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Carvedilol and rosuvastatin mitigate nephrotoxicity of sodium valproate through activation of Nrf2 pathway in rats.

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Abstract

Sodium valproate, one of the most widely used antiepileptic drugs, has been noted to induce nephrotoxicity through elevation of oxidative stress. Carvedilol, a non-specific beta-adrenergic blocker, and rosuvastatin, an anti-hyperlipidemic, possess antioxidant characteristics. This study was planned to estimate the promising defensive impacts of Carvedilol and/or rosuvastatin against valproate-induced nephrotoxicity. It was revealed that sodium valproate (SVP) markedly boosted serum creatinine (SCr) and blood urea nitrogen (BUN). Moreover, glutathione level (GSH) was decreased with a concomitant elevation in renal malondialdehyde level (MDA) and inducible nitric oxide synthase activities (iNOS). After SVP treatment, there were significant elevations in renal tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa B (NF-κB). Additionally, SVP-induced suppression of nuclear related factor 2 (Nrf2) pathways. Furthermore, histopathological examination showed prodigious inflammation, necrosis, congestion and degeneration. Coadministration of Carvedilol and/or rosuvastatin mitigated nephrotoxicity induced by SVP. Using the combination of Carvedilol and rosuvastatin presented additional intense renal protective effect when compared with each alone.

Key words: Carvedilol; Rosuvastatin; Sodium valproate; Nrf2 pathway; Nephroprotection.

1. INTRODUCTION

The kidney is a vital organ essential for the body to achieve critical functions such as detoxification, maintenance of homeostasis and excretion of toxic metabolites and drugs[1]. Drugs and exogenous toxicants can encourage renal harmfulness in which excretion does not go efficiently[2].

Nephrotoxic medicines are therapeutic agents that have the potential to compromise renal perfusion or cause direct renal injury[3]. Among these drugs, which induce nephrotoxicity, SVP which is noted as one of the most widely used antiepileptic drugs. It has a potent therapeutic effect against bipolar psychiatric syndromes, partial and generalized seizures, and migraine control[4]. Although, SVP is a safe drug, but it can induce severe side effects when it is used in higher concentrations[5]. Several studies have also confirmed that administration of SVP at therapeutic doses to neonatal rats for anticonvulsant action created toxic effects on cardiovascular[6] and reproductive system[7].

It has been reported that SVP-induced toxicity may be assisted by the increased yield of reactive oxygen species (ROS)[8]. ROS are involved in various cellular actions. These actions include gene expression of second messengers that are involved in the stimulation of several signaling transduction cascades eliciting the activation of transcription factors, mutagenesis and apoptosis initiation[9]. The elevated therapeutic use of SVP has been accompanied by renal tubular defects and hepatic dysfunction reports[10-12].

Oxidative stress can produce suppression of Nrf2 expression, a crucial transcription factor adjusting cellular antioxidant response. Nrf2 is essential in amending antioxidant genes expressions, encoding various antioxidant, like glutamate cysteine ligase catalytic subunit (GCLC), NAD(P)H quinine Oxidoreductase-1 (NQO-1) and hemoxygenease-1

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(HO-1) responding to oxidative stress[13]. The cytosolic repressor protein Kelch-like ECH-associated protein 1 (Keap1) is in charge of Nrf2 action. Antioxidant enzymes are the chief line for cellular protection that defends cellular constituents from oxidative injury. Encouragement of oxidative stress is related to marked depletion of the antioxidant protection arrangement including Nrf2, HO-1 with decline in total antioxidant capacity (TAC)[14].

A previous study showed that SVP-treated mice exhibited diminution of cytoplasmic Nrf2 and repression of Nrf2 nuclear translocation, compatible with an intensive mitigation of HO-1, NQO-1 and GCLC levels[15]. Additionally, SVP, in a dose dependent manner, exhibited Keap1 expression elevation and Nrf2 expression reduction[16]. Moreover, oxidative stress can lead to the activation of numerous transcription factors, for example NF-κB which plays a vital role in inflammation and activation of iNOS[17].

