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ACE2-Ang (1–7)/ SIRT1 is a Prospective Nephroprotective Panel of DIZE in CCl_4- Induced Kidney Injury

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Abstract

Carbon tetrachloride (CCl_4) causes oxidative stress and inflammation in rats' kidneys, leading to renal damage. Mortality from renal damage is alarmingly rising, necessitating coordinated efforts to create a viable treatment. Renin-angiotensin system (RAS) modulation by angiotensin-converting enzyme 2 (ACE2) is significant since the RAS is a key player in renal damage as a result of toxicity. Diminazene aceturate (DIZE), an ACE2 activator, has been shown to have positive benefits on models of renal disorders in earlier studies. In the current investigation, we sought to assess DIZE administration's therapeutic effects on rat experimental renal damage brought on by CCl_4. Male Albino rats were treated with either CCl_4 (0.5 mg/kg twice/week) and/or DIZE (15 mg/kg/day) for eight weeks. In this study, CCl_4 rats activated the RAS system as indicated by the downregulation of ACE2. The CCl_4-induced inflammatory response was demonstrated through increased levels of TNF-α, IL-6, IFN-γ, and NF-κB and suppression of SIRT1. Conversely, DIZE elicited protective action, upregulated the expression of ACE2, and suppressed the inflammation. In conclusion, DIZE inhibited the RAS system and provided renoprotection while suppressing the proinflammatory response caused by CCl_4.

Keywords: CCl_4; Diminazene; SIRT1; Angiotensin-converting enzyme 2 (ACE2); Renin-angiotensin system (RAS).

1. Introduction

Acute kidney damage always results in quickly progressing renal failure and is linked to high mortality rates. It is now known that certain hazardous substances cause oxidative stress, which causes kidney injury **(Khan et al., 2008)**. Numerous studies have used carbon tetrachloride (CCl_4) to mimic the oxidative stress in different rat tissues **(Naz et al., 2014)**. CCl_4 is widely used experimentally to induce liver, kidney, and central nervous system damage **(Scholten et al., 2015)**. Although the pathophysiology of renal failure brought on by carbon tetrachloride (CCl_4) is not fully understood, oxidative stress is still a major factor. The host defense system's initial response to oxidative stress is inflammation, which may be advantageous or harmful **(Medzhitov, 2008)**. A healing effect from inflammation is beneficial to the host. However, excessive and dysregulated inflammation can be harmful, Therefore, signals that initiate, maintain, and finally stop the cascade

when the insult is eliminated are necessary for the careful management of inflammatory processes **(Medzhitov, 2008)**. Renovascular toxicity is greatly impacted by the Renin-angiotensin system (RAS) axis components, angiotensin-converting enzyme1(ACE1) and angiotensin-converting enzyme2 (ACE2). In this sense, the generation of the peptide angiotensin II (Ang II) by ACE1 results in cellular differentiation, proliferation, hypertrophy, fibrosis, renal vasoconstriction, and increased tubular sodium reabsorption **(Ruggenenti et al., 2010)**. On the other hand, ACE2 is essential for converting Ang II (Ang II) into Ang-(1-7) (Ang-(1-7)), which has anti-inflammatory, antifibrotic, vasodilatory, anti-hypertrophic, and anti-apoptotic properties **(Ocaranza et al., 2014; Pandey and Gaikwad, 2017)**.

Diminazene aceturate (DIZE) has been the preferred therapy for animal trypanosomiasis since 1955. It is an aromatic diamidine that is composed of two amidinophenyl moieties connected by a triazene bridge **(Peregrine and Mamman, 1993)**.

It has been shown that DIZE protects against several pathologic diseases, such as ischemic stroke **(Mecca et al., 2011)** and pulmonary hypertension **(Kuriakose et al., 2013)**. Recent research has demonstrated that DIZE has antifibrotic characteristics by increasing ACE2 activity **(Rajapaksha et al., 2018)**, suggesting that it may also improve ACE2/Ang (1–7), the protective axis of RAS. In addition, DIZE has been found to suppress the pro-inflammatory cytokines production in septic shock **(Kuriakose et al., 2013)**. In contrast, ACE2 expression is known to be promoted by the histone deacetylase, silent information regulator 1 (SIRT1), which has a net negative impact on the coordinated inflammatory response **(Clarke et al., 2013)**. It is unclear, though, if DIZE's stimulation of ACE activity and consequent anti-inflammatory impact involve SIRT1 activation.

The goal of this work was to test the hypothesis that DIZE, an ACE2 activator, is a potent preventative measure against CCl_4-induced renal injury by activating the protective axis ACE2/Ang (1–7) and, eventually, interfering with the cytokine storm to promote renal injury recovery.

2. Materials and Methods

2.1. Drugs and chemicals

Diminazene aceturate was aquired from Sigma. Carbon tetrachloride (CCl_4) was sourced from El-Nasr Chemical Co (Egypt).

