Sitagliptin or Hesperidin Mitivates Cyclosporine-Induced Nephrotoxicity by Inhibiting Oxidative Stress and Apoptosis

Mustafa Ahmed Abdel-Reheim (mostafa011164@pharm.bsu.edu.eg)
Beni-Suef University

Sohayla Mahmoud Makram
Merit University

Basim Anwar Shehata
Beni-Suef University

Research Article

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Abstract

**Background and aims:** Immunosuppressive medication Cyclosporine A (CsA) is frequently used during organ transplantation. Additionally, rheumatoid arthritis and other autoimmune diseases have been treated with CsA. But it causes nephrotoxicity. Sitagliptin is a DPP-4 inhibitor that has been approved for the treatment of type 2 diabetes in adults. It is very effective. Citrus fruits contain the flavonone hesperidin, which is highly effective in treating a number of cardiovascular disorders. The study's goal was to clarify how sitagliptin or hesperidin prevented CsA's nephrotoxic effects.

**Main methods:** This study was done on 36 rats. The experimental design was designed into 6 groups that includes Group I (normal control): Rats received vehicle only for 14 days. Group II: Rats injected with a single daily i.p 20 mg/kg/day dosage CsA for the last 7 consecutive days. Group III: Sitagliptin was administered orally to rats once daily for 14 days at a dose of 10 mg/kg. Group IV: Rats received both CsA and sitagliptin treatments as previously indicated. Sitagliptin was administered 7 days before and 7 days after CsA that was administrated last 7 days. Group V: For 14 days straight, rats were given a single 200 mg/kg oral dosage of hesperidin formulated in distal water by oral gavage and Group VI: Rats received both CsA and hesperdin treatments as previously indicated. Blood and kidneys were taken on day 15. In addition to a histological analysis, markers of renal function, oxidative stress, inflammation and apoptosis were measured.

**Results:** Interestingly, sitagliptin or hesperidin attenuated CsA-mediated elevations of creatinine, Cystatin-C, glucose, MDA and MPO, while inhibiting CsA-induced decreases in albumin, catalase and GSH. Immunogenic assay of Nrf-2 and BAX and determination of tumor necrosis factor α (TNF-α) and western plot analysis among different groups also confirmed the safeguarding impact of sitagliptin or hesperidin on CsA-induced nephrotoxicity. Further confirmation of the reno-protective milieu provided by sitagliptin or hesperidin came from histological investigation.

**Conclusion:** our findings suggested that sitagliptin or hesperidin treatment along with CsA lowers CsA's nephrotoxicity, possibly acting by inhibiting oxidative stress, inflammation and apoptosis.

Introduction

In solid organ transplantation, allograft rejection is avoided by using the immunosuppressive medication cyclosporine A (CsA). Additionally, CsA has been utilized to treat autoimmune diseases such rheumatoid arthritis and psoriasis [1, 2, 3]. Inflammatory cell infiltration, tubular shrinkage, arteriolopathy, increased immunogenicity, and tubular interstitial fibrosis are the characteristics of the described CsA-evoked nephrotoxicity [4]. Although there are many different factors involved in the pathophysiology of CsA-induced nephrotoxicity, oxidative stress is crucial to the onset and advancement of this disease. The main signs of CsA renal injury are reactive oxygen species (ROS) overshooting and associated lipid peroxidation/oxidative aberrations [2]. In this regard, CsA-treated human renal mesangial cells have been used to define the overshooting of ROS [2, 5].
Additionally, oxidative stress and abnormalities caused by CsA have been revealed to be primarily caused by activated NADPH oxidase (NOX1). The NOX1 enzyme produces superoxide anion, which is the most prevalent ROS produced by CsA. Moreover, it has been observed that hypertension patients receiving CsA medication have higher plasma hydroperoxide levels [2, 6]. Additionally, lewis-lung cancer porcine kidney 1 (LLC-PK1) tubular cells have been identified as having decreased renal antioxidants like reduced glutathione [2, 7].

Sitagliptin is the first medication of its kind to be approved by the FDA for the treatment of persons with type 2 diabetes. It is an oral, extremely potent, and selective inhibitor of dipeptidyl peptidase-4 (DPP-4). In clinical trials, sitagliptin was usually well tolerated, had a negligible risk of hypoglycemia (albeit this is dependent on background therapy), and had no discernible impact on body weight [8].

