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Reno-protective Effect of *Citrus sinensis* by Regulating Antioxidant Capability and Gene Expression in Adenine-Induced Chronic Kidney in Rats

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Abstract: This study was performed to evaluate the reno-protective and antioxidant effects of aqueous Citrus sinensis (orange) peel extract in male Wistar rats administrated adenine. Adenine was administrated to male Wistar rats at a dose of 0.75 percent w/w in feed daily for four weeks. Rats previously fed adenine were treated with orange peel extract at a dose of 40 mg/kg BW/day by oral gavage during the same period of adenine administration. The results indicate that rats fed adenine-with aqueous orange peel extract significantly decreased urea, creatinine, and uric acid concentrations in the blood. The elevated kidney lipid peroxidation product, the lowered glutathione concentration, and the suppressed antioxidant enzyme activities were significantly improved. At the same time, the expressions of heme-oxygenase1 (HO-1), wnt7a, and β-catenin genes were actively regulated. Histological changes in the kidney were represented by congestion, hypertrophied glomerulus, vacuolization of the endothelial cells lining the glomerular tuft, and interstitial nephritis in adenine-administrated rats also remarkably improved. In conclusion, administering orange peel aqueous extract with adenine reduces the signs of adenine toxicity on renal function by enhancing the antioxidant defense system.

Keywords: chronic kidney disease (CKD); adenine; *Citrus sinensis* (orange) peel; oxidative stress; HO-1; Wnt7a.

1. Introduction

A deficiency in the function and structure of the kidneys is referred to as renal disease. High rates of kidney disease represent a considerable burden on healthcare systems, and they may advance to end-stage renal disease (ESRD), in which case transplantation or dialysis will be the only treatment choice, both of which are too expensive or unavailable in developing countries [1].

Many problems associated with renal disease are linked to oxidative stress [2]. Oxidative stress boosts endogenous antioxidants and cell-protective proteins, which limit or prevent tissue damage and dysfunction under normal circumstances. In various human and animal models of kidney disease, the heme oxygenase-1 (HO-1) system plays an important role in controlling oxidative stress and is protective [3]. Additionally, the Wnt/β-catenin pathway has important functions in nephrogenesis [4]. Also, Lin *et al*. [5] demonstrated that Wnt and β-catenin genes could be pivotal in promoting tubular repair and regeneration after kidney injury induced by either ischemia-reperfusion injury or nephrotoxins. Wnt signals were expressed at low levels in adult kidneys under normal conditions; therefore, they are reactivated in kidney disorders [6].

Traditional therapies only focus on the early repression of the principal source of kidney injury to maintain or postpone chronic renal failure [7]. The developments of several new renal protective treatments, with a particular focus on natural products with antioxidant activity and safety profiles, are urgently needed to either delay or reverse the decline in kidney function [8]. According to Newman and Cragg [9], herbs are used in 46% of the FDA. Several investigators reported that flavonoids are naturally occurring substances with various therapeutic and pharmacological activities [10–12]. Orange peel extracts are rich in flavonoids such as polymethoxylated flavones (PMF), C- or O-glycosylated flavones, O-glycosylated flavanones, flavonols, and a variety of other phenolic acids and derivatives, and exhibit potent free radical scavenging efficacy [13]. In this regard, several studies have demonstrated that orange fruit peel has various pharmacological benefits, including antioxidant, anti-inflammatory, and anti-diabetic [14]. As a result, the current study was planned to evaluate the effect of orange peel aqueous extract on the biochemical, molecular, and histopathological changes related to chronic kidney disease induced by adenine in a rat model.

2. Materials and Methods

2.1. Chemicals and plant materials.

Adenine was purchased from Alfa-Aeser Co. *Citrus sinensis* (orange) fruit was obtained from a local market in Mansoura City, Egypt. All other chemicals and reagents used in this investigation were of high analytical grade.

2.2. Experimental animals.

Twenty-four Wistar rats weighing 100–120 g were obtained from Theodore Bilharz Research Institute, Giza, Egypt. They were kept for 7 days for acclimatization having free admission to a feed composed of standard feeding. The experimental protocol was approved by University Animal Ethical Committee (code no. ph-ch-2020-9).

2.3. Preparation of the aqueous Citrus sinensis peel extract.

The aqueous extract was prepared according to the method of [16]. 350 g of fresh orange peel was extracted with 1.5 L of distilled water at 100 ℃ for 15 min, and the mixture was filtrated. 1.9 L of water was added to the filter and boiled for a further 20 min. The soluble aqueous extract was filtered and concentrated to dryness. The percentage yield was 2.77 g.

2.4. Free radical-scavenging activity on DPPH.

Free radical scavenging capacity of aqueous extract of orange peel extract was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity as described by Jo *et al*., [17]**.** Briefly, various concentrations of orange peel aqueous extract (1.0, 2.5, 5.0 µg/mL) were mixed with 1 mL of 0.3 mM methanol solution of DPPH and incubated at 27°C for 30 min. The absorption, A, of the sample was measured at 518 nm. DPPH radical-scavenging activity, expressed as a percentage, was assessed utilizing the following equation:

DPPH radical scavenging activity (%) =

$$= [1- (ABS_{sample}—ABS_{sblank})/ (ABS_{control}- ABS_{cblank})] \times 100$$

In the same concentration as the aqueous test extract, ascorbic acid was used as the standard material. All analyses were carried out in triplicate.

