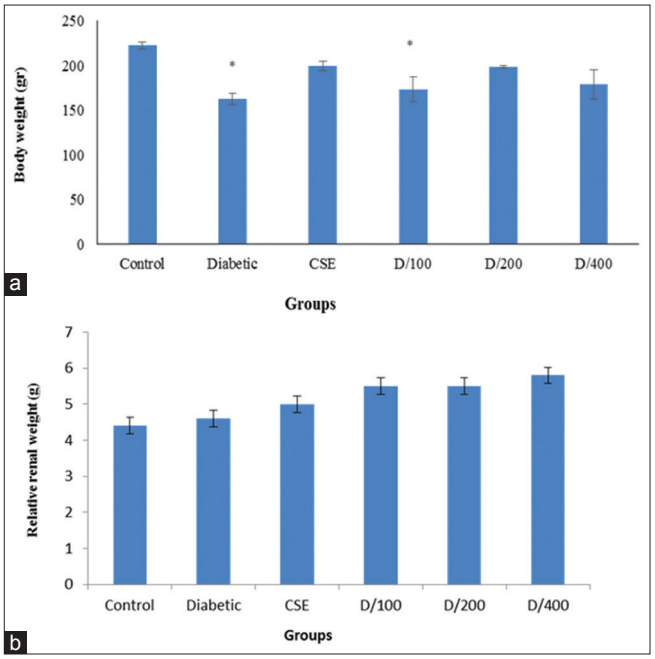

Figure 2: (a): Body weight, (b): renal/body weight ratio in control and experimental groups, data are expressed as the mean ± standard error (SE). Significant difference: *compared to control group, &compared to diabetic group ($P < 0.05$)

normal structure without signs of pathology (necrosis or inflammation) in the cortex and little changes in the medulla and interstitium (score 1). The renal tissue of diabetic rats treated with 400 mg/kg CSE reveals thickening of Bowman capsule (score 2) [Figure 4f].

Discussion

In this experimental model of early hyperglycemia-induced renal injury, urinary biomarkers of early DN were elevated, and CSE decreased FBG and urine NO and increased TAC significantly in all treated doses. We used the STZ rat model of severe hyperglycemia (type 1 diabetes) and 2-week time point because of our interest in the early events in diabetic renal injury.

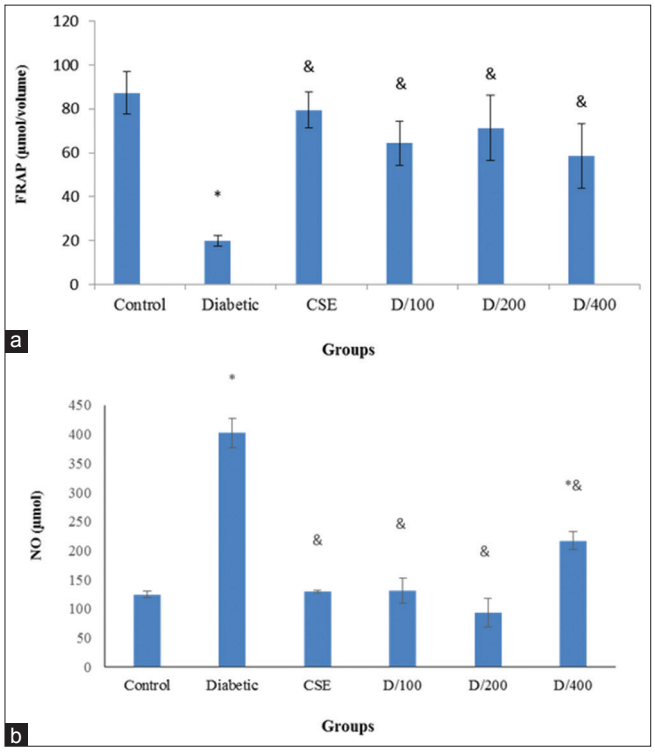

Figure 3: The effect of crab shell extract on ferric-reducing antioxidant power (FRAP) level of renal tissue (a) and nitric oxide (NO) level of urine (b). Data are expressed as mean ± standard error (SE). Significant difference: *compared to control group and &compared to diabetic group ($P < 0.05$)

There were no prominence histopathological changes among nondiabetic and diabetic-treated rats at this time point. Our finding of diabetic groups in severe histological changes in renal tissues is consistent with Zafar *et al*. report[23] and decreased TAC of renal tissue with Cakatay and Kayali report.[24] In kidneys of diabetic rats, lower activity of key antioxidant system, such TAC, was observed. Furthermore, a previous study showed that in the plasma of diabetic rats, TAC was decreased significantly in patients.[25] The present work indicated the same results and showed a significant reduction in kidney homogenate's FRAP in untreated diabetic rats. CSE treatment improved antioxidant power of kidney homogenate samples significantly.