People with type 2 diabetes have a high risk of both macrovascular and microvascular issues [9], due to the fact that type 2 diabetes is a major risk factor for the onset of chronic renal disease (CKD). Particularly in patients with documented cardiovascular disease, CKD raises the likelihood of negative cardiovascular (CV) outcome [10, 11, 12, 13], and a higher risk of cardiovascular events is connected separately with both microalbuminuria and macroalbuminuria [14].

As a result, a crucial factor in the long-term management of the condition is the possible impact of type 2 diabetes medicines on the outcomes of CV and CKD. The risk of CV disease in general and diabetic nephropathy in particular can be decreased by intensifying glucose management and using several CV risk factor medications [15]. However, there is a dearth of information regarding the efficacy of particular type 2 diabetes treatment plans with regard to these two outcomes [15].

According to the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS), sitagliptin did not reduce the incidence of serious CV outcomes, heart failure hospitalization, or adverse events in general when added to standard therapy in patients with type 2 diabetes and established CV illness. In this post hoc analysis, individuals in the TECOS study with type 2 diabetes and cardiovascular disease were evaluated for their CV and CKD outcomes based on the stage of their baseline estimated glomerular filtration rate (eGFR) [16].

Additionally, the flavonone hesperidin, which is largely present in citrus fruits, has been identified as a novel promising therapeutic agent capable of modifying a number of cardiovascular disease risk factors (CVDs). Furthermore, in diabetic mice, hesperidin showed that it might lower blood sugar and reduce inflammation [17].

TNF-α is a crucial immunoregulatory and proinflammatory cytokine having pleiotropic features, including the ability to trigger an inflammatory chain reaction that results in tissue damage. Numerous researches have examined the levels of IL-6, TNF-α, and TNF receptors in Systemic Lupus Erythematosus (SLE) [18]. To our knowledge only one study, Mahmoud et al. [19] concentrated on these in Egyptian patients, including nephritic patients. Last but not least, Nrf-2 and BAX protein histochemical immunoreactivity was used to gauge how much the chosen therapies contributed to the inflammatory responses.
The goal of this investigation is to determine how sitagliptin or hesperidin counteract the nephrotoxicity caused by CsA.

**Material And Methods**

**A- Animals:** 36 mature male Wistar albino rats, weighing 180 ± 20 g, were utilized in this investigation. They were purchased from the faculty of pharmacy- Assuit University, Assuit, Egypt. The institutional animal care and use committee of Beni-Suef University (BSU-IACUC) was in charge of overseeing the handling, nourishment, housing, acclimation, and experimental animal protocols. Their permission number is 022–360.

**B-Drug used:**

1. **Cyclosporine:** Sigma-Aldrich (St. Louis, MO) provided the cyclosporine, which was administered to the animals by IP injection at a dose of 20 mg/kg each day for the previous seven days.
2. **Sitagliptin:** The animals received sitagliptin orally for 14 days at a dose of 10 mg/kg/day from Sigma-Aldrich (St. Louis, MO).
3. **Hesperidin:** The animals were fed hesperidin orally at a rate of 200 mg/kg/day for 14 days straight, courtesy of Sigma-Aldrich (St. Louis, MO).

**C-experimental Design And Grouping Of Rats:**

- **Group I:** Rats were only given the vehicle for 14 days. The standard control group was this one.
- **Group II:** Rats given a single 20 mg/kg/day i.p. injection of CsA every day for the previous seven days.
- **Group III:** Sitagliptin was administered orally to rats once daily for 14 days at a dose of 10 mg/kg.
- **Group IV:** Rats received both CsA and sitagliptin treatments as previously indicated. Sitagliptin administered 7 days before and 7 days after CsA that was administrated last 7 days.
- **Group V:** For 14 days straight, rats were given a single oral dose of 200 mg/kg/day of hesperdin prepared in distal water by oral gavage.
- **Group VI:** Rats received both CsA and hesperdin treatments as previously indicated. CsA was administered 7th days after of hesperdin (that's was administrated 7 days before CsA and 7 days after CsA).