2.2. Animals and Experimental Design

This experiment was conducted on 32 rats (Male Albino) aged 6 weeks weighing 100-150 g were kept in cages (8 per cage) under regulated circumstances. The study was conducted at Ain Shams University's Laboratory Animals Research Center, Animals were fed without restrictions and acclimatized for two weeks prior to the study begins. They were kept at 22 ± 2°C with light/dark intervals. All animal medical procedures and supervision were carried out under the general standards of the Research Ethics Committee of the Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt, under authorization number 202101 MA1. This is following the FMASU-REC's general rules, the guiding principles of the ARRIVE guidelines, and the U.K. Animals (Scientific Procedures) Act, 1986 and accompanying recommendations; EU Directive 2010/63/EU on animal studies.

Rats were evenly divided into four groups. The first group was given a baseline diet and administered corn oil (0.5 ml/kg, i.p.) twice a week as a vehicle for CCl_4, and normal saline (15 ml/kg, intraperitoneally) daily as a medium for DIZE for 8 weeks; The second group of rats was administered daily DIZE (15 mg/kg/day in 15 ml/kg normal saline) for 8 weeks, whereas the third group received CCl_4 twice a week (0.5 mg/kg in 0.5 ml/kg corn oil, i.p.) for 8 weeks **(Sahreen et al., 2015)** and in the fourth group, rats were given CCl_4 twice a week (0.5 mg/kg in 0.5 ml/kg corn oil, i.p) and DIZE daily (15 mg/kg in 15 ml/kg normal saline) for 8 weeks **(Hasan et al., 2020)**. At the conclusion of therapy, rats were beheaded, blood was drawn, and kidney tissues were extracted.

2.3. Blood collection and tissue processing

Rats were sacrificed and their blood was extracted from the retro-orbital plexus for the investigation. Centrifugation was used to extract serum, and then kidney tissues were removed and submerged in a

cold solution. The kidney weight was used to calculate the nephrotoxicity index. Studies using transmission electron microscopy and histopathology were conducted on the samples. The residual kidney tissues were preserved at -80°C by freezing them. After homogenizing the kidneys in Tris–HCl buffer, aliquots were utilized to measure oxidative stress, inflammatory, ACE2, and SIRT1 indicators.

2.4. Quantitative Real-time PCR

RNeasy Plus Mini kits (Qiagen) were used to extract RNA from the kidney tissues. A volume of 60 µL was used to elute the RNA. The RNA was kept at -80°C after being evaluated for yield and purity using NanoDrop 2000 (Thermo Scientific, USA). RNA was reverse-transcribed into first-strand cDNA using the QuantiTect Reverse Transcription Kit (Qiagen) (cat. no. 205310) and RT Primer Mix (1 µg). Gene expression was examined using the Applied Biosystems StepOnePlus equipment and the QuantiTect SYBR Green PCR Kit (cat. no. 204141). According to the manufacturer's instructions, the HotStarTaq DNA polymerase was activated at 95°C for 15 minutes, then 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. The reactions were performed three times, with beta-actin serving as internal control. The findings were quantified using Ct values, which are the PCR cycle thresholds for the initial detection of the amplified product **(Table 1)**.

2.5. Assessment of renal function indices

Serum creatinine and urea were assessed using commercial kits obtained from Biodiagnostics (Giza, Egypt). The renal index was determined using the formula: (kidney weight/body weight) × 100.

2.6. Evaluation of oxidative stress markers

Superoxide dismutase (SOD) activity and reduced glutathione (GSH) levels were measured colorimetrically with commercially available kits (Biodiagnostics, Cairo, Egypt). Malondialdehyde (MDA) concentrations were measured as indicators of lipid peroxidation **(Mihara and Uchiyama, 1978)**.

2.7. Estimation of the inflammatory markers in the renal tissues

ELISA kits (Bioscience, USA) were used to measure tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ) in kidney homogenates in accordance with the manufacturer's instructions. A commercial kit (Spectrum Diagnostics, Cairo, Egypt) was used to evaluate the protein concentration colorimetrically.

2.8. Histopathological examination

Renal tissue samples were fixed for the whole night in 10% buffered formalin. They were then cleaned and dehydrated with a variety of ethanol grades (30–100%). After that, blocks of paraffin were used to implant the renal tissues. After slicing with a 5 µm thick rotatory microtome, the samples were deparaffinized in xylene, stained with haematoxylin, soaked in 70% alcohol, and stained with eosin. Sections were cleaned with xylene and cleansed with alcohol. Under a light microscope, the slices were inspected after being fixed with dibutylphthalate polystyrene xylene (DPX) **(Bancroft and Stevens, 1996)**.

