

## The Ameliorative Potential of Dexmedetomidine and Benincasa cerifera Extract in Renal Ischemia/Reperfusion Injury in a Streptozotocin-induced Diabetic Model

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<http://dx.doi.org/10.13005/bpj/1373>

(Received: February 08, 2018; accepted: March 13, 2018)

### ABSTRACT

Renal ischemia/reperfusion injury (IRI) represents the main reason for acute kidney injury (AKI). Dexmedetomidine (Dex) and Benincasa cerifera (BC) have wide benefits due to their anti-inflammatory and antioxidant properties. This study aims to illustrate the protective effects of BC and Dex on renal IRI in a diabetic model. Sixty adult male albino rats (Wistar strain), weighing 250–300 g, were included in the study. The rats were divided into four groups, as follows: sham group: (non-diabetic); diabetes mellitus (DM) + IRI group: streptozotocin (STZ)-induced diabetic rats exposed to renal IRI on day 30 after diagnosis of diabetes; DM + IRI + BC group: STZ-induced diabetic rats treated with BC (500 mg/kg) for 30 days after diagnosis of diabetes, then exposed to renal IRI; and DM + IRI + Dex group: STZ-induced diabetic rats treated with Dex (100 µg/kg intraperitoneally) 5 min before induction of ischemia on day 30 after diagnosis of diabetes, then exposed to renal IRI. Biochemical parameters, histopathological examination, and immunohistochemical markers were evaluated. A significant improvement in the biochemical, histopathological, and immunohistochemical parameters were observed in the DM + IRI + BC group, while the DM + IRI + Dex group showed improvements in renal IRI and dyslipidemia. The present study demonstrated that oxidative stress plays a chief role in renal IRI in the STZ-induced diabetic model. Treatment with BC achieved excellent ameliorative effects, while treatment with DEX improved renal IRI.

**Keywords:** Diabetes, Dexmedetomidine, Ischemia/reperfusion, Oxidative stress.

### INTRODUCTION

Diabetes mellitus (DM) is one of the most common diseases, with a high prevalence and incidence worldwide <sup>1</sup>. The International Diabetes Federation (IDF) reported that the prevalence of DM was 285 million people in 2010; this is expected to increase

by 50%, reaching up to 439 million, in 2030 <sup>2</sup>. Chronic kidney disease is a common consequence of DM that may lead to end-stage renal failure. Previous experimental trials have concluded that DM increases the kidneys' vulnerability to acute kidney injury (AKI), including renal ischemia/reperfusion injury (IRI) <sup>3</sup>.



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Renal IRI represents the main factor accounting for AKI; other causes include ureteral obstruction and kidney ischemia<sup>4</sup>. Renal IRI occurs spontaneously in many clinical situations, such as renal transplantation, partial nephrectomy, renal artery angioplasty, and ureteral obstruction. IRI is responsible for 30% of the total cases of delayed graft dysfunction after renal transplantation<sup>5</sup>. Multiple theories have been developed to explain the tissue damage occurring due to IRI, including hypoxic injury, inflammatory processes, and oxidative stress (OS) injury. All these mechanisms result in the insufficiency of both the renal endothelial and tubular cells and mitochondrial dysfunction, ending in tissue damage and necrosis<sup>6</sup>.

Dexmedetomidine (Dex) is a vastly selective and effective  $\alpha_2$ -adrenergic agonist used as a sedative, anxiolytic, analgesic, antioxidant, and anti-inflammatory agent. Dex is used for alleviating IRI conditions in several organs, including the heart, brain, and kidney<sup>7</sup>.

*Benincasa cerifera* (BC) Savi is a member of Cucurbitaceae family that has high biological value and is used in herbal medicine. It is mainly cultivated in East Asia, and its fruit is nutritive, acting as an immunopotentiator, diuretic, vasoconstrictor, and anti-anthelmintic. Moreover, it has been used in the treatment of many diseases, as it has anti-inflammatory, antipyretic, and antioxidant properties<sup>8</sup>. Phytochemically, BC contains lupeol and p-sitosterol and their acetates, as well as cucurbitin, cucurbitacin-B, isovitexin, rhamnose, alkali, fat, glucose, adenine, alnusenol, trigonelline, histidine, n-triaconatol, vitamin-B, pectic polysaccharide, mannitol, and amino acids<sup>8</sup>.

IRI is a common condition that has serious effects; thus, it is important to find components that can ameliorate these side effects. Thus, the present work aims to evaluate the beneficial effects of BC extract and Dex on renal IRI in STZ-induced diabetes.

## MATERIALS AND METHODS

This animal experimental study was conducted in King Fahd Research Center, King

Abdulaziz University (KAU), Jeddah, Saudi Arabia. The study protocol was approved by the Hospital Biomedical and Research Ethics Committee, Faculty of Medicine, King Abdulaziz University, Saudi Arabia, and the procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

## Drugs and Plants

BC was obtained from Taif City, Saudi Arabia. Dex was obtained from Hospira, Inc. (Lake Forest, USA). Furthermore, ketamine was obtained from King Abdulaziz University (Jeddah, Saudi Arabia). Finally, streptozotocin (STZ) was obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA).

## Experimental Animals

Sixty adult male albino rats (Wistar strain), weighing 250–300 g, were obtained from the Experimental Animal Care Center of King Fahad Medical Research Center, King Abdulaziz University. Animals were kept in special cages at 20–22°C and humidity (60%) under 12-h dark and light cycles. Rats were supplied with standard pellet chow with free access to tap water for 2 days for acclimatization before the experiment.

## METHODS

### Collection and Preparation of *Benincasa cerifera*

BC was collected from Taif City in the summer season. The freshly peeled rind was cut into small pieces and dried in a freeze-dryer device (IIShin Bio Base, Korea), then crushed using an electrical blender (Waring Commercial, USA) at King Fahd Center of Medical Research. The resulting powder was then extracted by dissolution with 70% v/v ethanol; the mixed solution was shaken for 24 h. The mixture was evaporated and dried using a gas-dryer device (Hahn vapor, Korea). Following this, the evaporated solution was placed in the first freeze-dryer device (IIShin Bio Base, Korea) to evaporate the water and obtain a solid powder form of the extracted plant<sup>9</sup>. The extracted powder was dissolved in water and administered orally in a dose of 500 mg/kg/day per group [3] for 30 days after diagnosis of STZ-induced DM via fasting blood glucose assessment<sup>10</sup>.

### Dexmedetomidine

Dex was administered as single dose (100 µg/kg intraperitoneally [i.p.]) 5 min before renal IRI induction <sup>11</sup>.

### Induction of Type 2 Diabetes Mellitus

Hyperglycemia was induced by a single i.p. injection of STZ at a dose of 55 mg/kg <sup>12</sup> into animals deprived of food for 18 h. STZ was dissolved in 0.1 M citrate buffer (pH 4.5) just prior to injection. Seventy-two hours later, hyperglycemia was confirmed by determining the blood glucose level using an Accu-check glucose meter (Roche Diagnostics, Basel, Switzerland). Blood samples were withdrawn from the retro-orbital venous plexus under light ether anesthesia. Rats with blood glucose levels less than 20 mmol/L were excluded from the study. The day on which hyperglycemia was confirmed was designated as day zero (72 h after STZ injection and confirmation of hyperglycemia) <sup>12</sup>.