Before scarification, blood samples were drawn from the retro-orbital plexus into clean, dry tubes. Following that, blood samples were centrifuged for 5 minutes at 4000 RPM. For the purpose of measuring the serum's parameters, the serum was separated and transferred to sterile screw-capped vials.
Animals were killed by cervical dislocation after blood assembly, and the kidneys were promptly removed and three times cleansed in ice-cooled normal saline [20]. To prepare 20% kidney homogenates, a portion of each kidney was homogenized (1/5 w/v) in ice-cold Tris-HCl buffer (pH 7.4, 0.1 M), and the homogenates were then stored at -20°C for biochemical examination [20].

**D-biochemical Analysis:**

1- **Determination of serum Albumin (g/dl)**

determined utilizing Dumas' techniques [21].

2. **Determination of serum creatinine (mg/dl):** That determined according to the method of Toora and Rajagopal [22].

3- **Determination of myeloperoxidase (MPO)**

According to the manufacturer's recommendations, serum MPO was calculated using the Quantikine ELIZA kit from R&D Systems, catalogue number DMYE00B, for the quantitative assessment of MPO concentration in serum.

4- **Determination of Cystatin-C (CYS-C)**

that was established using the technique of Finney et al. [23] and Erlandsen et al. [24]

5- **Determination of serum glucose**

that was established using the technique of Trinder [25].

6. **Determination of tissue malondialdehyde (MDA):** that was established using the technique of Satoh et al. [26]

7. **Determination of catalase (CAT) activity:** that was established using the technique of Fossati et al. [27]

8. **Determination of glutathione reduced (GSH):** that was established using the technique of Beutler et al. [28]

**E- Histopathological Examination**

All of the animals' kidneys underwent paraffin embedding and 10% formalin fixation. After that, specimens were embedded in blocks in order to slice them at five micron intervals. Following this, slices were prepared for hematoxylin and eosin (H&E) staining. The kidney was examined, and then photographs were taken.
F- Determination Of Tnf-α And Western Plot Analysis:

Measurement of TNF-α in sera

TNF-α was measured in sera using an ELISA kit according to Aderka et al. [29], employing ELISA kits according to Montero-Julian et al. [30]

Western Blot Analysis:

To achieve clear supernatant, tissue samples were centrifuged after being homogenized in RIPA buffer. The total protein content was determined using the Bradford reagent. SDS-PAGE was used to isolate 30 µg of protein per gel lane, and the protein was then transferred to a PVDF membrane. The membranes were then treated with primary antibodies against TNF-alpha Antibody after being blocked in Tris-buffered saline with Tween 20 (TBST) containing 5% non-fat milk powder (Novus Biologicals USA). By ensuring that protein loading is uniform throughout the gel, the housekeeping protein β-actin was utilized as a loading control to normalize the quantities of protein observed. The membranes were TBST-washed before being incubated for an hour with horseradish peroxidase-conjugated secondary antibodies from Novus Biologicals in Littleton, Colorado, USA. An improved chemi-luminescence kit was used to find immuno-labeling (BioRad, Hercules, CA). Finally, utilizing ImageJ to scan the acquired blots and quantify band intensities (NIH, Bethesda, Maryland, USA) [31].

G- Immunohistochemical determination of Bax and NrF-2.

Rehydrate and deparaffinize the tissue portion. Slide should be incubated for ten to fifteen minutes in hydrogen peroxide to decrease endogenous peroxidase's nonspecific background staining. Wash in the buffer twice. Incubate tissue in digesting enzyme if necessary.

To prevent nonspecific background staining, wash four times in buffer, apply block, and then incubate for 5–10 minutes at room temperature. Note: Don't wait longer than 10 minutes otherwise the intended stain may not be as strong. Once in the buffer, wash. Apply the primary antibody and incubate as directed by the manufacturer.

4 times in the buffer, wash. Apply the biotinylated link antibody, then let it sit at room temperature for 15 to 20 minutes. 4 times in the buffer, wash. Apply Streptavidin/HRP, and then let it sit at room temperature for 20 minutes. 4 times in the buffer, rinse. One 5ml vial of DAB Substrate should contain 8 drops of DAB chromogen. Mix thoroughly, then apply to tissue for five minutes. Rinse once in distilled water. Apply the DAB Chromogen/Substrate mixture, and then wait a further five minutes before continuing. Rinse the coverslip, counterstain, and DI water three times using a permanent mounting medium [32].