Carvedilol (CARV), a non-specific beta-adrenergic blocker, has been used safely for the management of congestive heart failure, hypertension and coronary disease[18]. CARV has been discovered to possess characteristic antioxidant effect with both ROS-suppressive and ROS-scavenging properties[19,20]. Furthermore, CARV has been reported to exert anti-inflammatory property[21].

Statins are engaged in the cure of hyperlipidemia. Nevertheless, they have added properties covering anti-oxidant and anti-inflammatory features, possibly due to disturbance of G-proteins[22]. ROSU has been extensively established because of its potency, efficacy and greater safety profile[23]. Some studies presented that a ROSU treatment can converse cardiac complaints, reduce biomarkers of oxidative stress (ROS) and has additional benefits such as immunomodulatory and thus anti-inflammatory properties[24-26]. Therefore, statins signify a probable oncoming line for the protection against nephrotoxicity by means of hindering the action of kidney damaging drugs[27,28].

Consequently, the existing work was designed to settle the possible protective influence of CARV, ROSU, or their engaged treatment on the nephrotoxicity gotten by SVP in rats and to examine their manipulative impact on Nrf2/HO-1 pathway and the sequential initiation of antioxidant enzymes together with their prospective cross talk with NF-***κ***B inflammatory pathway.

2. METHODS

2.1. Experimental rats

Forty two male Sprague–Dawley rats, with weight range 140-180 g., were utilized throughout the existing work. The animal house of Faculty of Pharmacy (girls) Al-Azhar University procured the required rats. Animals were left for one week for adaptation under ordinary research capability environments using adjusted temperature at 23 ± 2°C and allowing 12:12 light/dark cycle. The rats were allowed unrestricted admission to water and food. The regular strategies set by Faculty of Pharmacy (Girls) Al-Azhar University were followed and this was compatible with the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. Permit Number: 203 was given by the Ethical Committee of Faculty of Pharmacy (girls) Al-Azhar University, Egypt.

2.2. Chemicals

Sodium valproate was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). CARV was purchased from Multipharma, Cairo, Egypt and ROSU was procured from AstraZeneca Company (AstraZeneca, 6th of October City, Giza, Egypt). All extra chemicals used in the current work were of analytical rank. SVP and ROSU were dissolved in distilled water while CARV was dissolved in0.5 % carboxymethyl cellulose.

2.3. Design of the work

Rats were set into seven groups (6 rats/group) indiscriminately, and injected daily for 14 days as follows: the first group represented the control group and received 0.5 % carboxymethyl cellulose (10 ml/kg, p.o.), 0.2 ml saline p.o. and 0.2 ml saline i.p.; the second group served as the SVP group, and was injected by SVP (500 mg/kg i.p.)[29,30]; the third group served as the SVP+CARV group, and was treated by CARV (10 mg/kg; p.o.)[31] 1 h prior to the treatment with SVP (500 mg/kg i.p.)**;** the fourth group served as the SVP+ROSU group, and received ROSU (10 mg/kg; p.o.)[32] 1 h prior to the treatment with SVP (500 mg/kg i.p.); the fifth group served as the SVP+CARV+ROSU group, and was injected by CARV+ ROSU 1 h prior to the treatment with SVP; the sixth group served as the CARV group, and received CARV (10 mg/kg; p.o.); the seventh group served as the ROSU group, and received ROSU (10 mg/kg; p.o.).

At the completion of the 14 days of drug administration, rats were euthanized by decapitation.

The serum was separated then was centrifuged at 4000 rpm by means of a centrifuge with high speed (MPw-350, Warsaw, Poland) at 4°C for 20 min and put in storage at (−80°C) to assess SCr and BUN levels. The kidneys were homogenized in phosphate buffered saline (PBS) (10% w/v) and centrifuged (4000 rpm, 4°C, 15 min). Supernatants were frozen at −80°C till the evaluation of oxidative stress markers; MDA, GSH levels and iNOS, inflammatory mediators; TNF-α, as well as Nrf2 signaling pathway. For histopathological investigation and estimation of immunohistochemical expressions of nuclear factor kappa B (NF-κB) one more portion of renal tissue was preserved in 10% formol–saline.