### Induction of Renal Ischaemia Reperfusion Injury

The diabetic rats were anesthetized with ketamine (60 mg/kg, i.p.) <sup>13</sup> and then a midline laparotomy was performed, and the abdominal cavity was fully exposed. Body temperature was maintained at 37°C ± 0.5°C throughout the surgery. Bilateral renal pedicles were carefully isolated without damaging the ureter and clamped using nontraumatic microvascular clamps to result in complete cessation of the renal arterial blood flow. After 1 h, the clamps were removed to allow the return of blood flow to the kidneys, and the kidneys were observed while they underwent reperfusion for 1 h <sup>14-16</sup>.

### Experimental Design

The rats were divided into four main groups, as follows:

- Group 1: Sham: Sham non-diabetic rats were used as the negative control group, which was administered a single dose of saline daily for 30 days;
- Group 2: DM + IRI: STZ-induced diabetic rats exposed to renal IRI on days 30 after diagnosis of diabetes were used as the positive control group;
- Group 3: DM + IRI + BC: STZ-induced diabetic rats treated with BC for 30 days after diagnosis of diabetes, then exposed to renal IRI; and

- Group 4: DM + IRI + Dex: STZ-induced diabetic rats treated with Dex 5 min before induction of ischemia on day 30 after diagnosis of diabetes, then exposed to renal IRI.

### Physical Parameters

The body and kidney weights of each rat were measured.

### Preparation of Blood Samples and Serum

After 30 days of experimentation, the animals were fasted overnight (12–14 h), and 1 h after reperfusion initiation, blood samples were collected by eye puncture of the anesthetized rats. The blood sample obtained from each rat was divided into three tubes, one containing EDTA and mixed well for estimation of hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>), the second for glucose estimation, and the third for other biochemical markers. Following this, the samples from the second and third tubes centrifuged at 3,000 rpm for 20 min to obtain the serum and stored in a clean Eppendorf tube for biochemical marker measurement.

### Tissue Sampling

At the end of the experiment, the rats were sacrificed; the kidneys were obtained and perfused with phosphate-buffered saline (PBS) to remove all blood. The right kidney was kept in 10% neutral buffered formalin for histopathological studies, while the left kidney was stored at –80°C until the assay. Tissue homogenates were prepared in ice-cold phosphate-buffer (pH 7.5). Homogenates were centrifuged at 12,000 g for 10 min, and the supernatants were used for further biochemical analysis.

### Biochemical Investigations

#### Estimation of Fasting Serum Glucose, Insulin, and HbA<sub>1c</sub>

Serum glucose was quantitated via the glucose oxidase peroxidase method using the rat glucose assay kit (Cat. No. 81693, Crystal Chem Inc., Spain). Serum insulin was determined using an ultra-sensitive rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. 90060, Crystal Chem Inc, Spain) following the manufacturer's instructions. In addition, HbA<sub>1c</sub> was measured using a commercial kit (Cat. No. 80300, Crystal Chem Inc. Spain).

### Assessment of Renal Function

The serum creatinine (Creat) level was measured via the enzymatic colorimetric method using kits purchased from Crystal Chem (Cat. No. 80340, Crystal Chem Inc. Spain). Serum blood urea nitrogen (BUN) levels were measured using a rat BUN ELISA kit (Cat. No. MBS752119, My BioSource, Inc., San Diego, CA, USA) following the manufacturer's instructions.

### Assessment of Lipid Profile

Total cholesterol (TC) serum levels were measured using the rat TC ELISA kit (Cat. No. MBS752119, My BioSource, Inc., San Diego, CA, USA) following the manufacturer's instructions. Serum triglyceride levels were determined, using a commercially available kit (Cat. ab65336; Abcam, Cambridge, UK). High-density lipoprotein (HDL) and low-density lipoprotein (LDL)-very LDL (VLDL) serum levels were measured via the fluorometric method using a commercially available kit (Cat. ab65390; Abcam, Cambridge, UK) following the manufacturer's instructions.

### Assessment of Serum Lactate Dehydrogenase

Rat lactate dehydrogenase (LDH) was measured using an ELISA kit (Cat. No. MBS043166, My BioSource, Inc., San Diego, CA, USA) following the manufacturer's instructions.

### Assessment of Oxidative Stress Markers in Serum and Tissue Homogenate

Reduced glutathione (GSH) levels in serum and tissue homogenate were measured using the rat reduced GSH ELISA kit (Cat. No. MBS046356, My BioSource, Inc., San Diego, CA, USA) following the manufacturer's instructions. Malondialdehyde levels in serum and tissue homogenate were estimated using the rat malondialdehyde ELISA kit (Cat. No. MBS738685, My BioSource, Inc., San Diego, CA, USA) following the manufacturer's instructions.

Myeloperoxidase (MPO) levels were measured using an ELISA kit (Cat. No. CSB-E08722r, Cusabio Biotech Co. Ltd., Wuhan, Hubei Province, China) following the manufacturer's instructions. Plasma DNA oxidation levels were determined using the rat 8-hydroxy-deoxyguanosine (8-OHdG) ELISA kit (Cat. No. CSBE10526r, Cusabio Biotech Co. Ltd.) in accordance with the manufacturer's instructions.

### Histopathological Examination

The kidneys were dehydrated with a graded series of alcohol, cleared in xylene, and embedded in paraffin wax. Tissues were then cut into sections of 3–5  $\mu\text{m}$  in thickness using a microtome and stained with hematoxylin and eosin (H&E) for general observations, periodic acid-Schiff (PAS) for observation of glycogen, and Masson's trichrome (MT) for distinguishing collagen. For each specimen, at least three to five slides were examined using an Olympus BX53 microscope equipped with a DP73 camera (Olympus, Tokyo, Japan) <sup>12</sup>.

### Immunohistochemical Detection

Using the streptavidin-biotin-peroxidase technique, the endogenous peroxidase activity was eliminated using 10%  $\text{H}_2\text{O}_2$  for 15 min. Sections were then incubated for 1 h with the primary antibody against Bcl2-associated X (BAX) protein, a monoclonal antibody (Dako, Carpinteria, CA, USA; dilution: 1:200; cytoplasmic cellular site), as a marker for apoptotic death. They were similarly incubated with the primary antibody against tumor necrosis factor alpha (TNF- $\alpha$ ), a mouse monoclonal antibody (Dako; 5–10  $\mu\text{g}/\text{ml}$ ; cytoplasmic cellular site), as a marker for inflammatory cytokines. Sections were incubated for 20 min in 3,30-diaminobenzidine (DAB) chromogen and counterstained with Mayer's hematoxylin. Negative control sections were prepared by omitting the primary antibody. Positive control standard laboratory slides were used for all stains to demonstrate the technique's success. All the slides were examined under light microscopy, and the presence of labeled cells was documented. Absence of staining was recognized as a negative result (-), while the presence of brown staining was recognized as a positive result (+) <sup>17</sup>.