2.5. LD_{50} of aqueous extract of orange peel.

The animals were divided into 7 groups of four animals. Group 1: orange peel aqueous extract (100 mg/kg) once /oral, group 2: orange peel aqueous extract (200 mg/kg) once/ oral, group 3: orange peel aqueous extract (300 mg/kg) / oral, group 4: orange peel aqueous extract (400 mg/kg) once/ oral, group 5: orange peel aqueous extract (500 mg/kg) once/oral, group 6: orange peel aqueous extract (600 mg/kg) once/ oral and Group 7: orange peel extract (700 mg/kg) once/oral. All of the animals were kept under constant observation for 24 hours after the dose was administered, looking for any changes in behavior or physical activities and the mortality rate in each. The animals were counted at the end of the experiment. The Karber arithmetic method was used to calculate the LD_{50} [18]**.**

2.6. Experimental design.

Following a seven-day acclimation period, rats were randomly divided into four groups, six of each. Group 1 (Normal control group) was fed on a standard diet without any supplementation. Group 2 (aqueous orange peel extract): rats were given aqueous orange peel extract orally by gavage for four weeks at a 40 mg/kg/day dose. Group 3 (adenine group): rats were fed a diet containing adenine at a dose of 0.75% w/w/day for four weeks. Group 4 (adenine treated group): rats were given adenine at a dose in the third group and orange peel aqueous extract at a dose in the second group for 28 days. After 24 hours of the last dose, the rats were fasted overnight, anesthetized with halothane, and sacrificed. Blood samples were collected, and sera were separated by centrifugation for 10 minutes at 3000 g and kept at -20ºC. The kidney was immediately dissected, washed with ice-cold saline solution, blotted dry, and stored at -20 ℃ for the assay of biochemical parameters and histopathological examination.

2.7.Biochemical parameters.

Creatinine, Urea, Uric acid, Albumin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Superoxide dismutase (SOD) activity, Glutathione –S- transferase (GST) activity, Total antioxidant capacity, and Malondialdehyde (MDA) concentration were determined in serum and kidney using Biodiagnostic assay kits, Egypt.

2.8.Real-time Polymerase Chain Reaction.

RNAs were extracted from kidney samples using GeneJET RNA Purification Kit (Thermo Scientific). For RT-PCR, The HiSenScript ™ RH (-) cDNA synthesis Kit was used to convert RNAs to cDNA. qRT-PCR products were detected with SYBR Green (SensiFAST SYBR Hi-ROX Kit) with 20 µl reactions. GAPDH gene was included as an internal control. qPCR results were analyzed by the method of $2^{-\Delta\Delta C_T}$ [19]**.** PCR primers of HO-1, Wnt7a, and β-catenin genes were listed in Table (1).

Table 1. The primer arrangements for the target genes.

Gene	Primer sequence
Wnt7a	**F**:5-GCCCACCTTTCTGAAGATCAAG-3 **R**:5-TGGGTCCTCTTCACAGTAATTGG-3
β-catenin	**F**:5-TGAAGGTGCTGTCTGTCTGCTC-3 **R**:5-TGCATCGGACCAGTTTCTCAGA-3
HO-1	**F**: 5′TGCTTGTTTCGCTCTATCTCC-3 **R**: 5′CTTTCAGAAGGGTCAGGTGTC-3′
GAPDH	**F**: 5′-TATCGGACGCCTGGTTAC-3′, **R**: 5′-CTGTGCCGTTGAACTTGC-3′

2.9. Histopathological examination.

Kidney and liver tissues were fixed, embedded in paraffin wax, then 5- µm thick sections were prepared and stained with Hematoxylin and Eosin (H & E) stains for microscopic examination.

2.10. Statistical analysis.

All data were presented as mean S.E.M., and the statistical analysis was performed using SPSS version 14 (SPSS, Chicago, IL, USA). The statistical significance of the parameters in groups with and without a significant value was investigated using the one-way analysis of alteration (ANOVA) test. p-value < 0.05.

3. Results

The *in vitro* antioxidant potential of orange peel aqueous extract was determined with a DPPH assay (Table 2). DPPH radical increased significantly in a dose-dependent manner. The antioxidant abilities were established to be the highest inhibition value at nearly 65.2% for methanolic extract at a concentration of 5 mg/ml.

Table 2. DPPH radical scavenging activity of orange peel aqueous extract.

Sample concentration (mg/kg BW)	DPPH antioxidant activity (%)
1.0	0
2.5	3.04 ± 0.02
5.0	7.79 ± 0.004
10.0	12.46 ± 0.006
vitamin C	82.14±1.85

All data were represented as the mean ± SEM.

To assess the efficacy of aqueous extract of orange peel treatment in the KD induced by adenine, the lethal dose that kills 50% of mice (LD_{50}) was calculated. The data shows that the LD_{50} of oral supplementation of orange peel aqueous extract was 400 mg/kg body weight.

The serum creatinine, urea, and uric acid concentrations in control and treated rat groups are shown in Figure 1. In the adenine rat groups, serum creatinine, urea, and uric acid

concentrations were significantly ($p<0.05$) than in the control group. There was a significant decrease in serum creatinine, urea, and uric acid concentrations when comparing the adenine-treated rat groups to the adenine rat group. In addition, as compared to the control group, administration of an aqueous extract of orange peel did not result in a significant change ($p>0.05$) in serum creatinine, urea, and uric acid concentrations.