2.4. Estimation of nephrotoxicity markers

For the detection of SCr and BUN levels, colorimetric assay kits were used in this study (Biodiagnostics, Giza, Egypt). The process was performed following the instructions granted by the manufacturer.

2.5. Estimation of oxidative stress biomarkers

The kidney homogenate of treated groups was divided into two portions. Using Eagle Biosciences, Inc. USA kit, the first portion was used for MDA determination[33] and the second portion was used for GSH estimation. The process was performed following the instructions granted by the manufacturer.

2.6. Estimation of iNOS and TNF-α by ELISA

By means of rat enzyme-linked immunosorbent assay (ELISA) kits, the kidney homogenate of treated rats was used for measurement of iNOS protein content (MyBiosource, Inc, Southern California, San Diego, USA; Catalogue Number: MBS263618) and TNF-α (Cusabio Biotech Co., China; Catalogue Number: CSB-E11987r). The process was performed following the instructions granted by the manufacturer.

2.7. Estimation of NF-κB expression by immunohistochemical analysis

Immunohistochemical analysis was estimated by rehydration of paraffinized kidney sections in xylene and graded ethanol solutions. Citrate buffer was added (pH was adjusted to 6), sections were heated for exactly 20 minutes and chilled later (at 4°C) to be incubated with primary polyclonal rabbit anti-NF-κB antibody **(1:200; Invitrogen, Carlsbad, CA, USA)** all night. Phosphate buffered saline utilized for washing sections, and they were incubated for 30 min at 37°C jointly with biotinylated secondary antibody, and afterward with Avidin DH and biotinylated horseradish peroxidase H complex following Elite ABC kit instructions **(Vector Laboratories Inc., Burlingame, CA, USA).** Afterward an extra wash was done using phosphate buffered saline, the response was shown by diaminobenzidine tetrahydrochloride **(DAB Substrate Kit, Vector Laboratories Inc., Burlingame, CA, USA)** and the slices were additionally dyed with hematoxylin, dehydrated, and washed in xylene then slipped cover for light microscopic investigation.

2.8. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Nuclear related factor 2, NAD(P)H quinine Oxidoreductase-1 and hemoxygenease-1 genetic expression were estimated by RT-PCR procedure. Total RNA was isolated using the kit which was provided by Thermo Fisher Scientific Inc. Germany. (GeneJET , Kit,#K0732) . The superiority of RNA was certified by estimating the optical density (OD) at 260 nm. 4 µl of total RNA was processed to produce cDNA by the use of reverse transcription and a PrimeScript reverse transcription-PCR (RT-PCR) the kit was procured from Bioline, a median life science company, UK (SensiFAST™ SYBR® Hi-ROX One-Step Kit, catalog no. PI-50217 V). The PCR conditions and primers sequence from 5′- 3′ used were as follows:

Nrf2 forward, AGGACATGGAGCAAGTTTGG ; Nrf2 reverse,TTGCCCTAAGCTCATCTCGT; HO-1 forward, TCAGGTGTCCAGAGAAGGCTTT; HO-1 reverse, CTCTTCCAGGGCCGTGTAGA; NQO-1 forward, CATTCTGAAAGGCTGGTTTGA ; NQO-1reverse, TAGCTTTGATCTGGTTGTCAG ; β-actin forward, ATGGATGACGATATCGCTGC ; β-actin reverse , CTTCTGACCCATACCCACCA.

Real-time PCR was done with the following conditions: Reverse transcription step of 45°C for 10min ,Polymerase activation step of 95°C for 2 min, followed by Denaturation, Annealing and Extension for 40 cycles of 95°C for 5s,60°C for 10s and 72°C for 5s. All reactions were conducted in three replicates. β-actin was used as housekeeping reference, After the RT-PCR run the data were expressed in Cycle threshold (Ct) method ($2^{-\Delta\Delta Ct}$)to calculate modification in gene expression was calculated using the threshold cycle[34]

2.9. Histological examination

For histological inspection, the rat kidneys of treated groups were fixed in 10% neutral buffered formalin. Irrigation was finished by tap water. Next, dehydration was ended by a system of diluted alcohols (methyl, ethyl and absolute ethyl). Samplings were cleared in xylene and embedded in paraffin blocks. Then, tissue blocks were prepared and organized for sectioning following the method described by Bancroft and Stevens[35]. Finally, renal sections were ready to be examined by light microscopy via eosin–hematoxylin stain.