### Semi-Quantitative Morphometric Measurements

A minimum of ten non-overlapping fields for each animal were selected indiscriminately and histopathologically analyzed by a pathologist who was blind to the treatment given. The measurements were performed using Image-Pro Plus v6.0 (Media Cybernetics, MD, USA) and NIH ImageJ (v1.50; <http://rsb.info.nih.gov/ij/>) with an Olympus BX53 microscope. The renal cortex and outer medullary regions from control and other groups were examined for apoptosis (pyknotic nuclei), Erythrocyte extravasation, dilatation of Bowman's

capsule, tubular swelling, tubular dilatation, congestion, tubular cell degeneration, tubular necrosis, tubular hyaline casts and lymphocytic infiltration. Changes were scored as follows: no damage (-), mild (+), moderate (++) and severe (+++) damage<sup>18-23</sup>. Reaction to PAS was evaluated and scored semiquantitatively as follows: none (-), mild (+), moderate (++) and severe (+++). In addition, apoptotic cells (BAX-positive cells) were counted in an area of 20,000  $\mu\text{m}^2$  and selected randomly in the Bax-protein-stained sections at  $\times 400$  magnification. The optical density (OD) of TNF- $\alpha$  immunopositive cells was evaluated<sup>24</sup>.

### Statistical Analysis

Quantitative data were expressed as the mean  $\pm$  standard deviation (SD) of different parameters for the treated groups. The data were analyzed using one-way analysis of variance (ANOVA), followed by the LSD post hoc test. The statistical analysis was performed using SPSS version 23. The values were considered significant when  $p < 0.05$ <sup>25</sup>.

## RESULTS

### General Toxicity

No death or visible signs of toxicity were observed in any group during the experiment.

### Effects of BC and Dex on the Physical Parameters Effect on Body and Kidney Weight

There was a significant decrease in total body weight in all the study groups compared with the sham group. Kidney weight exhibited a significant increase in the DM + IRI group compared with the

sham group, while treatment with BC and Dex improved the kidney weight (Table 1).

### Effect of BC and Dex on the Serum Levels of Different Biochemical Parameters

#### Diabetic Indices

This study indicated a significant increase in the serum levels of fasting glucose and glycosylated hemoglobin, as well as a significant decrease in the serum levels of insulin, in all studied groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex). The DM + IRI + BC group showed an improved diabetic condition, as indicated by the significant decrease in the serum levels of fasting blood glucose and glycosylated hemoglobin and increase in serum insulin levels (Table 2).

#### Renal Functions and Serum Lactate Dehydrogenase

The results of this study showed significant elevation in serum Creat, BUN, and LDH in all groups (DM + IRI, DM + IRI + BC and DM + IRI + Dex). DM + IRI + BC and DM + IRI + Dex showed significant reductions in serum Creat, BUN, and LDH compared with the untreated group (DM + IRI; Table 2).

#### Lipid Profile

There were significant elevations in the serum levels of triglycerides, cholesterol, and LDL in all the study groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex). Treatment with BC (DM + IRI + BC) and Dex (DM + IRI + Dex) resulted in significant reductions in the serum levels of triglycerides, cholesterol, and LDL when compared with the untreated group DM + IRI (Table 2). Moreover, there was a significant decrease in serum levels

**Table 1: Effect of BC and Dexmedetomidine on body weight, Kidney**

Groups	Sham N=15	DM+IRI N=15	DM+IRI+BC N=15	DM+IRI+Dex N=15
Body weight (g)	417.00 $\pm$ 21.93	264.66 $\pm$ 4.16 <sup>1</sup> P= 0.000	318.66 $\pm$ 1.52 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	268.66 $\pm$ 1.52 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.674
Kidney weight (g)	1.34 $\pm$ 0.04	1.5 $\pm$ 0.08 <sup>1</sup> P= 0.001	1.38 $\pm$ 0.00 <sup>1</sup> P= 0.061 <sup>2</sup> P= 0.001	1.41 $\pm$ 0.02 <sup>1</sup> P= 0.001 <sup>2</sup> P= 0.001

Values are Mean $\pm$  SD: standard deviation. Comparison between groups done by ANOVA followed by LSD post hoc test. <sup>1</sup>P: compared to Sham group. <sup>2</sup>P: compared to DM+IRI groups.

of HDL cholesterol (HDL-C) in all the study groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex). Treatment with BC (DM + IRI + BC) resulted in a significant increase in the serum levels of HDL-C when compared with the untreated group (DM + IRI; Table 2). No significant difference regarding serum HDL-C levels were detected between the untreated group (DM + IRI) and Dex (DM + IRI + Dex).

#### Effects of BC and Dex on Oxidative Stress Markers in Serum and Renal Tissue Homogenate Lipid Peroxidation

There was a significant elevation in the serum levels of MDA and renal tissue homogenate MDA in all groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex). Treatment with BC (DM + IRI + BC) resulted in significant reductions in serum MDA

**Table 2: Effect of BC and Dex on serum biochemical parameters**

Groups	Sham N=15	DM+IRI N=15	DM+IRI+BC N=15	DM+IRI+Dex N=15
Glucose (GLU) (mmol/L)	5.80± 0.30	60.46±0.40 <sup>1</sup> P= 0.000	11.33±0.51 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	60.50±0.79 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.941
Insulin (INS) (mU/mL)	18.00±0.100	12.9000±0.100 <sup>1</sup> P= 0.000	16.7000±0.100 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	13.033±0.15275 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.195
Glycosylated Hemoglobin (HbA1c) (%)	5.33±0.83	12.00±9.07 <sup>1</sup> P= 0.000	7.3333±.0.90738 <sup>1</sup> P= .033 <sup>2</sup> P= 0.000	11.1667±1.04083 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.313
Creatinine (Creat) (mmole/L)	30.66±1.52	120.00±1.00 <sup>1</sup> P= 0.000	59.00±1.00 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	100.00±1.00 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000
Blood urea Nitrogen (BUN) (mmole/L)	5.86±0.25	25.56±0.92 <sup>1</sup> P= 0.000	9.03±0.152 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	23.23±0.65 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.001
Lactate Dehydrogenase (LDH) (U/L)	217.00±1.00	1492.6±6.42 <sup>1</sup> P= 0.000	406.66±6.11 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	1286.6±5.85 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000
Triglycerides (TG) (mmole/L)	1.35±0.05	7.50±0.26 <sup>1</sup> P= 0.000	2.03±0.05 <sup>1</sup> P= 0.001 <sup>2</sup> P= 0.000	6.23±0.15 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000
Cholesterol (Chol) (mmole/L)	1.93±0.15	8.06±0.15 <sup>1</sup> P= 0.000	3.20±0.10 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	7.00±0.20 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000
Low density lipoprotein (LDL) (mmole/L)	0.90±0.10	5.10±0.10 <sup>1</sup> P= 0.000	1.40±0.10 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	4.40±0.10 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000
High Density Lipoprotein (HDL) (mmole/L)	1.50±0.10	0.43±0.05 <sup>1</sup> P= 0.000	1.36±0.05 <sup>1</sup> P= 0.045 <sup>2</sup> P= 0.000	0.43±0.05 <sup>1</sup> P= 0.000 <sup>2</sup> P= 1.000

Values are Mean± SD: standard deviation. Comparison between groups done by ANOVA followed by LSD post hoc test. <sup>1</sup>P: compared to sham group. <sup>2</sup>P: compared to DM+IRI

levels, while treatment with Dex (DM + IRI + Dex) did not induce a significant difference (Table 3). Treatment with BC (DM + IRI + BC) and Dex (DM + IRI + Dex) resulted in significant reductions of MDA in renal tissue homogenate (Table 4).