Table 1. The sets of primers used in real-time q-PCR analysis

Gene	Forward Primer	Reverse Primer	Accession Number	Amplicon Size
SIRT1	ATCTCCCAGATCCTCAAGCCA	CTTCCACTGCACAGGCACAT	NM_001372090.1	189
ACE2	CCAGCAGGAGCTTGACATCT	TTGGTCCAGCAGCTTGTTTACT	NC_051356.1	188
NF-κB	TCTGTCATCCGTGCTTCCAG	CGTGGCCTGCCTAACTTCTG	NC_051337.1	128
β-actin	GTAAGCAGCCTTAGCCTGGA	CGCTCAGGAGGAGCAATGAT	NC_051347.1	144

2.9. Transmission electron microscopy (TEM)

Kidney specimens were cut into tiny pieces (1 mm^3) and incubated with 2.5% glutaraldehyde in 0.1 N PBS at room temperature for one hour. For an additional hour, osmium tetraoxide was used to fix the specimen. Renal tissues were hydrated twice in 70, 90, and 100% acetone, as well as in a 1:1 (acetone:resin) solution for a few minutes. Renal tissue samples were then implanted in epoxy resin. Ultrathin slices (90 nm) were examined using a transmission electron microscope (Jeol Jem-1400, USA Inc.).

2.10. Statistical analysis

The data is provided as mean ± SD. Statistical analyses were performed using one-way ANOVA, with Tukey-Kramer as a post-hoc test. Significance is defined as a probability threshold of 0.05. All statistical analyses were done using the Instant version 3 software program. Graph Pad Prism (ISI® software, USA) version 8.4.3 was used to create graphs.

3. Results

3.1. DIZE reversed CCl_4-induced renal damage

The current investigation found that CCl_4 significantly increased blood urea and creatinine levels by 3.1 and 4.2 folds, respectively, compared to the control group ($P < 0.05$). When CCl_4-intoxicated rats were cotreated with DIZE, blood urea and creatinine levels increased only by 1.5-fold and 1.75-fold, respectively. Similarly, CCl_4-intoxicated rats exhibited a considerably larger renal index than the control group (2.4 times), whereas there was no significant difference in kidney weight between the rats simultaneously treated with DIZE and the control groups **(Fig.1i)**.

The detrimental effects of CCl_4 on renal cell function were also evident in renal architecture. The gross appearance of the kidney is exhibited in Fig.1ii where in the CCl4 group, the kidney surface is greatly irregular and rough and the tissues are hardened. However, the surface of the kidney in the control, DIZE, and CCl4 groups cotreated with DIZE groups was intact, regular, and smooth.

3.2. DIZE decreased CCl_4-induced oxidative stress

MDA levels increased significantly by 2.5-fold following chronic CCl_4 injection compared to the control group. Furthermore, CCl_4 treatment resulted in a substantial 2.3-fold reduction in GSH levels and a 4.2-fold drop-in SOD activity. After cotreatment with DIZE, MDA levels recovered to normal levels (2.1-fold drop), GSH was replenished (1.5-fold rise), and SOD activity was maintained (3.6-fold increase) **(Fig. 2)**.

3.3. DIZE reduced CCl_4- induced inflammatory response

The levels of inflammatory cytokines TNF-α, IL-6, IFN-γ and mRNA levels of nuclear factor-κB (NF-κB) were significantly elevated by 3-fold, 3.3-fold, 4-fold, 2.85-fold respectively in the CCl_4-intoxicated rats compared to the control group. After cotreatment with DIZE, TNF-α was decreased by 2.4 folds, IL-6 by 2.5-folds, IFN-γ by 2.5-folds and NF-κB by 1.9-folds compared to the CCl_4-intoxicated group **(Fig. 3)**.

3.4. DIZE improved the gene expression of SIRT1 and ACE2 after CCl_4 intoxication

CCl_4 administration induced a significant decrease in the gene expression of SIRT1 and ACE2 in the renal tissues compared with the control group. Interestingly, when CCl_4 administration was combined with DIZE, there was a significant increase in the gene expression of SIRT1 and ACE2 in the renal tissues **(Fig. 4)**.

3.5. Histopathological examination of renal tissue using H&E stain

Histopathological examination of renal tissue showed normal tubular cells **(Fig. 5A, 5B)** architecture in the control and negative control groups. While renal tissues from CCl_4-intoxicated rats showed atrophied and degenerated tubules **(Fig. 5C)**. Cotreatment with DIZE considerably ameliorated the tubular damage **(Fig. 5D)**.