2.10. Statistical analysis

Statistical significance of differences between means of groups was achieved by operating the prism computer program (GraphPad software Inc. V5, San Diego, CA). One-way analysis of variance (ANOVA) was applied to calculate the statistical significance followed by Tukey's multiple comparison test. The 0.05 level of probability was considered of statistical significance.

3. RESULTS

3.1. Nephrotoxicity markers assessment

The effect of SVP on BUN and SCr levels were presented in Figures. 1A and 1B. SVP induced a significant increase in BUN and SCr levels by 61% and 327%, respectively, as compared with the control group. Nonetheless, CARV/SVP-administered group showed a significant reduction in these parameters by 25% and 48%, respectively, when compared with SVP group. On the other hand, BUN and SCr levels considerably decreased in ROSU/ SVP-injected rats by 19% and 48%, respectively, with respect to SVP group .In CARV/ROSU/SVP-injected rats, BUN and SCr levels were significantly decreased by 41% and 49%, respectively, with respect to SVP group.

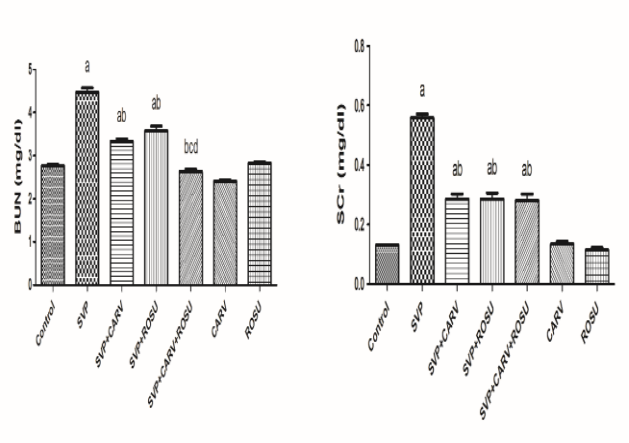

Figure 1. Carvedilol and /or rosuvastatin mitigated valproate-induced elevations in blood urea nitrogen (BUN) and serum creatinine levels (SCr).
Values are mean ± SD using n = 6. a, b, c, or d: Statistically significant when compared to the control, SVP, SVP+CARV or SVP+ROSU group, respectively, $P < 0.05$ using ANOVA followed by Tukey's as post-hoc test.
BUN: blood urea nitrogen; SCr: serum creatinine; SVP: sodium valproate; CARV: carvedilol; ROSU: rosuvastatin

3.2. Determination of oxidative stress markers

Administration of SVP greatly increased the renal content of MDA with respect to the control group values. However, renal MDA content was significantly decreased in CARV/SVP-treated rats, ROSU/SVP-treated group and in CARV/ROSU/SVP-treated rats by 34%, 46% and 86% respectively, when compared with SVP group values. On the other hand, the renal content of GSH was significantly decreased in SVP-treated rats by 67% with respect to the control group values.

However, CARV/SVP-treated rats, ROSU/SVP treated group and CARV/ROSU/SVP-treated rats showed a significant increase in the renal content of GSH, by 55%, 107% and 210% respectively, when compared with SVP group values (Figure 2).

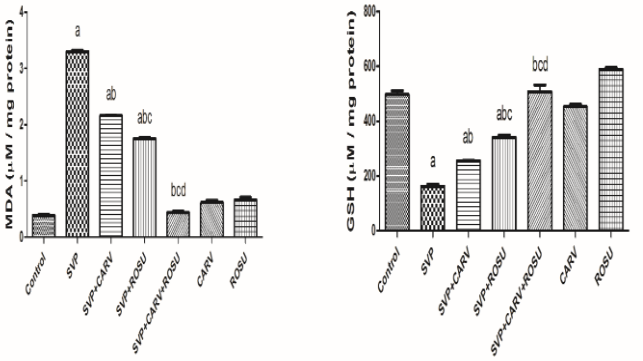

Figure 2. Carvedilol and /or rosuvastatin attenuated valproate-induced oxidative stress.
Values are mean ± SD using n = 6. a, b, c, or d: Statistically significant when compared to the control, SVP, SVP+CARV or SVP+ROSU group, respectively, $P < 0.05$ using ANOVA followed by Tukey's as post-hoc test.
MDA: malondialdehyde; GSH: glutathione; SVP: sodium valproate; CARV: carvedilol; ROSU: rosuvastatin