### 8-Hydroxy-Deoxyguanosine

There were significant elevations of the serum 8-OHdG levels in all groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex). Treatment with BC (DM + IRI + BC) and Dex (DM + IRI + Dex) resulted in

**Table 3: Effect of BC and Dex on oxidative stress markers in serum**

Groups	Sham N=15	DM+IRI N=15	DM+IRI+BC N=15	DM+IRI+Dex N=15
Malondialdehyde (MDA) (nmole /mL)	1.70±0.01	4.14±0.15 <sup>1</sup> P= 0.000	2.99±0.01 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	4.09±0.17 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.585
8- Hydroxy-Desoxyguanosine (8-OHdG) (ng/mL)	0.19±0.01	0.88±0.01 <sup>1</sup> P= 0.000	0.31±0.01 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	0.83±0.01 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000
Myeloperoxidase (MPO) (ng/mL)	11.30±0.55	41.70±0.75 <sup>1</sup> P= 0.000	13.86±1.73 <sup>1</sup> P= 0.014 <sup>2</sup> P= 0.000	16.36±0.45 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.002
Glutathione (GSH) (micromole/L)	19.20±0.20	4.73±0.25 <sup>1</sup> P= 0.000	14.96±0.20 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	5.76±0.25 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.001

Values are Mean± SD: standard deviation. Comparison between groups done by ANOVA followed by LSD post hoc test. <sup>1</sup>P: compared to Sham group. <sup>2</sup>P: compared to DM+IRI

**Table 4: Effect of BC and Dex on oxidative stress markers in renal tissue homogenate**

Groups	Sham N=15	DM+IRI N=15	DM+IRI+BC N=15	DM+IRI+Dex N=15
Malondialdehyde (MDA) (Nano mole /gm renal tissue)	3.5033 ±0.05508	11.2420±0.17880 <sup>1</sup> P= 0.000	7.9471±0.32443 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	7.6667±0.34512 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000
8- Hydroxy-Desoxyguanosine (8-OHdG) (ng/mg renal tissue)	8.1000 ±0.72111	20.5200±2.85517 <sup>1</sup> P= 0.000	10.3857±2.51690 <sup>1</sup> P= 0.200 <sup>2</sup> P= 0.000	8.9000±2.58147 <sup>1</sup> P= 0.655 <sup>2</sup> P= 0.000
Myeloperoxidase (MPO) (ng/mg renal tissue)	3.5667 ±0.41633	19.0600±1.35757 <sup>1</sup> P= 0.000	9.1429±1.47970 <sup>1</sup> P= 0.000 <sup>2</sup> P= 0.000	5.8333±1.63666 <sup>1</sup> P= 0.037 <sup>2</sup> P= 0.000
Glutathione (GSH) (micromole/gm renal tissue)	15.9667 ±1.30512	1.3000±0.59582 <sup>1</sup> P= 0.000	15.4143±1.57208 <sup>1</sup> P= 0.539 <sup>2</sup> P= 0.000	14.4667±1.57208 <sup>1</sup> P= 0.115 <sup>2</sup> P= 0.000

Values are Mean± SD: standard deviation. Comparison between groups done by ANOVA followed by LSD post hoc test. <sup>1</sup>P: compared to Sham group. <sup>2</sup>P: compared to DM+IRI

significant reductions in the serum levels of 8-OHdG in the untreated group (DM + IRI; Table 3). The renal tissue homogenate levels of 8-OHdG showed a significant increase compared with the untreated group (DM + IRI). Treatment with BC (DM + BC + IR) and Dex (DM + Dex + IR) resulted in significant reductions in the renal tissue homogenate levels of 8-OHdG (Table 4).

#### Myeloperoxidase Activity

There was a significant elevation in the serum levels and renal tissue homogenate of MPO in all groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex; Tables 3 and 4). Treatment with BC (DM

+ IRI + BC) and Dex (DM + IRI + Dex) resulted in significant reductions of serum and renal tissue homogenate levels of MPO when compared with the untreated group (DM + IRI; Tables 3 and 4).

#### Reduced Glutathione

There were significant reductions in serum GSH in all groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex). Treatment with BC (DM + IRI + BC) and Dex (DM + IRI + Dex) resulted in significant increases in serum GSH when compared with the untreated group (DM + IRI; Table 3). Concerning renal tissue homogenate, GSH showed a significant decrease in the untreated group (DM + IRI).

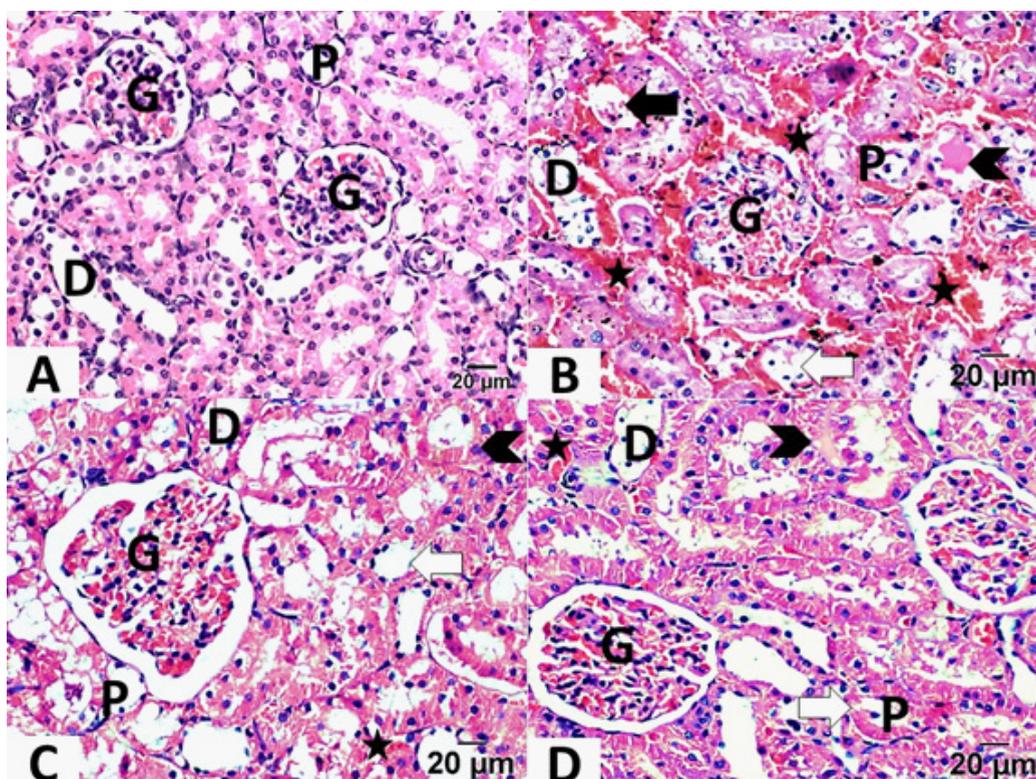


Fig 1(A): Section of sham group showed normal histoarchitecture. Note glomerulus (G), proximal tubule (P), distal tubule (D). Fig 1(B). Section of DM+IRI group showed degeneration and necrosis of proximal tubule epithelial cells (white-arrow) that poured into the lumen (black-arrow), severe erythrocyte extravasation surrounding the glomeruli and the tubules (star) and accumulation of proteinaceous material in the tubules (arrowhead) vacuolar degeneration of the tubules, and some tubules are dilated with distorted cellular boundaries. Fig 1(C). Sections of DM+IRI+BC showed reduced pathological findings with minimal degeneration of the tubules (white-arrow), minimal erythrocyte extravasation (star) and no apparent proteinaceous material (arrowhead). Fig 1(D). Sections of DM+IRI+Dex showed slight degeneration of the tubules (white-arrow) and mild erythrocyte extravasation (star) and reduced appearance of proteinaceous material at tubules (arrowhead) (H&E, Scale bar 20  $\mu$ m).