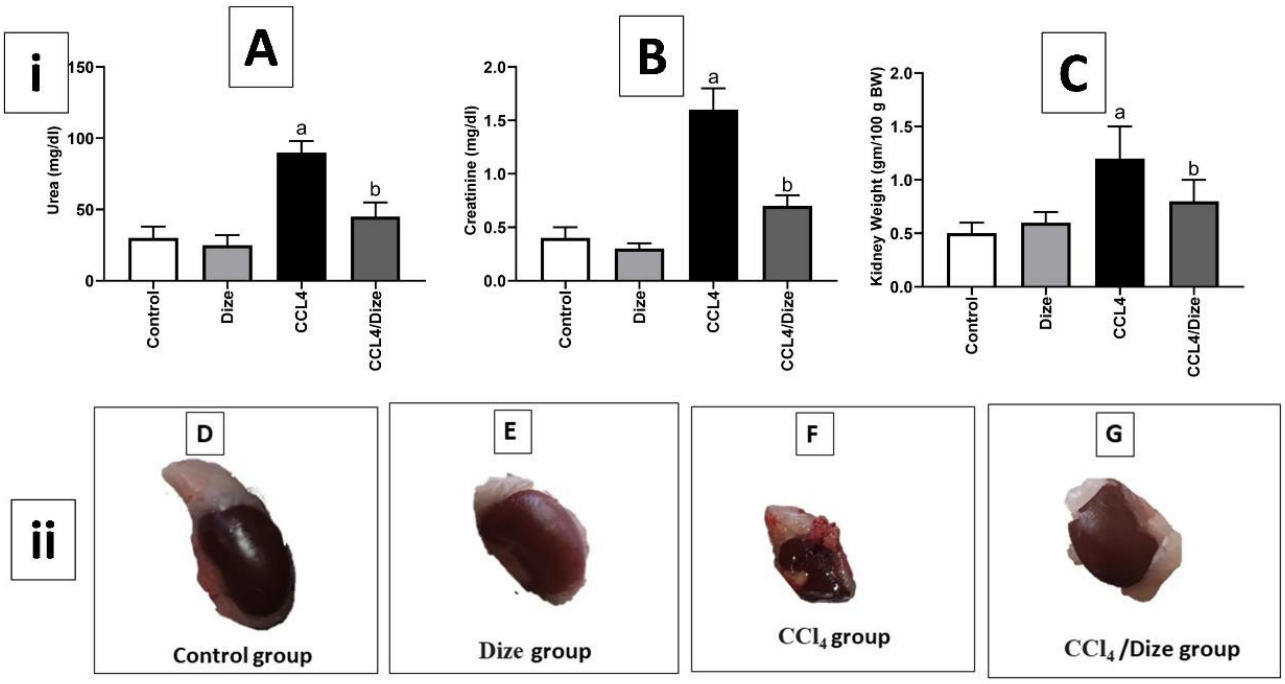

Figure 1: The effects of CCl_4 and DIZE administration on (i): Serum levels of urea, creatinine and kidney weight (ii): Kidney gross appearance.

(A): Urea serum levels (mg/dl) in different groups**. (B)**: Creatinine serum levels (mg/dl) in different groups**. (C)**: Kidney weight (gm/100 g BW) in different groups**. (D)**: Kidney gross appearance in control groups**. (E)**: Kidney gross appearance in DIZE only groups**. (F)**: Kidney gross appearance in CCl_4 groups**. (G)**: Kidney gross appearance in CCl_4/ DIZE groups**.** Data are presented as the means ± SD. $P < 0.05$ is considered statistically significant; a, significant ($P < 0.05$) versus control; b, significant ($P < 0.05$) versus CCl_4 group; one-way ANOVA followed by Bonferroni-corrected post hoc tests were conducted. DIZE, Diminazene aceturate.

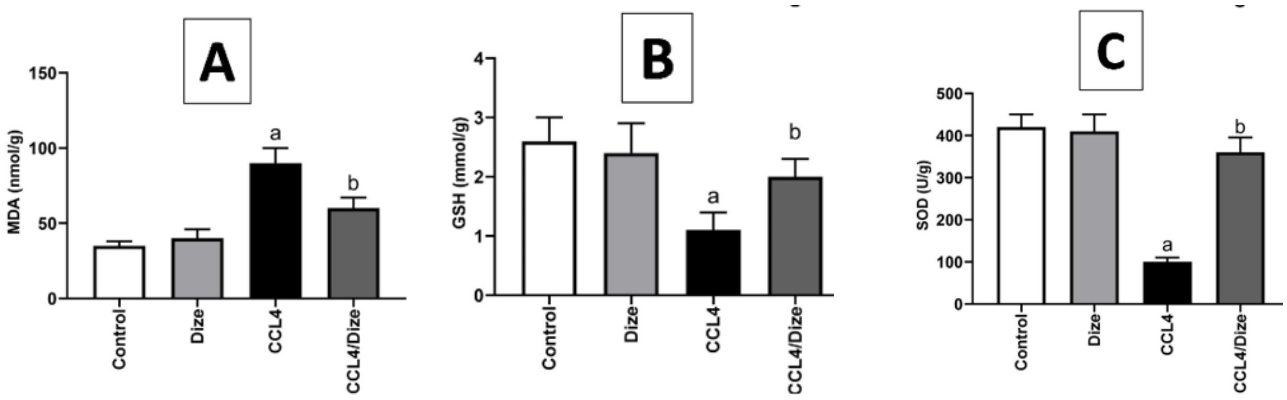

Figure 2: The effects of CCl_4 and DIZE administration on renal levels of oxidative stress markers.