3.3. Analysis of iNOS and TNF-α content

It became obvious that SVP treatment triggered a significant increase in iNOS content by 597% with respect to the control values. However, the renal content of iNOS was significantly decreased in CARV/SVP-treated rats, ROSU/SVP-treated group and in CARV/ROSU/SVP-treated rats by 59%, 71%

and 90% respectively, when compared with SVP group values (Figure 3).

The tissue level of TNF-α in the different treated rat groups were presented in Figure 4. SVP exhibited a significant increase in TNF-α content by 677% with respect to the control group values. However, the renal TNF-α content was significantly decreased in CARV/SVP-treated rats, ROSU/SVP-treated group and in CARV/ROSU/SVP-treated rats by 51%, 62% and 84% respectively, when compared with SVP group values.

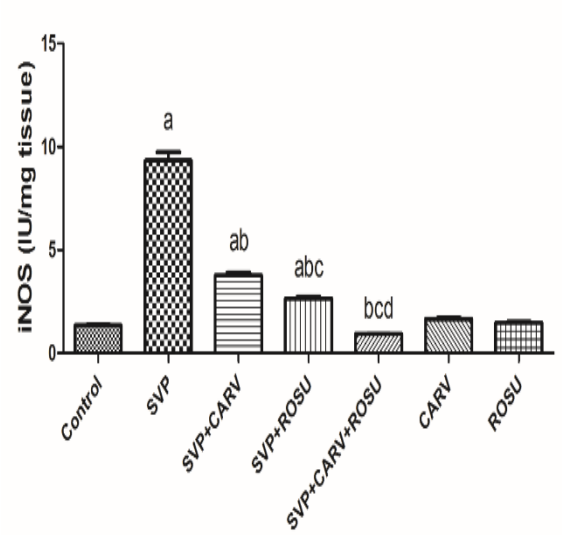

Figure 3. Carvedilol and /or rosuvastatin attenuated valproate-induced modifications in inducible nitric oxide synthase (iNOS) content.
Values are mean ± SD using n = 6. a, b, c, or d: Statistically significant when compared to the control, SVP, SVP+CARV or SVP+ROSU group, respectively, P < 0.05 using ANOVA followed by Tukey's as post-hoc test.
iNOS: inducible nitric oxide synthase; SVP: sodium valproate; CARV: carvedilol; ROSU: rosuvastatin

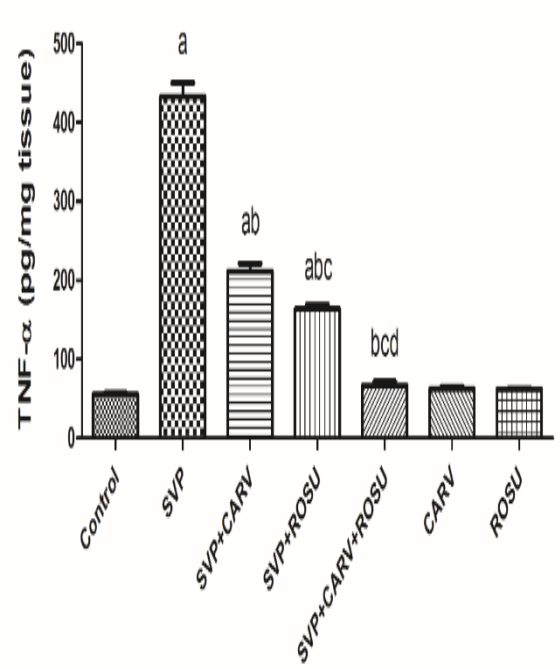

Figure 4. Carvedilol and /or rosuvastatin diminished valproate-induced alterations in tumor necrosis factor-alpha (TNF-α) content.
Values are mean ± SD using n = 6. a, b, c, or d: Statistically significant when compared to the control, SVP, SVP+CARV or SVP+ROSU group, respectively, P < 0.05 using ANOVA followed by Tukey's as post-hoc test.
TNF-α: tumor necrosis factor-alpha; SVP: sodium valproate; CARV: carvedilol; ROSU: rosuvastatin