**Table 5: Histopathologic assessment of Renal Cortex and outer medullary regions of the different groups**

Groups	sham	DM+IRI	DM+IRI+BC	DM+IRI+Dex
Apoptosis (pyknotic nuclei)	--	++	+	+
Erythrocyte extravasation	--	+++	+	+
dilatation of Bowman's capsule	--	+++	+	++
Tubular cell swelling	--	+++	+	+
Tubular dilatation	--	+++	++	++
Congestion	--	+++	++	+
Tubular cell degeneration				
Tubular Necrosis	--	+++	+	+
Tubular hyaline casts	--	+++	+	++
Lymphocytic infiltration	--	++	+	+

Note: None (--); mild (+), moderate (++), severe (+++). N= (15)

Treatment with BC (DM + IRI + BC) and Dex (DM + IRI + Dex) resulted in significant increase of renal tissue homogenate levels of GSH when compared with the untreated group (DM + IRI; Table 4).

### Histopathological Examination

#### Histological Results

The normal histological architecture was demonstrated in the sham group (Fig. 1A). The DM + IRI group showed varying degrees of deterioration in the histological architecture, including degeneration and necrosis of the proximal tubule epithelial cells that poured into the lumen; severe erythrocyte extravasation surrounding the glomeruli and the tubules and accumulation of proteinaceous material at tubules; vacuolar degeneration of the tubules; and dilation of some tubules, with distorted cellular boundaries (Fig. 1B). The DM + IRI + BC group displayed minimal histological alterations of the glomeruli and tubules (Fig. 1C). The DM + IRI + Dex group revealed a slight degeneration of the tubules, mild erythrocyte extravasation, and reduced appearance of proteinaceous material at the tubules (Fig. 1D; Table 5).

#### Histological Findings for Masson's Trichrome

The sham group showed minimal collagen fiber deposition (Fig. 2A). The DM + IRI group revealed an apparently thickened parietal layer of the Bowman's capsule; proximal and distal tubules

with some collagen fiber condensation; obvious blood congestion; and a vacant area between some tubular cells and basal lamina (Fig. 2B). The DM + IRI + BC group displayed mild collagen deposition in the glomeruli surrounding the Bowman's capsules and near the convoluted tubules (Fig. 2C). The DM + IRI + Dex group revealed minimal intraglomerular collagen fibers, with no remarkable changes in terms of glomerular basal lamina thickness or change in the basal lamina of convoluted tubules (Fig. 2D).

#### Histological Findings for PAS

The sections from the sham group showed a normal PAS reaction, with the presence of regular brush borders of the proximal convoluted tubules and basement membranes (Fig. 3A). The DM + IRI group sections showed an increased PAS reaction (Fig. 3B), while the DM + IRI + BC group showed an obvious reaction (Fig. 3C). Finally, DM + IRI + Dex showed an apparent PAS reaction (Fig. 3D; Table 6).

#### Immunohistochemical Detection of BAX Expression

An immunohistochemical study using the anti-BAX antibody showed an increase in the intensity of the glomerular and tubular epithelial cell staining in the DM + IRI group, with obvious apoptosis, in comparison with the sham group, which showed minimal BAX expression. The DM

+ IRI + BC group showed minimal cytoplasmic Bax expression, and the DM + IRI + Dex group showed mild cytoplasmic Bax expression (Figure 4).

#### Apoptotic Cell Number

The mean number of apoptotic cells in the DM + IRI group was significantly increased compared with the sham group. In addition, the apoptotic cell number improved significantly in the DM + IRI + BC and DM + IRI + Dex groups compared with the DM + IRI group (Table 7).

#### Immunohistochemical Detection of TNF- $\alpha$ Expression

The DM + IRI group showed strong inflammatory cytokine expression for TNF- $\alpha$  in the

glomeruli and renal tubular cells in comparison with the sham group, which showed weak cytoplasmic inflammatory cytokine expression. Furthermore, the DM + IRI + BC group showed minimal cytoplasmic inflammatory cytokine expression, and the DM + IRI + Dex group showed mild cytoplasmic inflammatory cytokine expression (Figure 5). The OD supported the immunohistochemical findings (Table 8).

#### DISCUSSION

The transient cessation of renal blood flow occurs in numerous clinical conditions, such as renal transplantation and partial nephrectomy, as well as in major vascular and cardiothoracic surgeries<sup>26</sup>. This cessation leads to renal IRI, which causes a