Statistical analysis:
ANOVA (Analysis of Variance) - One Way Analysis of Variance and Duncan Multiple Range Test (DMRT) were used in the statistical analysis to compare the effects of the various treatment groups on the various variables under investigation. The statistical analysis was made using SPSSPC+ version 28 – Computer program.

Results

A- The effects of sitagliptin or hesperidin treatments the serum concentrations of albumin, creatinine, myeloperoxidase (MPO), Cystatin-C (CYS-C) and glucose (Glu) in cyclosporine A (CsA)-treated rats.

As shown in table (1), CsA significantly elevated serum creatinine, MPO, CYS-C and Glu levels and significantly decreased serum albumin level in CsA-treated rats as respect to the corresponding levels in the healthy control rats.

Using sitagliptin as a pretreatment greatly reduced the rise in serum levels of creatinine (by ~56%), MPO (by ~75%), CYS-C (by ~58%), and Glu (by ~50%) and significantly increase CsA-induced decrease in serum level of albumin (by ~36%) compared to CsA-treated rats.

Hesperidin pretreatment considerably reduced the increase in blood levels of creatinine (by ~23%), MPO (by ~43%), CYS-C (by ~21%), and Glu (by ~24%) and significantly increase CsA-induced decrease in serum level of albumin (by ~12%) compared to rats given CsA.

Furthermore, compared to the control group, neither sitagliptin nor hesperidin significantly changed the levels of the aforementioned biomarkers except for sitagliptin which significantly decrease serum glucose level (by ~18%). Such results explain the reno-protective activities of sitagliptin or hesperidin against CsA-induced damage of kidney cells.

B- Sitagliptin or hesperidin therapy reduced renal oxidative stress and slowed renal lipid peroxidation in rats receiving CsA.

As shown in table (2), CsA significantly elevated renal MDA content and significantly decreased renal CAT and GSH content in CsA-treated rats as compared to their comparable levels in the normal control rats.

Pretreatment with sitagliptin effectively reduced the rise in renal MDA content brought on by CsA (by ~64%) and significantly increase CsA-induced decrease in renal CAT (by ~106%) and GSH (by ~103%) content compared to CsA-treated rats.

Hesperidin pretreatment considerably reduced the rise in renal MDA caused by CsA (by ~17%) and significantly increase CsA-induced decrease in renal CAT (by ~51%) and GSH (by ~51%) content compared to CsA-treated rats.

The improvement in oxidative stress parameters that occur with sitagliptin or hesperidin explain the reno-protective activities of these drugs against kidney injury induced by CsA.
C-determination Of Tnf-α And Western Plot Analysis:

The findings in Table (3) showed that the TNF-α substantially increased in the cyclosporine-treated group compared to the healthy control group. While the levels of TNF-α were dramatically lowered by hesperidin + cyclosporine or sitagliptin + cyclosporine when compared to the CsA-treated group (Fig. 1).

In this work, western blot analysis was used to find TNF-α expression. The expression of TNF-α was significantly higher in the CsA-treated group of rats compared to the control group. Hesperidin + CsA or Sitagliptin + CsA significantly reduced TNF-α expression in comparison to those in the CsA-treated group (Fig. 2).

D- Hesperidin or sitagliptin reduced the kidney tissues' histopathological/inflammatory changes brought on by CsA (Fig. 3).

The current investigation used hematoxylin-eosin (H and E) staining to determine the pathologic changes in the renal tissues removed from rats exposed to the various treatments (Fig. 3A–E). Microscopic examinations of the kidney tissues from control rats that received the vehicle revealed that the renal glomeruli and proximal and distal convoluted tubules had normal histological appearances and that inflammatory cells had penetrated them, as shown in Fig. 3A (score = 0). The glomerular basement membrane was disrupted and infiltrated with mononuclear inflammatory cells in CsA-treated rats (Fig. 3B) (score = 2). In rats treated with sitagliptin alone (Fig. 3C), renal tissues showed glomeruli with normal lining epithelium and renal tubules (score = 0). Prior sitagliptin administration (Fig. 3D) significantly lessened the severity of the pathological changes caused by CsA in the kidneys, and the renal glomeruli and proximal and distal convoluted tubules appeared normal histologically with inflammatory cell infiltration. (score = 1). In rats treated with hesperidin alone (Fig. 3E), microscopic examination of the kidney tissues revealed normal glomerular basement membrane with few RBCs (higher protective effect of hespiridin than sitagliptin) (score = 0). Rats given hesperidin/CsA treatment for their kidneys had better-organized morphological architecture, thicker basement membrane, and less blood vessel congestion (Fig. 3F) (score = 1).