Natural compounds and medicinal plants have been used for the treatment of DM and associated complications; our review[26] showed that natural compounds such as royal jelly and CSE confer tissue protection of STZ-induced diabetic rats which might be due to active antioxidant components of CS.

The prevalence of diabetes has been increasing worldwide and is estimated to continue in the future; hyperglycemia-induced DN is a serious complication and is the most common cause of end-stage renal disease. Several mechanisms have been reported to contribute to the development and progression of DN.[27] Impairment of kidney function is a prominent feature of DM characterized by proteinuria, loss of renal function, and hypertension in the end stage of disease. DM can affect kidney histology including thickening of glomerular basement membrane, mesangial expansion, and global glomerulosclerosis at the end stage of disease.[4]

Hyperglycemia and insulin resistance may also have accompanied by OS.[28] A study has demonstrated OS roles in DM and uncontrolled production of ROS, NO,

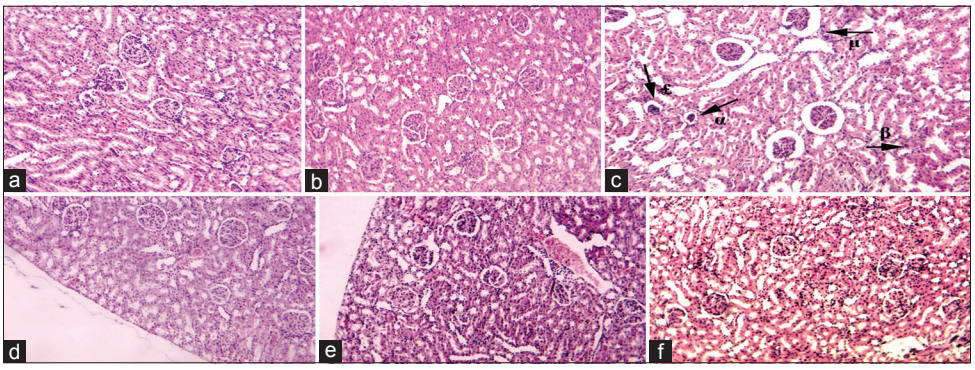

Figure 4: Light micrograph of kidney sections (H and E, ×10). (a) Control groups and (b) CSE (100 mg/kg) showed the normal renal histological structure (score 0), (c) diabetic group showed thickening of Bowman capsule (£) and retraction of glomerular tuft (α), and necrosis in more than 25% of the proximal (µ) and distal tubules (β) (score 4), (d) diabetic + 100 mg/kg and (e) diabetic + 200 mg/kg crab shell extract showed normal structure without signs of pathology (necrosis or inflammation) in the cortex and little changes in the medulla and interstitium (score 1), (f) Diabetic + 400 mg/kg of crab shell extract reveals thickening of Bowman capsule (score 2)

and other oxygen substances are often lead to damage of macromolecule such as DNA and ultimately develop DN microvascular complication. The antioxidant effect of CS can be attributed to the presence of chitin derivative (COS and chitosan), selenium, and astaxanthin through reduction of free radicals. A study showed that at a concentration of 500 μg/ml, COS reduced NO and prostaglandin E2 (PGE2) production by inhibiting the iNOS and COX-2 expression.[29] In the present study, different doses of crude CSE improved kidney tissue damages by diabetes; the doses were lower than studies[21,23,30] with one of the CSE components which means that it contains more than one antidiabetic constitute.

DM was characterized by peripheral insulin resistance which was followed by failure of compensation for pancreatic β-cells and amelioration of hyperglycemia.[2] Some natural components such as chitin, COS, and astaxanthin have generally shown antidiabetic effects.[31] Furthermore, these elements have been found in CS. All concentrations of COS not only decrease FBG levels but also increase viability of pancreatic islet cells, and exposure of primary-cultured pancreatic cells to COS continuously increased the secretion of insulin significantly.[16]

Our previous study[14] showed a similar protective effect of CSE on serum biochemical markers and histological changes of pancreas in diabetic rats. CSE induced a significant reduction in FBG and serum levels of NO and a significant increase in TAC (FRAP). The extract improved pancreatic tissue changes caused by diabetes. Here, we find protective and antidiabetic effects of CSE on renal tissue histology and TAC and urine NO.

Conclusion

CSE improved antioxidant status and diabetic histological changes of rat kidney; it showed antidiabetic properties and could be considered as a suitable supplement in diabetic treatments. Further research recommended opening new perspective in its treatment and prevention.

Financial support and sponsorship

This study originated from M.D thesis and was financially supported by Kermanshah University of Medical Science, Kermanshah, Iran.

Conflicts of interest

There are no conflicts of interest.

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