(A): MDA serum levels (nmol/g) in different groups**. (B)**: GSH serum levels (mmol/g) in different groups**. (C)**: SOD serum levels (U/g) in different groups**.** Data are presented as the means ± SD. $P < 0.05$ is considered statistically significant; a, significant ($P < 0.05$) versus control; b, significant ($P < 0.05$) versus CCl_4 group; one-way ANOVA followed by Bonferroni-corrected post hoc tests were conducted. DIZE, Diminazene aceturate; MDA, Malondialdehyde; GSH, Reduced glutathione; SOD, Superoxide dismutase.

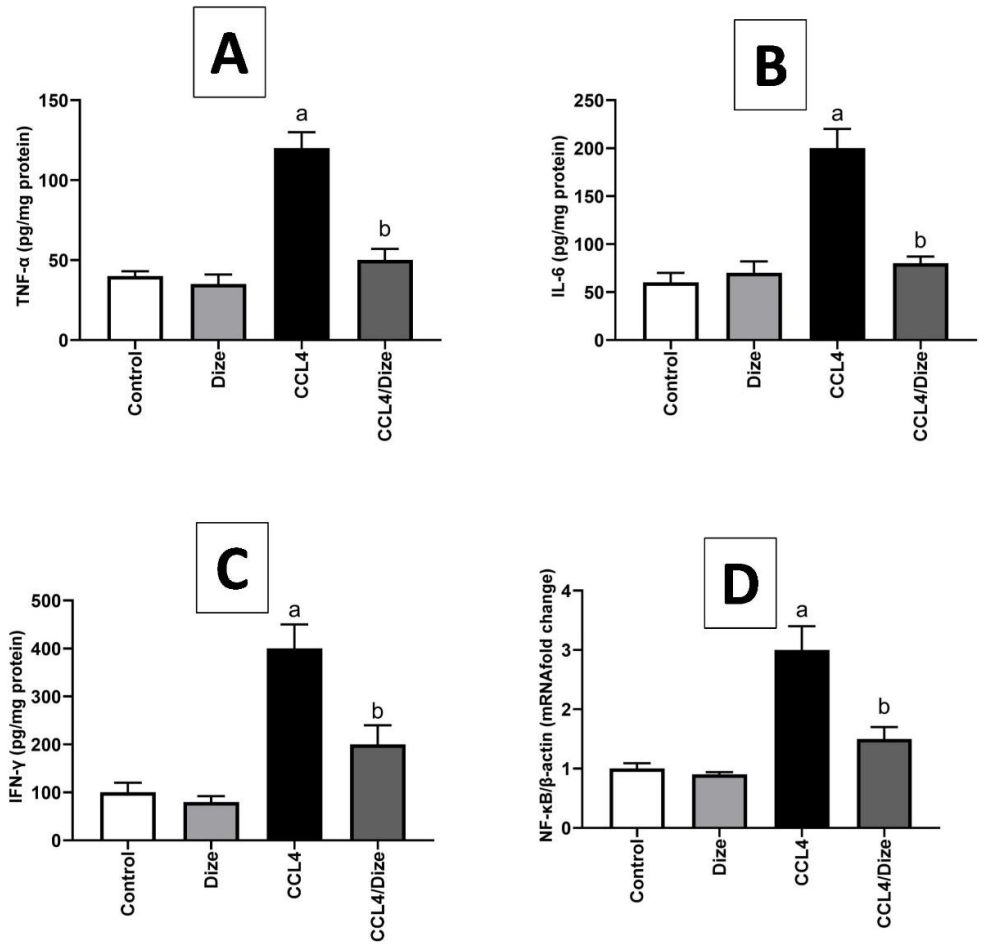

Figure 3: The effects of CCl_4 and DIZE administration on renal levels of inflammatory markers.
(A): TNF-α serum levels (pg/mg protein) in different groups**. (B)**: IL-6 serum levels (pg/mg protein) in different groups**. (C)**: IFN-γ serum levels (pg/mg protein) in different groups**. (D)**: NF-κB mRNA fold change relative to β-actin in different groups**.** Data are presented as the means ± SD. $P < 0.05$ is considered statistically significant; a, significant ($P < 0.05$) versus control; b, significant ($P < 0.05$) versus CCl4 group; one-way ANOVA followed by Bonferroni-corrected post hoc tests were conducted. DIZE, Diminazene aceturate.