E- Effects of sitagliptin or hesperidin therapy on Nrf-2 and BAX immunoexpression levels in the kidneys of rats receiving CsA.

1- Immunoexpression Levels Of Nrf-2 (Fig. 4)

Immunostaining pictures demonstrating average levels of Nrf-2 immunoexpression in 6 kidney tissue sections from 6 rats in each of the control (A), CsA (B), sitagliptin (C), sitagliptin + CsA (D), hesperidin (E), and hesperidin + CsA (F) groups. Cytoplasm or nuclei that were brownish yellow or dark brown served as indicators of positive staining. (A) Sections of the kidney from the control showed little brown staining. (B) Sections of CsA group showed that renal tubular cells' cytoplasm and nucleus exhibit intense brown staining. (C) Sections of sitagliptin alone, renal tissues showed very little brown staining. (D) Sections of
sitagliptin/CsA group showed very little brown staining. (E) Sections of hesperidin alone revealed faintly stained immune-reactive cells. (F) Sections of hesperidin/CsA group showed that brown staining was reduced in intensity and was only visible in the cytoplasm.

2- Immunoexpression Levels Of Bax (Fig. 5)

Using six kidney tissue sections from six rats in each of the control (A), CsA (B), sitagliptin (C), sitagliptin + CsA (D), hesperidin (E), and hesperidin + CsA (F) groups, immunostaining pictures were created to illustrate the mean immunoexpression levels of BAX. Cytoplasm or nuclei that were brownish yellow or dark brown served as indicators of positive staining. (A) Sections of the kidney from the control showed no cells with immunological reactions. (B) Sections of CsA group showed immune-reactive cells that are widely dispersed and highly stained in the renal tubules. (C) Sections of sitagliptin alone, renal tissues showed no immune-reactive cells. (D) Sections of sitagliptin/CsA group showed no cells with immunological reactions. (E) Sections of hesperidin alone revealed immune-reactive cells that are barely stained. (F) Sections of hesperidin/CsA group showed that the brown staining was reduced in intensity and was only visible in the cytoplasm.

Discussion

The current research demonstrated pronounced sitagliptin or hesperidin ameliorative effects on CsA-induced nephrotoxicity. These effects explained by improving the renal injury and oxidative parameters and decreasing TNF-α level. In addition, sitagliptin or hesperidin counteracted the apoptotic effect of BAX and reduce Nrf-2 activity [33].

Cyclosporine A (CsA) consumption has been linked to nephrotoxicity, which leads to chronic kidney failure after prolonged use, as is the case in patients who have undergone organ transplantation [2, 34]. Therefore, it has become essential to look for new therapeutic approaches that lessen the hazardous effects of CsA.

The increase in serum levels of creatinine, MPO, and CYS-C as well as the decrease in serum albumin levels in the rats given CsA (20 mg/kg/day) were evidence of nephrotoxicity in the current investigation [7, 33]. As demonstrated by the suppression of CsA-mediated elevations in blood levels of creatinine, MPO, and CYS-C as well as the decrease in serum albumin level, our current data demonstrated the renoprotective characteristics of sitagliptin or hesperidin. Sitagliptin improved the renal function, renal hypertrophy and renal macrophage infiltration that reduce the level of creatinine, albumin, cystatin and increase the level of catalase. This results in accordance with Gangadharan et al. [35] where they reported that, Sitagliptin may be utilized as an extra reno-protective medication in diabetic nephropathy because it has been shown to ameliorate renal hypertrophy, renal tissue macrophage infiltration, and histological indicators of tubulointerstitial injury.
In the development of CsA's pathogenesis and nephrotoxicity, oxidative stress is a significant factor [3, 36, 37].