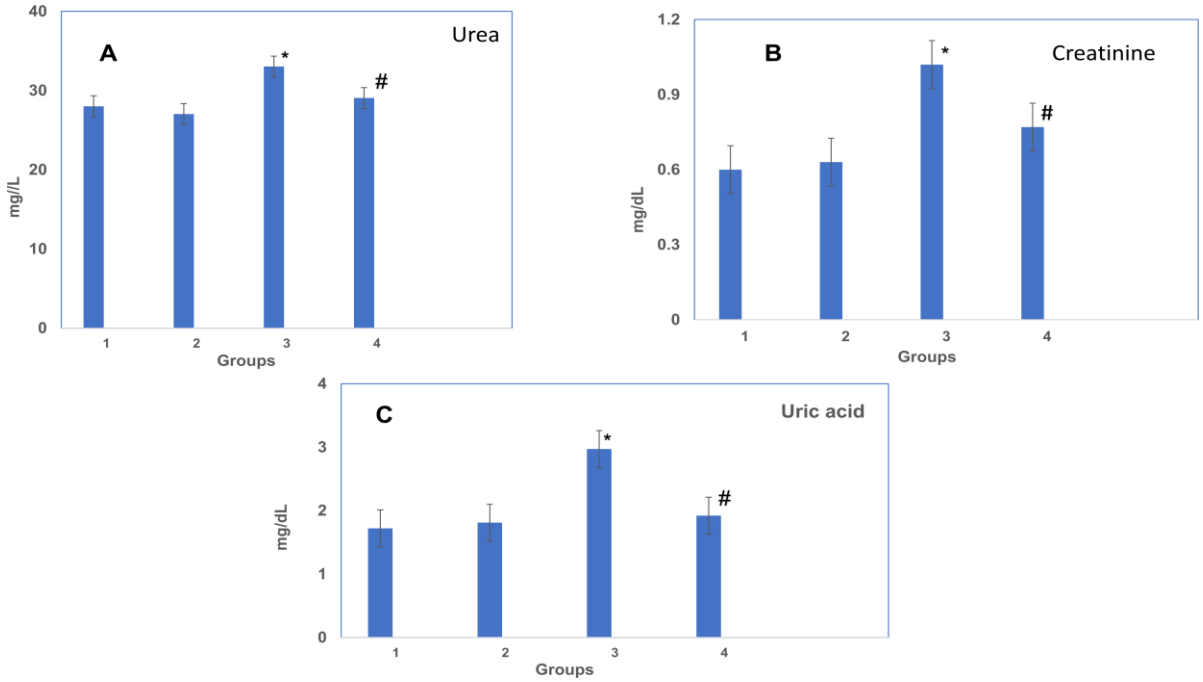

Figure 1. Concentrations of (**A**) serum urea, (**B**) creatinine, and (**C**) uric acid in control and treated rat groups. Each chart showed mean ± SEM (n=6). The differences among all groups have been assessed by one-way ANOVA with ($p < 0.05$) considered significant, (*) significant when compared to the control group, (#) significantly when compared to the adenine treated group.

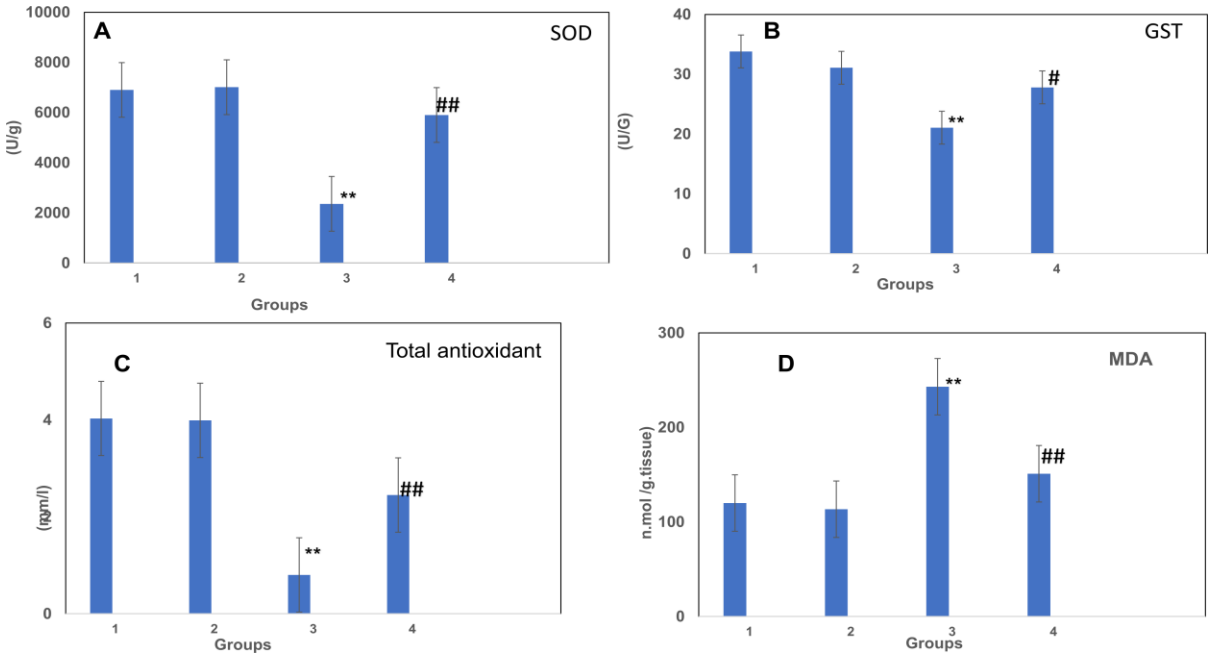

Figure (2). (**A**) Superoxide dismutase (SOD), (**B**) Glutathione –S –transferase (GST), (**C**) Total antioxidant capacity, and (**D**) Lipid peroxidation product (MDA) in the kidney of different groups of rats. Each chart showed mean ± SEM (n=6). The differences among all groups have been assessed by one-way ANOVA with ($P < 0.05$) considered significant, (*) significant when compared to the control group, (#) significant when compared to adenine treated group.