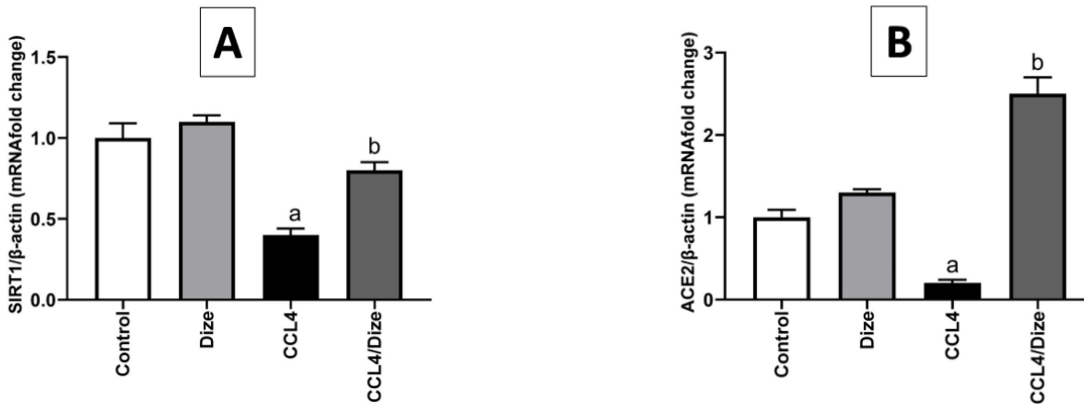

Figure 4: The effects of CCl_4 and DIZE administration on SIRT1 and ACE2 gene expression.
(A): SIRT1 mRNA fold change relative to β-actin in different groups**. (B)**: ACE2 mRNA fold changes relative to β-actin in different groups**.** Data are presented as the means ± SD. $P < 0.05$ is considered statistically significant; a, significant ($P < 0.05$) versus control; b, significant ($P < 0.05$) versus CCl_4 group; one-way ANOVA followed by Bonferroni-corrected post hoc tests were conducted. DIZE, Diminazene aceturate.

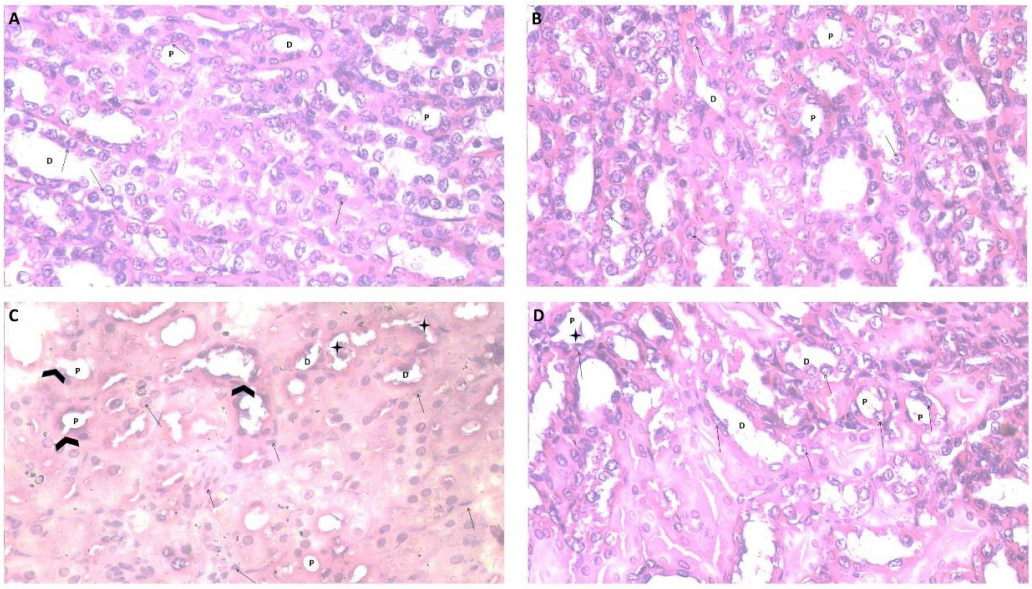

Figure 5: Examination of the effects of CCl_4 and DIZE administration on the histopathology of the renal tissues using H&E stain.

(A), (B): In the control and DIZE-only groups, normal renal cortex was observed with normal proximal convoluted tubules (P) and distal convoluted tubules (D) which have vesicular nuclei (arrow). (**C):** In CCL_4 intoxicated group, the renal tubules were malformed or degenerated as the distal convoluted tubules were small in size and degenerated, displaying atrophied vacuolated cytoplasmic epithelium (D) with pyknotic nuclei (arrow), and the proximal convoluted tubules were malformed, exhibiting atrophied vacuolated cytoplasmic epithelium (P) with pyknotic nuclei (arrow) with abundance of cellular debris in tubular lumina (stars) and loss of brush border (arrowhead). (**D):** Upon cotreatment with DIZE in the CCL_4, the renal tubules were ameliorated, as both the proximal convoluted tubules (P) and the distal convoluted tubules (D) were mostly intact with normal size and vesicular nuclei (arrow), with little cellular debris (stars).