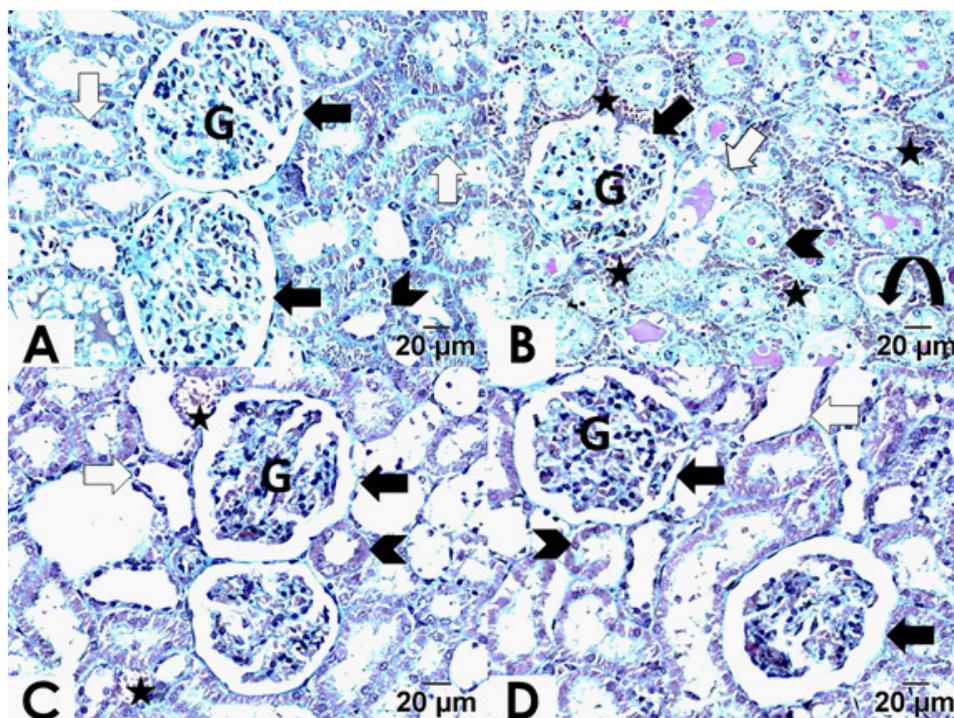
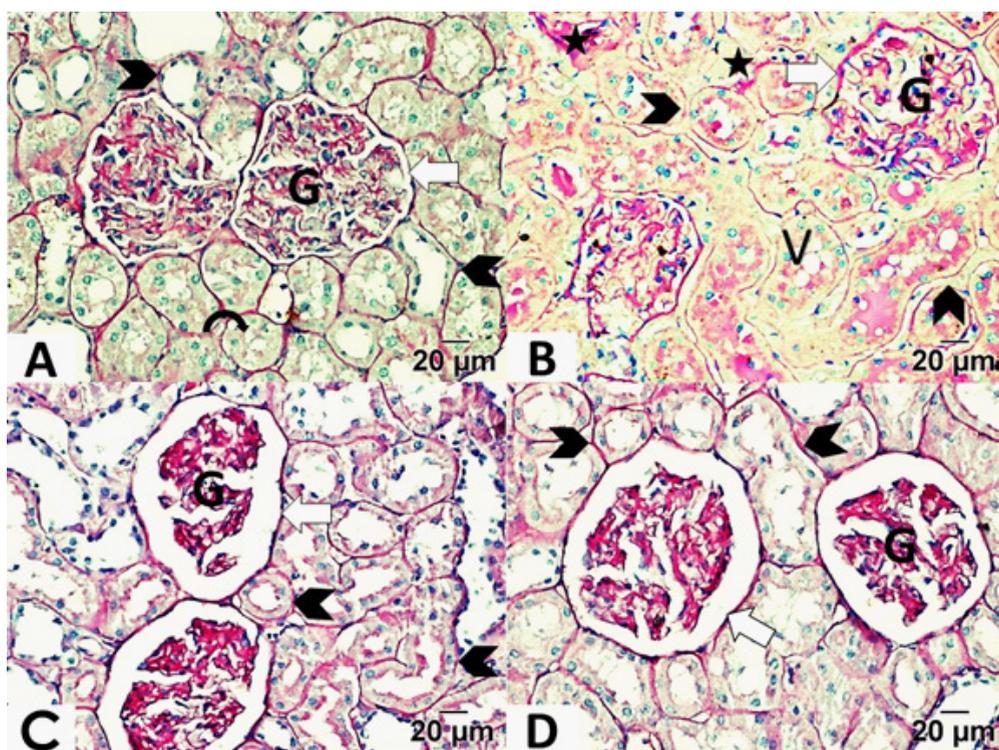


Fig 2(A): Section of sham group showed minimal collagen fibers in the glomeruli (G), around Bowman's capsule (black arrow) and around the proximal (black arrowhead), distal tubules (white arrow); Fig 2(B). Section of DM+IRI group revealed some condensation of collagen fibers in the glomeruli (G), around the apparently thickened parietal layer of Bowman's capsule (black arrow), the proximal (black arrowhead) and distal tubules (white arrow). Note congested blood (star) and vacant area between some tubular cells and basal lamina (curved arrow); Fig 2(C). Sections of DM+IRI+BC showed mild collagen in the glomeruli (G), surrounding Bowman's capsule (black arrow) and the proximal (black arrowhead), distal tubules (white arrow). Note congested blood (star); Fig 2(D). Sections of DM+IRI+Dex revealed minimal intraglomerular (G) collagen fibers with no change in the basal lamina thickness (black arrow) and no notable changes in the proximal (black arrowhead), distal tubules (white arrow) (Masson's trichrome, Scale bar 20  $\mu$ m).

decrease in the renal glomerular filtration and blood flow<sup>5, 23, 27</sup>.

In the current study, there was a disturbance in the diabetic profiles of the STZ-induced diabetic groups (DM + IRI, DM + IRI + BC, and DM + IRI + Dex); these results are in line with previous findings, which were explained in relation to multiple factors, such as improper insulin secretion or insulin resistance, along with the alteration in carbohydrate, lipid metabolism, and OS markers<sup>12, 28-30</sup>. The present work demonstrated that BC ameliorated the diabetic condition; these results are in agreement with

previous reports, which attributed the hypoglycemic effects of BC to insulin release from the remnant pancreatic  $\beta$ -cells and enhancement of peripheral glucose metabolism<sup>31</sup>. Researchers have analyzed the contents of BC extract and found that it is rich in catechins, carotenes, tannins, uronic acids, and polyphenol compounds, one of the most important of which is gallic acid<sup>32</sup>. BC extract normalized the blood glucose level and restored the insulin level due to its enrichment of gallic acid<sup>33</sup>. In contrast, Dex did not have any ameliorative effect on the diabetic condition, which may be explained by the inhibitory effects of Dex on insulin secretion<sup>34</sup>.



**Fig 3(A):** Section of sham group showed normal reaction of a glomerulus (G), boundaries of Bowman's capsule (white-arrow), and basement membranes of proximal and distal convoluted tubules (arrowhead). Note regular curved brush border (curved arrow). **Fig 3(B):** Section of DM+IRI group showed increased reaction of the glomerulus (G), boundaries of Bowman's capsule (white-arrow), and basement membranes around proximal and distal convoluted tubules (arrowhead) with the presence of vascular congestion (star). Note vacuoles inside and around the tubules (V). **Fig 3(C):** Sections of DM+IRI+BC showed a mild reaction in the glomerulus (G), boundaries of Bowman's capsule (white-arrow), and basement membranes around proximal and distal convoluted tubules (arrowhead). **Fig 3(D):** Sections of DM+IRI+Dex showed a mild reaction in the glomerulus (G), boundaries of Bowman's capsule (white-arrow), and basement membranes around proximal and distal convoluted tubules (arrowhead) (PAS, Scale bar 20  $\mu$ m).

Renal IRI is more frequent in diabetics and considered a complex inflammatory process that is initiated with ischemia and aggravated during reperfusion of ischemic renal tissue. It results a complex series of cellular events, inflammatory processes, apoptosis, and OS <sup>29</sup>.