As shown in Figure 2, in comparison to the control group, the adenine group showed a significant increase ($p<0.05$) in MDA concentration in kidneys, which was correlated with a significant ($p<0.05$) decrease in SOD, GST, and total antioxidant capacity. In comparison to the adenine group, administration of orange peel aqueous extract resulted in a significant ($P > 0.05$) decrease in MDA concentration in the kidneys, as well as an increase in SOD and GST activity and a significant ($p<0.05$) increase in total antioxidant concentration. There was no significant ($p>0.05$) difference in MDA concentration, total antioxidant capacity, SOD, and GST activity between the orange peel group rats and the control rats.

Adenine decreased HO-1 renal mRNA expression significantly ($p<0.05$) in the adenine group compared to the normal control group. At the same time, there was a significant ($p<0.05$) increase in their expression related to the adenine group after administration of orange peel aqueous extract (Figure 3).

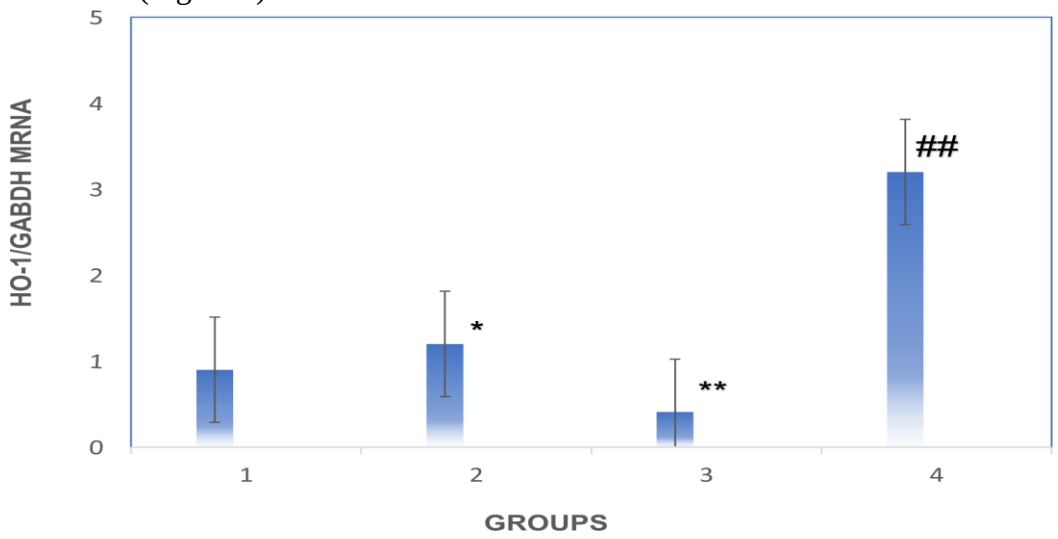

Figure (3): HO-1 gene in kidneys of different groups of rats. Each chart showed mean ± SEM (n=6). The differences among all groups have been assessed by one-way ANOVA with ($P < 0.05$) considered significant, (*) significant when compared to the control group, (#) significantly when compared to the adenine treated group.

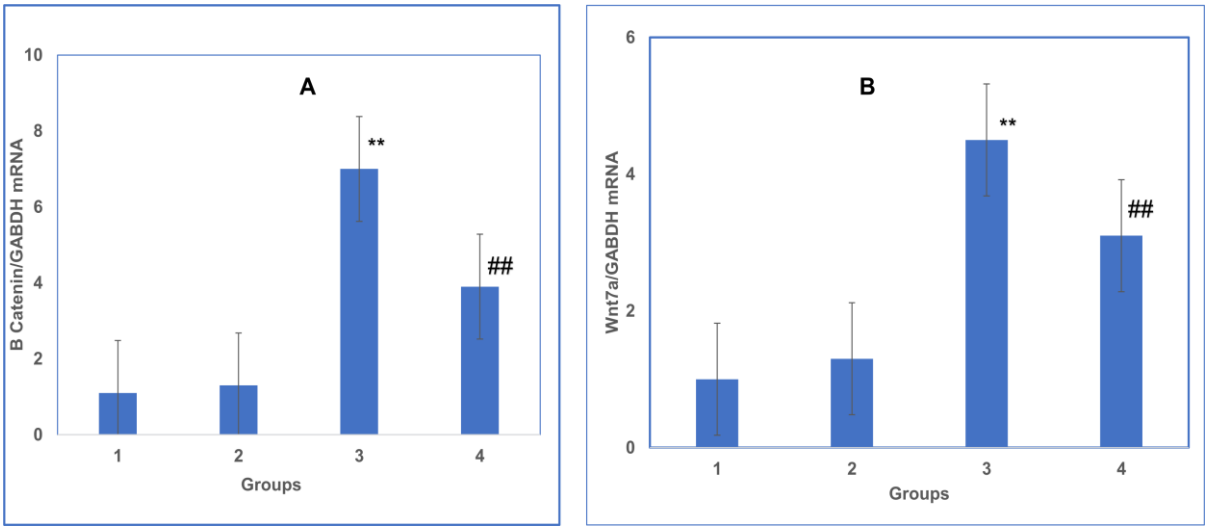

Figure 4. (**A**): β-catenin, and (**B**) Wnt7a genes in kidneys of different groups of rats. Each chart showed mean ± SEM (n=6). The differences among all groups have been assessed by one-way ANOVA with ($P < 0.05$) is considered significant, (*) significant when compared to the control group, (#) significant when compared to adenine treated group

The administration of rats with adenine resulted in a highly significant increase in the expression of Wnt7a and β-catenin genes in the kidneys of rats from the adenine group compared to the control group ($P< 0.001$). In comparison, treatment with orange peel aqueous extract showed a significant ($p<0.05$) decrease in the expression of kidney Wnt7a and β-catenin genes in the adenine-treated group (Figure 4 A-B).