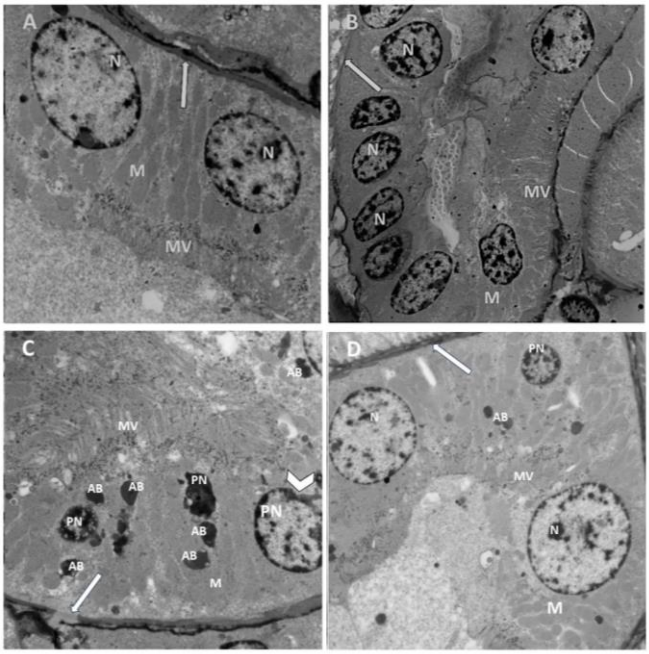

Figure 6: Examination of the effects of CCl_4 and DIZE administration on the renal ultrastructure using TEM imaging technique.

(A): In the control group and **(B)** DIZE only group, the renal tubular cells displayed long abundant apical microvilli (MV), elongated intact mitochondria (M), euchromatic nuclei (N), intact basal membrane (arrow). **(C):** In the CCL_4 intoxicated group, the renal tubular cells demonstrated fused mitochondria with solubilized cristae (M), basal lamina was broken (arrows), the nuclei were shrunken and pyknotic (PN), sometimes with a broken nuclear membrane (arrowhead). The nuclear remnants and components were enclosed in vesicles termed apoptotic bodies (AB). (D): After treatment with DIZE in the CCL_4 group, the renal tubular cells showed distinctive mitochondria with defined cristae, multiple euchromatic nuclei, only a few were pyknotic, scarce apoptotic bodies (AB) and unbroken basal membrane (arrow).

3.6. Electron microscopical examination of renal tissue

The ultra-structure of the rats' kidneys is shown in **Fig. 6**. The control group **(Fig. 6A)** and negative control **(Fig. 6B)** displayed normal ultra-renal structure in the form of intact tubular cells. Structural abnormalities after CCl_4 involved extensive apoptosis, which has the hallmark of disintegrated cellular components, and pyknotic nucleus **(Fig. 6C)**. Contrarily, cotreatment with DIZE **(Fig. 6D)** led to the survival of renal cells, with only a subset of renal tubular cells displaying minimal damage.

4. Discussion

Toxicant-induced kidney damage is traditionally believed to be inevitably progressive, frequently leading to fibrosis and organ failure **(Lafrance and Miller, 2010)**. A clinical illness known as acute kidney injury (AKI) develops when there is structural damage caused by the injury, which manifests as a rapid fall in glomerular filtration rate (GFR) and the elimination of nitrogenous waste **(Schrier et al., 2004)**. A superior animal model of AKI that simulates oxidative stress in numerous physiological settings is CCl_4-induced AKI in rats **(Ozturk et al., 2003)**. In the current study, CCl_4-induced renal injury was further evident by elevated urea and creatinine serum levels. Histologically, we found that the renal tubules were malformed as both the proximal and distal convoluted tubules had atrophied vacuolated cytoplasmic epithelium with pyknotic nuclei. Examination of the cellular components by TEM revealed extensive apoptosis where cellular and nuclear remnants were enclosed in apoptotic bodies. Also, the gross appearance of the kidney showed multiple fibrotic areas.

A key enzyme in CCl_4-induced nephrotoxicity, P450 cytochrome, is localized in cortical tubule cells, where it causes many free radicals to develop due to a sequence of electron transfers **(Ronis et al., 1998)**. These radicals attach to macromolecules or attack polyenoic fatty acids in cellular membranes, which eventually disintegrate to produce a variety of stable end products, such as malondialdehyde (MDA) **(Recknagel et al., 1989)**. In this work, CCl_4-treated rats had high MDA levels and decreased GSH and SOD content, which is consistent with earlier studies describing CCl_4-induced membrane lipid damage.