Wu et al. [2] and Vangaveti et al. [5] claimed that lipid peroxidation, ROS production, and GSH depletion are responsible for the advancement of CsA-induced damage in rats' kidneys. In the current study, we found that renal MDA content was significantly increased by CsA administration, while renal CAT and GSH content were significantly decreased when compared to their corresponding levels in normal control rats. These findings are consistent with the findings of the previous studies. Furthermore, our findings demonstrated that sitagliptin or hesperidin cotreatment counteracts the aforementioned effects of CsA as seen by a notable drop in kidney MDA and a corresponding rise in renal CAT and GSH production as compared to their respective levels in the CsA group. Both El-Agamy et al. [38] and Kelleni et al. [39] reported that Sitagliptin, a DPP4 inhibitor, has been linked to beneficial antioxidative, anti-apoptotic, and anti-inflammatory properties against doxorubicin-induced cardiotoxicity in male Wistar rats. According to reports, sitagliptin protected against the cardiomyopathy caused by doxorubicin [40]. Effects of sitagliptin that are protective come in accordance with Sally et al. [41] which demonstrated that by lowering oxidative stress, sitagliptin defends against gentamicin-induced nephrotoxicity in rats. Also, protective consequences of hesperidin come in keeping with Walaa et al. [42] which proved that hesperidin protects against doxorubicin-induced hepatotoxicity by boosting the antioxidant defense system and reducing oxidative stress.

Our results on western plot analysis cleared that, the TNF-α increased in the group treated with cyclosporine that indicated that cyclosporine causes nephrotoxicity. While, in the groups treated with Sitagliptin and the addition of sitagliptine and hesperidine to cyclosporine reduce the level of TNF-α so they can be used successful to reduce and treat the nephrotoxicity side effect of cyclosporine. This results agreed with the outcomes of Aringer et al. [18] and Rahim et al. [43] where they reported that, TNF-α is a crucial immunoregulatory and proinflammatory cytokine having pleiotropic features, including the ability to trigger a series of inflammatory processes that result in tissue damage and the reduction of this effect using proinflammatory drugs causes decrease the incidences of the proinflammatory effect of TNF-α. Also Makdissi et al. [44] reported that sitagliptin has anti-inflammatory properties by inhibition of CD26 expression and Subramanian et al. [45] proved that hesperidin has anti-inflammatory action which indicated by inhibition of TNF-α level in gentamicin-induced nephrotoxicity.

The current immunohistochemical responses' findings have provided additional support for sitagliptin's or hesperidin's ameliorative effects against CsA-induced inflammation provided by inhibition of the Nrf-2 expression. Nephrotoxicity induced by CsA caused increased the expression of Nrf-2, these results compatible with the results obtained by Arab et al. [33] Interestingly, prior to CsA, sitagliptin or hesperidin therapy markedly reduced Nrf-2 signaling. The outcomes line up with the results obtained by Arab et al. [33] which proved that camel milk decrease Nrf-2 expression induced by CsA on kidneys of male albino rats.
Bax is one of the most significant apoptosis indicators and is crucial for the progression of the intrinsic apoptotic pathway [46]. In order to better understand the reno-protective mechanisms of sitagliptin or hesperidin, we investigate the potential involvement of apoptosis modification following treatment. After CsA administration with or without sitagliptin or hesperidin administration, the level of Bax mRNA was measured in the rats’ kidneys, and the results showed that CsA created a proapoptotic mechanism by boosting the renal Bax expression. The preceding administration of sitagliptin or hesperidin dramatically reduced this impact. The results agreed with the findings obtained by Abdelrahman et al. [47] where they reported that, nephrotoxicity of cisplatin was manifested by elevation in creatinine in serum, blood urea nitrogen and Bax. The effects of cisplatin-induced changes in inflammatory, oxidative stress, and apoptotic parameters were reduced by the administration of sitagliptin or hesperidin.

In conclusion, sitagliptin or hesperidin coadministration with CsA led to a considerable preservation of the renal tissue, possibly by control of Nrf-2 signaling, modulation of tissue inflammation, and modulation of apoptosis, as shown by histological analyses.

**Abbreviations**

BAX, Bcl-2-associated X protein
CAT, catalase
CKD, chronic kidney disease
CsA, cyclosporine A
CYS-C, Cystatin-C
DPP-4, dipeptidyl peptidase-4
Glu, glucose
GSH, reduced glutathione
H&E, haematoxylin
eosin
IL-6, interleukin-6
LLC-PK1, lewis-lung cancer porcine kidney 1
MDA, malondialdehyde
MPO, myeloperoxidase
NOX1, activated NADPH oxidase
Nrf-2, nuclear factor erythroid 2-related factor 2
ROS, reactive oxygen species
TNF-α, tumor necrosis factor α.