Histological examinations of the control, aqueous extract of orange peel, adenine group, and adenine-treated groups kidney and liver (A, B, C, and D, respectively) revealed normal hepatocytes with dominant situated nuclei, normal sinusoids, portal areas, and hepatic cords radially stetted around the central vein in the control group receiving (40 mg/kg/ day) dose of orange peel aqueous extract. The liver sections of the adenine group exhibited degeneration in hepatocytes, multifocal areas of necrosis, fibrosis, and congested blood vessels. The liver sections of the adenine group that were treated with orange peel aqueous extract (40 mg/kg) showed slight enhancement in a histological graph, with only minor hydropic deterioration in hepatocytes (Figure 5A). The kidney sections of group 1 showed normal architecture of glomeruli and tubules. However, adenine administration to rats resulted in severe lesions, including glomerular atrophy, glomerular capsule expansion, interstitial congestion, clear necrosis in epithelial cells within renal tubules, and pyknotic nuclei. Alternatively, except for a slight widening in the renal space of the glomerular capsules, marked recovery of most of the renal architecture and even histology is observed in the adenine-treated with orange peel aqueous extract group (Figure 5 B).

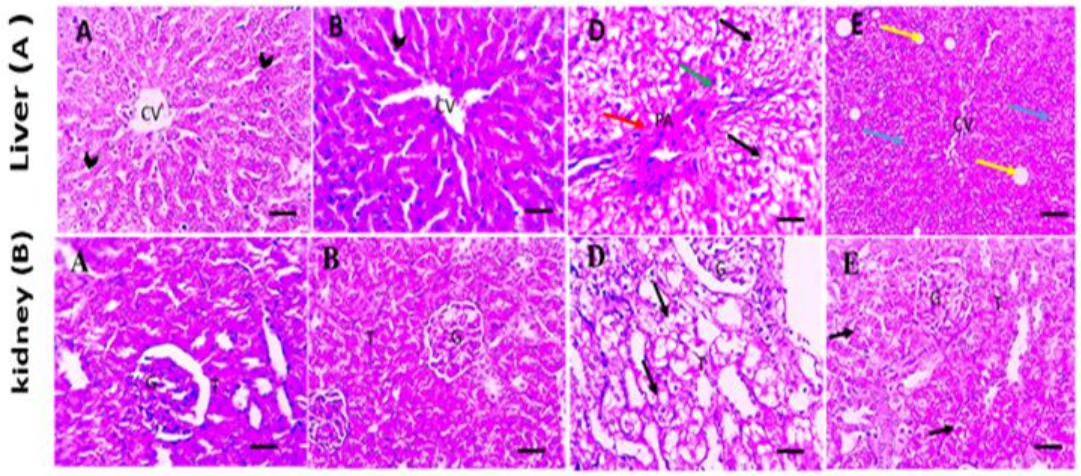

Figure 5. Photomicrograph of (**A**) liver sections and (**B**) kidney sections of studied groups stained with H&E showing: normal radiating hepatic cords around normal central veins (CV) with normal sinusoids (black arrowheads) in the control group 1 (A) and the group received orange peel aqueous extract 2 (B), Adenine group 3 (D) showing diffuse macrovesicular steatosis (yellow arrows) and hydropic degeneration (black arrows) in hepatocytes, occluded sinusoids and markedly congested central veins (CV) (red arrows), severe portal fibrosis (green arrow and adenine group treated with orange peel aqueous extract 4 (E) showing moderate intermingled micro-vesicular steatosis (blue arrows) with few macrovesicular fat vacuoles (yellow arrows) in hepatocytes and occluded sinusoids. Markedly congested central veins (CV) (red arrows), mild portal fibrosis (blue arrows). Kidney sections (B) show normal glomeruli (G), tubules (T), and interstitial tissue in the control group 1 (A), and the group received orange peel aqueous extract 2 (B); Kidney sections from the adenine group 3 (D) group showed marked tubular hydropic degeneration (black arrows) and perivascular inflammation (violet arrow), Kidney sections from the adenine- treated group 4 (E) showing mild tubular degeneration and congested blood vessels (black arrows). Low magnification X: 100 bar 100 and high magnification X:400 bar 50.

4. Discussion

The kidneys are essential in removing toxins, metabolic products, and other foreign substances from the body [19]. Renal fibrosis is closely related to the synthesis of mediators and signaling pathways, such as dysregulated uremic toxins, oxidative stress, and Wnt/β-catenin [4,21,22]. This study investigated the effect of aqueous orange peel extract on adenine-induced chronic kidney disease. The current results demonstrated that orange peel aqueous extract has antioxidant activity consistent with the findings of Rao *et al*. [23]. The present study showed that rats receiving adenine had higher creatinine, urea, and uric acid levels. These results were in accordance with Diwan *et al*. [24] and may be attributed to adenine metabolites accumulating in renal tubules, resulting in interstitial inflammation, tubular injury, and fibrosis-inducing CKD [25]. These renal histological abnormalities produced changes in the glomerular and renal tubules, leading to reduced renal function that may increase these findings [26]. The administration of aqueous orange peel extract to the adenine group resulted in significantly lower blood concentrations of creatinine, urea, and uric acid, indicating an improved renal excretion of these compounds. These results were obtained by Ahmed *et al*. [27] and may be attributed to orange peel extract, which inhibits hepatic xanthine oxidase and xanthine dehydrogenase activity and lowers serum uric acid levels, and/or antioxidative and antihyperuricemic properties of flavanone constituent in the extract [28].