3.4. Immunohistochemical analysis of NF-κB protein expression

Immunohistochemical modifications in kidney tissue of different treatment rat groups were shown in Figure 5. The expression of NF-κB protein was highly increased after SVP administration with respect to the control group. Conversely, there was a significant decrease in NF-κB protein expression in CARV/SVP-treated rats, ROSU/SVP-treated group and in CARV/ROSU/SVP-treated rats by 29%, 43% and 57% respectively, when compared with SVP group values.

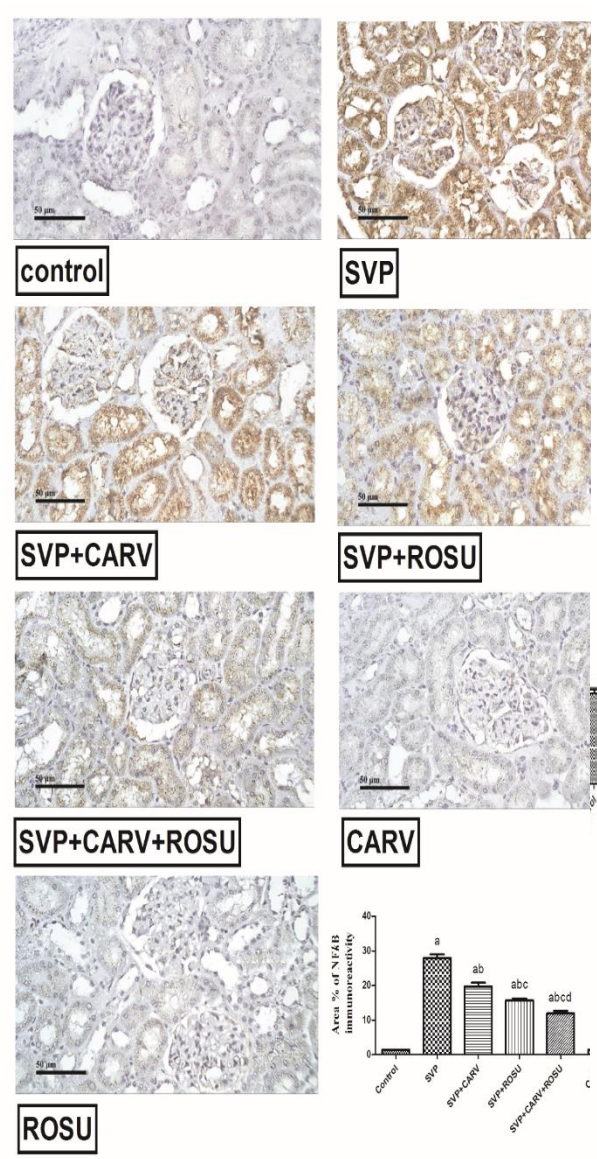

Figure 5. Carvedilol and /or rosuvastatin decreased valproate-induced variations in nuclear factor-kappa B (NF-***κ***B) protein expression.
Values are mean ± SD using n = 6. a, b, c, or d: Statistically significant when compared to the control, SVP, SVP+CARV or SVP+ROSU group, respectively, P < 0.05 using ANOVA followed by Tukey's as post-hoc test.
NF-***κ***B: nuclear factor-kappa B; SVP: sodium valproate; CARV: carvedilol; ROSU: rosuvastatin