**Declarations**

Ethics approval and consent to participate
All methods were carried out in accordance with ARRIVE guidelines and in compliance with the National Research Council’s Guide for the Care Use of Laboratory Animals. All experimental protocols were approved by Beni-Suef University (BSU-IACUC), Faculty of Pharmacy research ethics committee. Their permission number is 022-360.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. (mostafa011164@pharm.bsu.edu.eg)

Competing interests:

The authors declare that they have no competing interests.

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Author contributions:

MAA, SMM and BASM designed and conducted the experiments, evaluated the findings, wrote and reviewed the main manuscript text. All authors read and approved the final manuscript.

Acknowledgments:

We appreciate the assistance in this research from our colleague Dr. Ahmed M. Abd-Eldayem, department of pharmacology, faculty of medicine, Assiut University.

References


Tables

Table (1): The effects of sitagliptin or hesperidin treatments the serum concentrations of albumin, creatinine, myeloperoxidase (MPO), Cystatin-C (CYS-C) and glucose (Glu) in cyclosporine A (CsA)-treated
The information gives the averages ± standard deviations for the parameters' concentrations in the serum of the rats from the specified groups (N = 6). ANOVA was used in the statistical analysis, which was followed by the Tukey multiple-comparison test.

\( a \ p < 0.05, \) vs standard control rats.

\( b \ p < 0.05, \) vs rats given CsA.

**Table (2):** Sitagliptin or hesperidin therapy reduced renal oxidative stress and slowed renal lipid peroxidation in rats receiving CsA.
<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA</th>
<th>CAT</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±S.D</td>
<td>Mean±S.D</td>
<td>Mean±S.D</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>0.90±0.07</td>
<td>1.81±0.06</td>
<td>1.82±0.06</td>
</tr>
<tr>
<td>Cyclosporine (20 mg/kg/day)</td>
<td>2.35±0.05</td>
<td>0.88±0.04a</td>
<td>0.87±0.03a</td>
</tr>
<tr>
<td>Sitagliptin (10 mg/kg/day)</td>
<td>0.80±0.04</td>
<td>1.91±0.05</td>
<td>1.89±0.05</td>
</tr>
<tr>
<td>Cyclosporine + Sitagliptin</td>
<td>0.85±0.04b</td>
<td>1.81±0.04b</td>
<td>1.77±0.06b</td>
</tr>
<tr>
<td>Hesperidin (200 mg/kg/day)</td>
<td>1.93±0.06</td>
<td>1.53±0.06</td>
<td>1.51±0.06</td>
</tr>
<tr>
<td>Cyclosporine + Hesperidin</td>
<td>1.96±0.11b</td>
<td>1.33±0.07b</td>
<td>1.31±0.07b</td>
</tr>
</tbody>
</table>

The information gives the averages ± standard deviations for the parameters' concentrations in the serum of the rats from the specified groups (N = 6). ANOVA was used in the statistical analysis, which was followed by the Tukey multiple-comparison test.

a p < 0.05, vs standard control rats.

b p < 0.05, vs rats given CsA.

Table (3): TNF-α among different groups.

<table>
<thead>
<tr>
<th>TNF-α</th>
<th>Area</th>
<th>Percent</th>
<th>Relative Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>1053.783</td>
<td>13.666</td>
<td>1</td>
</tr>
<tr>
<td>Cyclosporine (20 mg/kg/day)</td>
<td>5432.981a</td>
<td>57.678</td>
<td>4.22</td>
</tr>
<tr>
<td>Sitagliptin (10 mg/kg/day)</td>
<td>953.783ab</td>
<td>12.632</td>
<td>0.92</td>
</tr>
<tr>
<td>Cyclosporine + sitagliptin</td>
<td>998.843ab</td>
<td>12.997</td>
<td>0.95</td>
</tr>
<tr>
<td>Hesperidin (200 mg/kg/day)</td>
<td>3778.742ab</td>
<td>40.762</td>
<td>2.98</td>
</tr>
<tr>
<td>cyclosporine + hesperidin</td>
<td>3489.375ab</td>
<td>37.875</td>
<td>2.77</td>
</tr>
</tbody>
</table>
a $p < 0.05$, vs standard control rats.

b $p < 0.05$, vs rats given CsA.