Adenine induced oxidative stress and depleted kidney antioxidant levels [29]. The obtained results showed that lipid peroxidation was significantly raised in the kidney of rats treated with adenine, which was linked to an increase in MDA levels as well as a decrease in tissue SOD, GST, and total antioxidant in rats that had only been administered adenine as compared to healthy control rats. These results are consistent with Nemmar *et al*. [30]. Meanwhile, the administration of aqueous orange peel extract resulted in a significant increase in antioxidant markers, as evidenced by a decrease in MDA and an increase in antioxidant agents in kidney tissue compared to the adenine-treated group [31].

Under normal circumstances, oxidative stress generates endogenic antioxidants and cytoprotective proteins, which limit or prevent tissue damage and malfunction. Because of the toxic effects of heme deficiency on renal tissues and its pro-inflammatory activity, as well as the inflammation caused by renal failure pathology, HO-1 has physiological roles in cytoprotection, inflammatory response modulation, and antioxidative functions [32]. The enzyme HO-1 is involved in heme breakdown, producing carbon monoxide (CO), ferrous iron (Fe++), and biliverdin (BV), which is promptly converted to bilirubin (BR) by biliverdin reductase [33]. Our findings revealed that adenine-treated rats significantly decreased HO-1 expression, indicating oxidative stress caused by adenine. The phenolics-related bioactive compounds in orange peel aqueous extract (40 mg/kg/day) boosted the expression of the HO-1 gene in the rats' kidneys, resulting in the maintenance of the normal redox status of cells, according to [34,35].

Wnt 7a and β-catenin signaling have been linked to the development of kidney disease [21]. The current data show that renal adenine rats had higher Wnt7a expression, similar to Qin *et al*.'s findings [36]. Inhibition of canonical Wnt 7a and β-catenin signaling by orange peel extract lowers kidney disease progression, consistent with increased blood uremic toxins, oxidative stress, and pathological alterations in the kidney. The existence of pentacyclic triterpenoids as antagonists could explain these findings [37].

Histological examinations of kidney sections showed that rats in the control group showed normal histoarchitecture, glomerulus, proximal convoluted tubules, distal convoluted tubules, and Bowman's space. In contrast, rats treated with adenine alone had several histopathological changes, including deterioration in hepatocytes, multifocal areas of necrosis, fibrosis, congested blood vessels, and enlargement of adenine deposit. The treatment of adenine rats with orange peel aqueous extract (40 mg/kg body weight) alleviated these alterations. Our results are consistent with those of Nasution *et al*. [38]**.** Finally, adenine-induced renal dysfunction revealed that adenine crystals accumulation in the kidney might cause renal cell inflammation, renal tubular regeneration, and renal failure. The reno-protective effects of orange peel aqueous extract may be due to phytoconstituents such as polyphenolic compounds, particularly the characteristic flavanone glycosides hesperidin, neohesperidin, naringin, rutin, and narirutin [14,15]**.**

5. Conclusion

The *Citrus sinensis* (orange) peel aqueous extract has brought potential renal protective effects against adenine-induced chronic kidney disease in Wistar rats which were proved by improvement of the kidney and liver functions and histological integrity. Extracts' antioxidant and anti-inflammatory mechanisms were attributed to suppressing renal oxidative damage and inflammatory gene expressions, thereby reducing renal myopathy and detrimental structural alterations. Of note, the reno-protective role of the orange peel aqueous extract is not only due to their antioxidant activity but also their up-regulation effect on HO-1, wnt 7a, and β-catenin signaling, an important regulator of the antioxidant response. Accordingly, the orange peel aqueous extract can be recommended as an adjunct to prevent the progression of chronic kidney diseases.

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Conflicts of Interest

The authors declare that they have no competing interests.

References

1. Yan, M.T.; Chao, C.T.; Lin, S.H. Chronic Kidney Disease: Strategies to Retard Progression. *Int J Mol Sci* **2021**, *22*, 10084, https://doi.org/10.3390/ijms221810084.
2. Ebert, T.; Neytchev, O.; Witasp, A.; Kublickiene, K.; Stenvinkel, P.; Shiels, P.G. Inflammation and Oxidative Stress in Chronic Kidney Disease and Dialysis Patients. *Antioxid Redox Signal* **2021**, *35*, 1426–1448, https://doi.org/10.1089/ars.2020.8184.
3. Lever, J.M.; Boddu, R.; George, J.F.; Agarwal, A. Heme Oxygenase-1 in Kidney Health and Disease. *Antioxid Redox Signal* **2016**, *25*, 165–183, https://doi.org/10.1089/ars.2016.6659.