3.5. Manifestation of Nrf2, HO-1 and NQO-1 mRNA expression in the kidney

As presented in Figure 6, Nrf2 mRNA expression in the kidney of SVP-induced rats indicated a significant down-regulation by 69% when compared with control rats group. However, there is significant increase in expression of Nrf2 mRNA expression in CARV/SVP-treated rats, ROSU/SVP-treated group and in CARV/ROSU/SVP-treated rats by 120%, 136% and 530% respectively, when compared with SVP group values. As for HO-1 mRNA, a significant down-regulation in expression by 59% when compared with control rats group. On the other hand, there is significant increase in expression of HO-1 mRNA expression in CARV/SVP-treated rats, ROSU/SVP-treated group and in CARV/ROSU/SVP-treated rats by 116%, 176% and 324% respectively, when compared with SVP group values. NQO-1 mRNA expression in the kidney of SVP-induced rats indicated a significant down-regulation by 69% when compared with control rats group. However, there is significant increase in expression of NQO-1 mRNA expression in CARV/SVP-treated rats, ROSU/SVP-treated group and in CARV/ROSU/SVP-treated rats by 115%, 154% and 510% respectively, when compared with SVP group values.

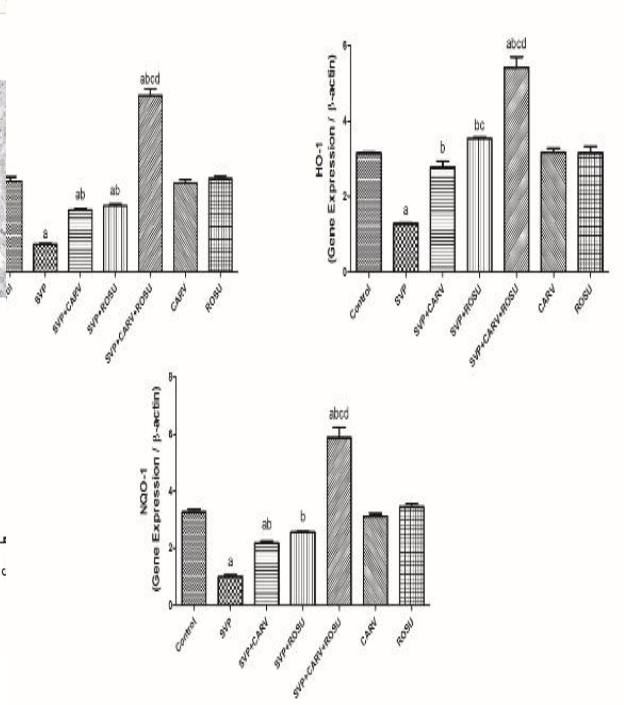

Figure 6. Carvedilol and /or rosuvastatin attenuated valproate-induced modifications in Nrf2, HO-1 and NQO-1 mRNA expressions.
Values are mean ± SD using n = 6. a, b, c, or d: Statistically significant when compared to the control, SVP, SVP+CARV or SVP+ROSU group, respectively, P < 0.05 using ANOVA followed by Tukey's as post-hoc test.
Nrf2: nuclear related factor 2; HO-1: hemoxygenease-1; NQO-1: NAD(P)H quinine Oxidoreductase-1; SVP: sodium valproate; CARV: carvedilol; ROSU: rosuvastatin

3.6. Histopathological estimation

The demonstrative histopathological outcomes are shown in Figure 7. No marked pathological variations were detected in renal tissues of the control group, the group received CARV alone and the other group received ROSU alone (Fig. 7A, F and G). However, renal tissues of SVP group showed severe congestion of glomerular tuft and periglomerular

mononuclear inflammatory cells infiltration (Fig. 7B); these pathological variations were obviously diminished in the CARV/ SVP, ROSU/ SVP and in CARV/ ROSU/ SVP groups (Fig. 7C, D and E).