There was an improvement of serum Creat and serum BUN in this study that demonstrated the beneficial effects of both BC extract and Dex. These results agree with previous studies indicating that

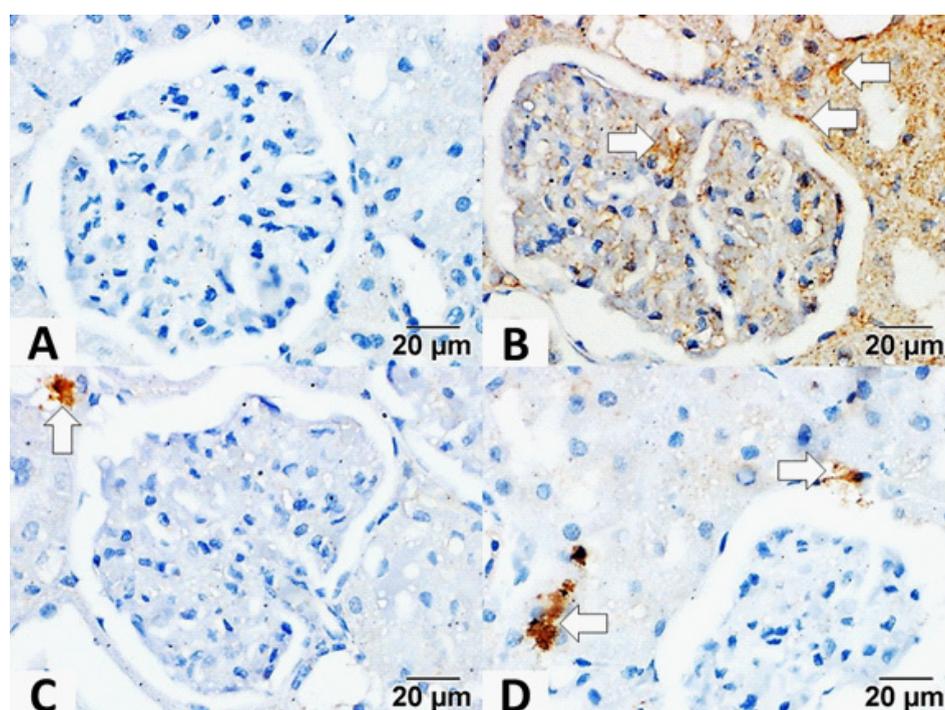
STZ-induced diabetes resulted in renal impairment, as evidenced by significant increases in the serum Creat and serum BUN <sup>12, 28-30</sup>.

This study showed that treatment with BC resulted in a significant improvement in renal functions, in accordance with a previous study that attributed these results to the antihyperglycemic and antioxidant effects of the polyphenol content of BC <sup>1</sup>. In addition, the renal functions improved due to treatment with Dex; these results agree

**Table 6: Reaction to PAS of the different experimental groups**

Histopathological findings	Sham	DM+IRI	DM+IRI+BC	DM+IRI+Dex
PAS (+) granules in tubules	-	++	+	+

None (-); mild (+), moderate (++) , severe (+++).



**Fig 4(A):** Section of sham group showed minimal cytoplasmic Bax (BCL2-associated X) immunoreactivity. **Fig 4(B):** Section of DM+IRI group showed strong positive cytoplasmic Bax immunoreactivity lining the glomeruli, cortical and in the tubular epithelial cells (white-arrow). **Fig 4(C):** Sections of DM+IRI+BC showed minimal cytoplasmic Bax immunoreactivity in the cytoplasm of some renal tubular cells (white-arrow). **Fig 4(D):** Sections of DM+IRI+Dex showed mild cytoplasmic Bax immunoreactivity (white-arrow) in most renal tubular cells (BAX, Scale bar 20 µm).

with previous studies that attributed these results to reduced vasoconstriction, promotion of renal arterial vasodilation, decreased vascular resistance, and increased glomerular filtration rate and filtration fraction <sup>7, 11, 35</sup>, thereby improving renal IRI <sup>36</sup>.

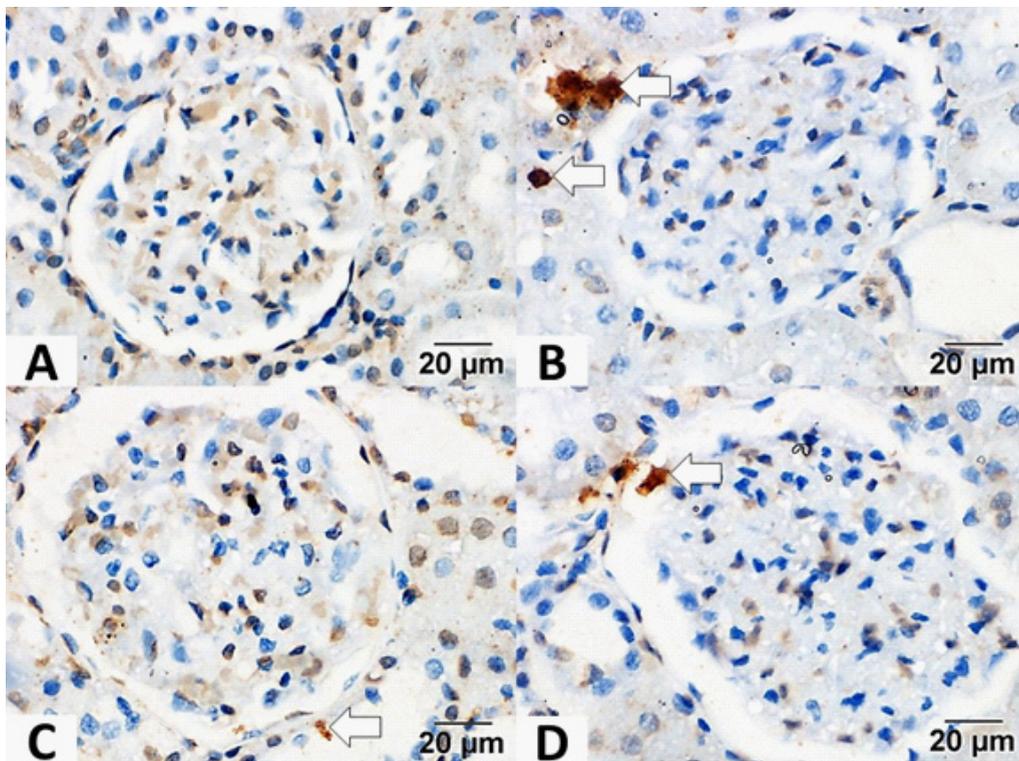
In this study, there was a significant increase in serum LDH in the untreated group, which

indicated tissue necrosis and tissue damage due to renal IRI; protection with BC and Dex resulted in significant decreases in the serum LDH levels, mainly due to their renoprotective effects, which resulted from their antioxidant properties <sup>37</sup>. This study showed that untreated groups demonstrated dyslipidemia; these results are in accordance with previous studies stating that, due to disturbances in

**Table 7: Mean  $\pm$  SD of apoptotic cell number/20000  $\mu\text{m}^2$  of the different groups**

Groups	Sham	DM+IRI	DM+IRI+BC	DM+IRI+Dex
Apoptotic cell number	3.64 $\pm$ 0.76	9.57 $\pm$ 0.24 <i>P</i> <sup>1</sup> < 0.001	4.36 $\pm$ 0.59 <i>P</i> <sup>1</sup> < 0.01 <i>P</i> <sup>2</sup> < 0.001	6.02 $\pm$ 0.62 <i>P</i> <sup>1</sup> < 0.001 <i>P</i> <sup>2</sup> < 0.001

SD, standard deviation. *P* < 0.05 is significant. *P*<sup>1</sup> Significance versus sham-operated. *P*<sup>2</sup> Significance versus ischemia reperfusion.