4. Li, S. S.; Sun, Q.; Hua, M.R.; Suo, P.; Chen, J.R.; Yu, X.Y.; Zhao, Y.Y. Targeting the Wnt/β-Catenin Signaling Pathway as a Potential Therapeutic Strategy in Renal Tubulointerstitial Fibrosis. *Front Pharmacol* **2021**, *12*, 719880, https://doi.org/10.3389/fphar.2021.719880.
5. Lin, S.L.; Li, B.; Rao, S.; Yeo, E.J.; Hudson, T.E.; Nowlin, B.T.; Pei, H.; Chen, L.; Zheng, J.J.; Carroll, T.J.; *et al*. Macrophage Wnt7b Is Critical for Kidney Repair and Regeneration. *Proc Natl Acad Sci U S A* **2010**, *107*, 4194–4199, https://doi.org/10.1073/pnas.0912228107.
6. Surendran, K.; Schiavi, S.; Hruska, K.A. Wnt-Dependent Beta-Catenin Signaling Is Activated after Unilateral Ureteral Obstruction, and Recombinant Secreted Frizzled-Related Protein 4 Alters the Progression of Renal Fibrosis. *J Am Soc Nephrol* **2005**, *16*, 2373–2384, https://doi.org/10.1681/ASN.2004110949.
7. Wouters, O.J.; O'Donoghue, D.J.; Ritchie, J.; Kanavos, P.G.; Narva, A.S. Early Chronic Kidney Disease: Diagnosis, Management and Models of Care. *Nat Rev Nephrol* **2015**, *11*, 491–502, https://doi.org/10.1038/nrneph.2015.85.
8. Xu, H.; Wu, T.; Huang, L. Therapeutic and Delivery Strategies of Phytoconstituents for Renal Fibrosis. *Adv Drug Deliv Rev* **2021**, *177*, 113911, https://doi.org/10.1016/j.addr.2021.113911.
9. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs from 1981 to 2014. *J Nat Prod* **2016**, *79*, 629–661, https://doi.org/10.1021/acs.jnatprod.5b01055.
10. Chen, X.-M.; Kitts, D.D. Flavonoid Composition of Orange Peel Extract Ameliorates Alcohol-Induced Tight Junction Dysfunction in Caco-2 Monolayer. *Food Chem Toxicol* **2017**, *105*, 398–406, https://doi.org/10.1016/j.fct.2017.04.009.
11. Guerra, A.R.; Duarte, M.F.; Duarte, I.F. Targeting Tumor Metabolism with Plant-Derived Natural Products: Emerging Trends in Cancer Therapy. *J Agric Food Chem* **2018**, *66*, 10663–10685, https://doi.org/10.1021/acs.jafc.8b04104.
12. Ayalew, H.; Tewelde, E.; Abebe, B.; Alebachew, Y.; Tadesse, S. Endemic Medicinal Plants of Ethiopia: Ethnomedicinal Uses, Biological Activities and Chemical Constituents. *J Ethnopharmacol* **2022**, *293*, 115307, https://doi.org/10.1016/j.jep.2022.115307.
13. Razola-Díaz, M.D.C.; Guerra-Hernández, E.J.; García-Villanova, B.; Verardo, V. Recent Developments in Extraction and Encapsulation Techniques of Orange Essential Oil. *Food Chem* **2021**, *354*, 129575, https://doi.org/10.1016/j.foodchem.2021.129575.
14. Bureš, M.S.; Maslov Bandić, L.; Vlahoviček-Kahlina, K. Determination of Bioactive Components in Mandarin Fruits: A Review. *Crit Rev Anal Chem* **2022**, 1–26.
15. Mostafa, M.; Amer, N.; Serag, M.; Khedr, A.-H.; Abdel-Mogib, M. Phytochemical Constituents and Antibacterial Activity of the Medicinal Herb Deverra Tortuosa (Desf.) DC. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2020**, *11*, 108–115, https://doi.org/10.33887/rjpbcs/2020.11.2.13.
16. Jo, Y.-J.; Cho, H.-S.; Chun, J.-Y. Antioxidant Activity of β-Cyclodextrin Inclusion Complexes Containing Trans-Cinnamaldehyde by DPPH, ABTS and FRAP. *Food Sci Biotechnol* **2021**, *30*, 807–814, https://doi.org/10.1007/s10068-021-00914-y.
17. Kyhoiesh, H.A.K.; Al-Hussainawy, M.K.; Waheeb, A.S.; Al-Adilee, K.J. Synthesis, Spectral Characterization, Lethal Dose (LD50) and Acute Toxicity Studies of 1,4-Bis(Imidazolylazo)Benzene (BIAB). *Heliyon* **2021**, *7*, e07969, https://doi.org/10.1016/j.heliyon.2021.e07969.
18. Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real- Time Quantitative PCR and the 2(-Delta Delta C(T)) Method. **2001**, *408*, 402–408, https://doi.org/10.1006/meth.2001.1262.
19. Kalantar-Zadeh, K.; Jafar, T.H.; Nitsch, D.; Neuen, B.L.; Perkovic, V. Chronic Kidney Disease. *Lancet* **2021**, *398*, 786–802, https://doi.org/10.1016/S0140-6736(21)00519-5.
20. Malik, S.A.; Modarage, K.; Goggolidou, P. The Role of Wnt Signalling in Chronic Kidney Disease (CKD). *Genes (Basel)* **2020**, *11*, E496, https://doi.org/10.3390/genes11050496.
21. Zhang, Y.; Jin, D.; Kang, X.; Zhou, R.; Sun, Y.; Lian, F.; Tong, X. Signaling Pathways Involved in Diabetic Renal Fibrosis. *Front Cell Dev Biol* **2021**, *9*, 696542, https://doi.org/10.3389/fcell.2021.696542.
22. Rao, M.J.; Wu, S.; Duan, M.; Wang, L. Antioxidant Metabolites in Primitive, Wild, and Cultivated Citrus and Their Role in Stress Tolerance. *Molecules* **2021**, *26*, 5801, https://doi.org/10.3390/molecules26195801.
23. Diwan, V.; Brown, L.; Gobe, G.C. Adenine-Induced Chronic Kidney Disease in Rats. *Nephrology (Carlton)* **2018**, *23*, 5–11, https://doi.org/10.1111/nep.13180.
24. Boon, A.-C.; Lam, A.K.; Gopalan, V.; Benzie, I.F.; Briskey, D.; Coombes, J.S.; Fassett, R.G.; Bulmer, A.C. Endogenously Elevated Bilirubin Modulates Kidney Function and Protects from Circulating Oxidative Stress