In addition to its immediate negative consequences, oxidative stress can also trigger inflammatory pathways by activating redox-sensitive transcriptional factors like NF-κB. NF-κB is a transcription factor that controls the host's inflammatory and immune responses (**Oeckinghaus and Ghosh, 2009)**. The expression of a variety of different proinflammatory mediators, including TNF-α, IFN-γ is regulated by NF-κB. In the present study, heightened levels of TNF-α, IL-6, and IFN-γ in the kidneys, along with increased expression of NF-κB after CCl_4 injection, indicated a pronounced inflammatory response in the kidney. Previously, the features of antagonistic crosstalk were found between NF-κB. and SIRT1, with NF-κB promoting a pro-inflammatory phenotype and glycolytic metabolism and SIRT1 supporting oxidative respiration and anti-inflammatory responses **(Kauppinen et al., 2013)**. In the current work, SIRT1 expression was suppressed in CCl_4 rats that had active NF-κB expression. NF-κB as a transcription factor can bind to and hence promote multiple binding sites in the promoter sequences of the Sirt1 gene **(Voelter-Mahlknecht and Mahlknecht, 2006)**. Also, NF-κB was shown to bind to the miR-34a promoter, activating its expression **(Li et al., 2012)**. SIRT1's 3′ UTR is the target of miR-34a, which suppresses SIRT1 expression (Yamakuchi et al., 2008). Additionally, oxidative stress decreases the amount of NAD+ in the cell, which is a necessary substrate for SIRT1 activity, and therefore suppresses the SIRT1-mediated signaling **(Furukawa et al., 2007)**. According to Li et al., IFN-γ increased the transcriptional expression of CIITA and HIC1, a well-known SIRT1 transcription inhibitor, thereby decreasing the expression of SIRT1 **(Li et al., 2011)**. This is in accordance with the elevated level of IFN-γ and reduced expression of SIRT1 in our study.

Recently, SIRT1 was found crucial in the regulation of ACE2 expression **(Clarke et al., 2013)**. The current study found that reduced SIRT1 expression was accompanied by a decrease in ACE2 expression.

ACE2 acts as a carboxypeptidase, eliminating the solitary C-terminal amino acid from Ang II to produce Ang-(1–7). Therefore, ACE2 is considered a counterbalance for the action of Ang II. In contrast, ACE1 primarily functions as a carboxydipeptidase (peptidyldipeptidase), eliminating the C-terminal dipeptide from Ang I to generate Ang II **(Turner and Nalivaeva, 2022)**. AngII can cause cellular hypertrophy in renal

tubular cells by producing reactive oxygen species (ROS) among other mechanisms **(Wen et al., 2012)**. Also, AngII emerges as a key profibrogenic cytokine that regulates the proliferation of renal cells and the production of the extracellular matrix via a variety of fibrotic mechanisms **(Rüster and Wolf, 2011)**. As a result of the role played by RAS in the pathophysiology of kidney disorders, the antioxidant and nephroprotective effects of RAS inhibitors have been explored in recent years and have proven successful as renoprotective.

In this study, we probed the use of DIZE in activating ACE2 as an off-target effect for Dize aside from its traditional antitrypanosomal action. In support of Dize's action as an ACE2 activator, we found an upregulated expression of ACE2 in the DIZE/ CCl_4 group. In the present study, the increase in ACE2 causes a decrease in AngII and its consecutive ROS generation in renal tissues, therefore mitigation of inflammation and fibrosis is anticipated. In the current study, coadministration of Dize in CCl_4 rats impeded oxidative stress as shown by the increase in the renal levels of SOD and GSH and the decrease in MDA levels. Also, the Dize/ CCl_4 group had extenuated inflammation as the levels of TNF-α, IL-6, and IFN-γ were decreased as well as downregulated expression of NF-κB. These findings were in concurrence with previous studies professing the antioxidant **(Abdelrahman et al., 2023)** and the anti-inflammatory actions of Dize **(Hasan et al., 2020)**. Amelioration of renal injury in the DIZE/ CCl_4 group was further evident by reduced urea and creatinine serum levels. Histologically, the integrity of renal tubules was preserved, and the cellular components were intact. Also, the gross appearance of the kidney showed a smooth non-fibrotic surface.

5. Conclusion

Following cotreatment with DIZE, there was an improvement in renal cell survival as well as a reduction in oxidative stress and inflammation, all of which were linked to the significantly increased expression of SIRT1 and ACE2.

Conflict of Interests: The authors declare that there is no conflict of interest.

Data availability statement: The data supporting this study's findings are available from the corresponding author upon reasonable request.

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