**Figures**

Figure 1

TNF-α among different groups
Western blot analysis for TNF-\(\alpha\) among different groups

Western blot analysis for TNF-\(\alpha\) among different groups Relative to \(\beta\)-actin in kidney tissues of all groups

**Group I**: Rats were only given the vehicle for 14 days. The standard control group was this one.

**Group II**: Rats given a single 20 mg/kg/day i.p. injection of CsA every day for the previous seven days.

**Group III**: Sitagliptin was administered orally to rats once daily for 14 days at a dose of 10 mg/kg.

**Group IV**: Rats received both CsA and sitagliptin treatments as previously indicated. Sitagliptin administered 7 days before and 7 days after CsA that was administrated last 7 days.

**Group V**: For 14 days straight, rats were given a single oral dose of 200 mg/kg/day of hesperidin prepared in distal water by oral gavage.

**Group VI**: Rats received both CsA and hesperidin treatments as previously indicated. CsA was administered 7th days after of hesperidin (that was administrated 7 days before CsA and 7 days after CsA). (N=6 Rats).
Hesperidin or sitagliptin reduced the kidney tissues' histopathological/inflammatory changes brought on by CsA.

Microscopic examinations of the kidney tissues from control rats that received the vehicle revealed that the renal glomeruli and proximal and distal convoluted tubules had normal histological appearances and that inflammatory cells had penetrated them, as shown in Fig. 3A (score = 0). The glomerular basement membrane was disrupted and infiltrated with mononuclear inflammatory cells in CsA-treated rats (Fig. 3B) (score = 2). In rats treated with sitagliptin alone (Fig. 3C), renal tissues showed glomeruli with normal lining epithelium and renal tubules (score = 0). Prior sitagliptin administration (Fig. 3D) significantly lessened the severity of the pathological changes caused by CsA in the kidneys, and the renal glomeruli and proximal and distal convoluted tubules appeared normal histologically with inflammatory cell infiltration. (score = 1). In rats treated with hesperidin alone (Fig. 3E), microscopic examination of the kidney tissues revealed normal glomerular basement membrane with few RBCs (higher protective effect of hespiridin than sitagliptin) (score = 0). Rats given hesperidin/CsA treatment for their kidneys had better-organized morphological architecture, thicker basement membrane, and less blood vessel congestion (Fig. 3F) (score = 1).
Figure 4

Immunooxpression levels of Nrf-2

Immunostaining pictures demonstrating average levels of Nrf-2 immunooxpression in 6 kidney tissue sections from 6 rats in each of the control (A), CsA (B), sitagliptin (C), sitagliptin + CsA (D), hesperidin (E), and hesperidin + CsA (F) groups. Cytoplasm or nuclei that were brownish yellow or dark brown served as indicators of positive staining. (A) Sections of the kidney from the control showed little brown staining (Magnification 100X). (B) Sections of CsA group showed that renal tubular cells’ cytoplasm and nucleus exhibit intense brown staining (Magnification 400X). (C) Sections of sitagliptin alone, renal tissues showed very little brown staining (Magnification 100X). (D) Sections of sitagliptin/CsA group showed very little brown staining (Magnification 100X). (E) Sections of hesperidin alone revealed faintly stained immune-reactive cells (Magnification 400X). (F) Sections of hesperidin/CsA group showed that brown staining was reduced in intensity and was only visible in the cytoplasm (Magnification 400X).
Figure 5

Immunostaining pictures were created to illustrate the mean immunoexpression levels of BAX. Cytoplasm or nuclei that were brownish yellow or dark brown served as indicators of positive staining. (A) Sections of the kidney from the control showed no cells with immunological reactions. (B) Sections of CsA group showed immune-reactive cells that are widely dispersed and highly stained in the renal tubules. (C) Sections of sitagliptin alone, renal tissues showed no immune-reactive cells. (D) Sections of sitagliptin/CsA group showed no cells with immunological reactions. (E) Sections of hesperidin alone revealed immune-reactive cells that are barely stained. (F) Sections of hesperidin/CsA group showed that the brown staining was reduced in intensity and was only visible in the cytoplasm.