**Fig 5(A):** Section of sham-operated group showed weak cytoplasmic TNF- $\alpha$  protein expression. **Fig 5(B).** Section of ischemia reperfusion group showed strong positive cytoplasmic immunoreaction for TNF- $\alpha$  in the glomeruli and in the renal tubular cells (white-arrow). **Fig 5(C).** Sections of reperfusion treated with benincasa cerifera showed minimal cytoplasmic TNF  $\alpha$  immunoreactivity in the cytoplasm of some renal tubular cells (white-arrow). **Fig 5(D).** Sections of reperfusion treated with dexmedetomidine showed mild cytoplasmic TNF  $\alpha$  immunoreactivity (white-arrow) in renal tubular cells (TNF- $\alpha$ , Scale bar 20  $\mu\text{m}$ ).

**Table 8: OD of TNF- $\alpha$  expression of the different groups, mean  $\pm$  SD, (n=15)**

Groups	Sham	DM+IRI	DM+IRI+BC	DM+IRI+Dex
OD of TNF $\alpha$	0.521 $\pm$ 0.813	5.03 $\pm$ 1.166	3.85 $\pm$ 0.124	4.23 $\pm$ 0.089
		$P^1 < 0.001$	$P^1 < 0.001$	$P^1 < 0.001$
			$P^2 < 0.001$	$P^2 < 0.05$

TNF- $\alpha$ , tumor necrosis factor  $\alpha$ , SD, standard deviation.  $P < 0.05$  is significant.  $P^1$  Significance versus sham-operated.  $P^2$  Significance versus ischemia reperfusion.

metabolism associated with STZ-induced diabetes as result of glucose intolerance, decreased insulin levels enhance lipolysis and increase LDL synthesis<sup>38, 39</sup>. Treatment with BC resulted in a significant improvement of all parameters of the lipid profile, in agreement with a previous study<sup>40</sup>, which attributed this finding to the antihyperlipidemic properties of BC. The authors explained that the antihyperlipidemic effects of BC arise from multiple mechanisms, including its regenerative effects on pancreatic  $\beta$ -cells, which result in increasing the serum insulin levels, inhibitory effects on PPAR signaling and HMG-CoA reductase, and antioxidant properties<sup>41</sup>. In this study, Dex administration improved dyslipidemia, which may have been due to its antioxidant activity<sup>36</sup>.

OS plays a critical role in renal IRI, as with initiation of ischemia, renal tissues are exposed to hypoxia and the depletion of adenosine triphosphate (ATP); then, during the reperfusion phase, the OS continues via the release of reactive oxygen species (ROS) and free radicals<sup>36</sup>. ROS activate inflammation and vasoconstrictors, resulting in the damage of tubular cells<sup>42</sup>. The results of the current work confirmed the role of OS in renal IRI, as evidenced by the significant elevation of MDA, 8-OHdG, and MPO and decrease of GSH in serum and renal ischemic tissue homogenate in the untreated group (DM + IRI) as indicators of OS<sup>43, 44</sup>.

The results of this study agree with those of previous studies showing a significant increase in the MDA levels in renal IRI groups, which is used as an indicator of lipid peroxidation causing damage to the cellular and mitochondrial membranes, thereby leading to cellular disturbance and possibly ending with cell necrosis<sup>3, 42, 45-48</sup>. The renal IRI

group showed a significant increase in serum and renal tissue homogenate levels of 8-OHdG, which is considered a sensitive index for oxidative DNA damage resulting from OS produced due to renal IRI; these results agree with previous studies<sup>49, 50</sup>. Moreover, the MPO levels were increased in both serum and renal tissue homogenate; MPO is considered a marker of neutrophil infiltration in the renal tissue, leading to the release of multiple inflammatory cytokines; these results are in accordance with previous studies<sup>51-53</sup>. Studies have demonstrated that neutrophil infiltration plays a critical role in initiating the inflammatory response as a consequence neutrophil degranulation, which exacerbates MPO, cytokine, and ROS release, leading to a cellular disturbance and loss of function in renal tissues<sup>54</sup>.

The results of the current work showed a significant decrease in the level of GSH in the untreated group, indicating the consumption of endogenous antioxidants like GSH. These findings agree with those of previous studies<sup>55, 56</sup>.

The antihyperglycemic and antioxidants effects of BC and its power in stabilizing cellular membranes through the inhibition of lipid peroxidation, cellular infiltration, and restoration of reduced GSH levels mean that BC delays renal complications stemming from DM<sup>10, 41, 57</sup>. The antioxidant effects of BC are due to its high content of polyphenols and flavonoids, which act as hydrogen donors for free radicals. They break the chain reaction and inhibit lipid peroxidation, promote the endogenous antioxidant defense system, and modulate the oxidant/antioxidant balance; hence, there is a positive correlation between phenolic content and antioxidant activity<sup>57-59</sup>.

In this study, the administration of Dex significantly ameliorated OS conditions, as found in other studies<sup>60</sup>. Similar results documented that pretreatment with Dex results in the improvement of MDA and MPO levels in lung tissue homogenate after IRI<sup>61, 62</sup>. These authors explained that the renoprotective action of Dex makes it an effective  $\alpha_2$ -adrenergic receptor agonist, producing effects on the kidney by decreasing the efferent sympathetic outflow of the renal nerve, resulting in increased diuresis and natriuresis<sup>60</sup>. Moreover, by stabilizing cellular membranes to prevent lipid peroxidation, Dex antioxidant activity reduces malondialdehyde levels, restores total GSH levels, free radical scavenging activity, activates antioxidant enzymes, and maintains the integrity of DNA and cellular protein<sup>36</sup>.

In the current study, pretreatment with BC protected the kidney's architectural and cytological structure from lipid peroxidation and OS, coinciding with previous findings<sup>10</sup>. BC's renoprotective effect on renal IRI can be explained by its antioxidant activity<sup>63</sup>.

A significant decrease was detected in BAX immunoreactivity in the groups treated with Dex and BC, suggesting that apoptosis, a major pathway of IRI, could be reduced or prevented by both substances. This coincided with other researchers' findings<sup>64</sup>.

Kocoglu *et al.*<sup>65</sup> reported a significant decrease in the histopathological injury in a renal IRI model, supporting the current findings. Dex's renoprotective effect of Dex may be attributed to the increase of renal blood flow and glomerular filtration by reducing the release of noradrenaline<sup>66</sup>. Villela *et al.*<sup>67</sup> reported that Dex decreased the urinary osmolality and plasma vasopressin level and produced free water diuresis. Previous researchers found that Dex improves renal function, urine flow, and glomerular filtration<sup>68</sup>. In addition, it has been reported to increase renal arterial vasodilation via direct vascular effects<sup>65</sup>. The renoprotective effect of Dex has been attributed to the increase in antiapoptotic Bcl-2 and (Mouse double minute 2 homolog) Mdm-2 expression; this increase has been associated with a decrease in the levels of proapoptotic Bax<sup>69</sup>. This coincided with the current BAX immunohistochemistry findings. In the present study, the renoprotective role of Dex, was revealed by an improvement in post-ischemic attenuated histological damage, decreased number of apoptotic cells, and reduction of TNF- $\alpha$ <sup>70</sup>.

In conclusion, the present study demonstrated that oxidative stress plays a chief role in renal IRI in an STZ-induced diabetic model. Treatment with BC extract achieved excellent ameliorative effects on both diabetes and renal IRI, while treatment with Dex improved renal IRI, renal functions, and dyslipidemia, but it did not affect the diabetic condition.

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