in a Rat Model of Adenine-Induced Kidney Failure. *Sci Rep* **2015**, *5*, 15482, https://doi.org/10.1038/srep15482.
25. Johnson, R.J.; Nakagawa, T.; Jalal, D.; Sánchez-Lozada, L.G.; Kang, D.-H.; Ritz, E. Uric Acid and Chronic Kidney Disease: Which Is Chasing Which? *Nephrol Dial Transplant* **2013**, *28*, 2221–2228, https://doi.org/10.1093/ndt/gft029.
26. Ahmed, O.; Fahim, H.; Ahmed, H.; Morsy, B.; Aljohani, S.; Abdelazeem, W. The Nephropreventive and Antioxidant Effects of Navel Orange Peel Hydroethanolic Extract, Naringin and Naringenin in N-Acetyl-P- https://doi.org/10.17582/journal.aavs/2019/7.2.96.105.
27. Haidari, F.; Ali Keshavarz, S.; Reza Rashidi, M.; Mohammad Shahi, M. Orange Juice and Hesperetin Supplementation to Hyperuricemic Rats Alter Oxidative Stress Markers and Xanthine Oxidoreductase Activity. *J Clin Biochem Nutr* **2009**, *45*, 285–291, https://doi.org/10.3164/jcbn.09-15.
28. Inami, Y.; Hamada, C.; Seto, T.; Hotta, Y.; Aruga, S.; Inuma, J.; Azuma, K.; Io, H.; Kaneko, K.; Watada, H.; *et al*. Effect of AST-120 on Endothelial Dysfunction in Adenine-Induced Uremic Rats. *Int J Nephrol* **2014**, *2014*, 164125, https://doi.org/10.1155/2014/164125.
29. Nemmar, A.; Al-Salam, S.; Beegam, S.; Zaaba, N.E.; Yasin, J.; Hamadi, N.; Ali, B.H. Cardiac Inflammation, Oxidative Stress, Nrf2 Expression, and Coagulation Events in Mice with Experimental Chronic Kidney Disease. *Oxid Med Cell Longev* **2021**, *2021*, 8845607, https://doi.org/10.1155/2021/8845607.
30. Okokon, J.E.; Simeon, J.O.; Umoh, E.E. Hepatoprotective Activity of the Extract of Homalium Letestui Stem against Paracetamol-Induced Liver Injury. *Avicenna J Phytomed* **2017**, *7*, 27–36, https://doi.org/10.22038/AJP.2016.6950.
31. Campbell, N.K.; Fitzgerald, H.K.; Dunne, A. Regulation of Inflammation by the Antioxidant Haem Oxygenase 1. *Nat Rev Immunol* **2021**, *21*, 411–425, https://doi.org/10.1038/s41577-020-00491-x.
32. Amata, E.; Pittalà, V.; Marrazzo, A.; Parenti, C.; Prezzavento, O.; Arena, E.; Nabavi, S.M.; Salerno, L. Role of the Nrf2/HO-1 Axis in Bronchopulmonary Dysplasia and Hyperoxic Lung Injuries. *Clin Sci (Lond)* **2017**, *131*, 1701–1712, https://doi.org/10.1042/CS20170157.
33. Li, G.-J.; Wang, J.; Cheng, Y.-J.; Tan, X.; Zhai, Y.-L.; Wang, Q.; Gao, F.-J.; Liu, G.-L.; Zhao, X.; Wang, H. Prophylactic Effects of Polymethoxyflavone-Rich Orange Peel Oil on Nω-Nitro-L-Arginine-Induced Hypertensive Rats. *Applied Sciences* **2018**, *8*, 752, https://doi.org/10.3390/app8050752.
34. Grunenwald, A.; Roumenina, L.T.; Frimat, M. Heme Oxygenase 1: A Defensive Mediator in Kidney Diseases. *Int J Mol Sci* **2021**, *22*, 2009, https://doi.org/10.3390/ijms22042009.
35. Qin, S.; Taglienti, M.; Cai, L.; Zhou, J.; Kreidberg, J.A. C-Met and NF-KB-Dependent Overexpression of Wnt7a and -7b and Pax2 Promotes Cystogenesis in Polycystic Kidney Disease. *J Am Soc Nephrol* **2012**, *23*, 1309–1318, https://doi.org/10.1681/ASN.2011030277.
36. Szakiel, A.; Pączkowski, C.; Pensec, F.; Bertsch, C. Fruit Cuticular Waxes as a Source of Biologically Active Triterpenoids. *Phytochem Rev* **2012**, *11*, 263–284, https://doi.org/10.1007/s11101-012-9241-9.
37. Nasution, M.; Ginting, C.N.; Fachrial, E.; Lister, I.N.E. Potency of Sunkist Orange (Citrus SinensisL. Osbeck) against Kidney Histology of White Wistar Rats Induced by Gentamicin. *Majalah Obat Tradisional* **2020**, *25*, 42–48, https://doi.org/10.22146/mot.49107.