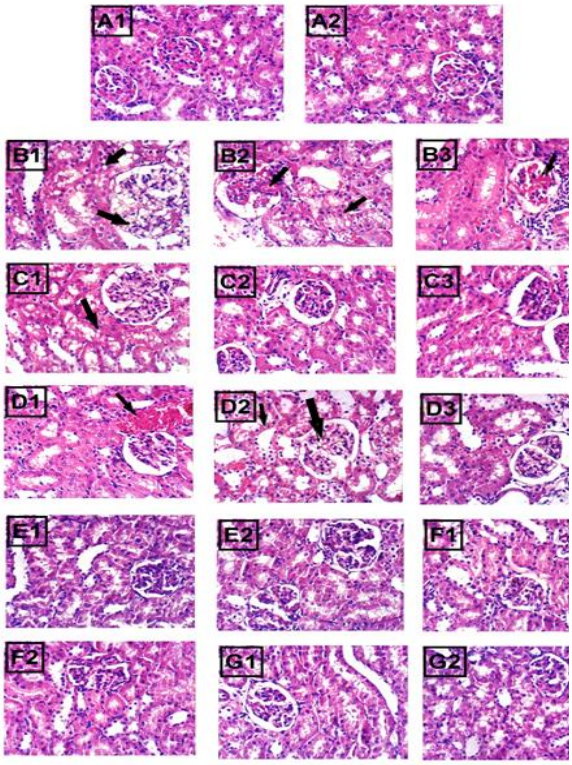

Figure 7. Representative Photomicrograph of kidney section stained by (H & E X 400). (A1, A2, F1, F2, G1, G2) section of a control rats, rats received CARV alone and rats received ROSU alone respectively, showing the normal histological structure of the tissue. (B) section of SVP rats, (B1) showing sever vacuolar degeneration of epithelial lining renal tubules and endothelial lining glomerular tuft while (B2) showing sever necrobiosis of epithelial lining renal tubules and congestion of glomerular tuft (B3) showing moderate periglomerular mononuclear inflammatory cells infiltration and congestion of glomerular tuft. (C) section of rats received SVP + CARV (C1) showing slight vacuolation of epithelial lining renal tubules (C2,C3) showing no histopathological alterations. (D) section of rats received SVP + ROSU (D1) showing mild congestion of renal blood vessel (D2) showing moderate vacuolar degeneration of epithelial lining renal tubules and endothelial lining glomerular tuft (D3) showing mild periglomerular mononuclear inflammatory cells infiltration. (E1,E2) section of rats received SVP + CARV + ROSU showing the normal histological structure of the tissue.SVP: sodium valproate; CARV: carvedilol; ROSU: rosuvastatin

4. DISCUSSION

Nephrotoxic agents recurrently make irritation in glomerulus, proximal tubules and surrounding cellular matrix[36]. The irritation as well as cytotoxicity follows the increase of ROS production and oxidative stress, the impaired mitochondria in tubules and the troubled tubular transport system[37-40]. Studies have confirmed that around 20% of nephrotoxicity is made by drugs[41-44].

In this investigation, we estimated the possible protective effects of CARV and/or ROSU on sodium SVP-induced nephrotoxicity in rats. We established that administration of CARV or ROSU alone and in combination before SVP, safeguard kidney from injury through up-regulating the Nrf2 pathway and detracting oxidative stress and inflammation.

As opposed to previous conclusions that certified that SVP guard against kidney damage by way of its histone deacetylase-inhibiting action[45], Tabatabaei and Abbott described that the cytotoxic effect of SVP is developed because of hydrogen peroxide generation and high ROS production[46]. This could be the cause behind the modification of glomerular filtration rate, which was revealed by the elevation in SCr and BUN levels. The current results presented that SVP produced nephrotoxicity evidenced by the increased BUN and SCr levels. Both CARV and ROUS mitigated BUN and SCr levels when administered separately with SVP; an observation supported by the conclusions of other studies[47-49].

In the present study, co-treatment of CARV and SVP significantly decreased SCr level compared with the administration of the toxicant alone. This provides an indication to the nephroprotective activity of CARV. ROSU similarly reduced SCr level in a significant manner with subsequent exposure to SVP.

There is no clear advantage in SCr level reduction upon combining of two drugs but there is a great decrease in BUN level when compared with each drug alone. Likewise, the dramatic changes in the kidney histological architecture confirmed the nephrotoxicity by SVP where it caused direct damage to the kidney. The improvement caused by CARV and/or ROSU treatment in renal function was braced by the amendments seen in the histological investigation that was achieved in our study. These outcomes are consistent with the conclusion of Al-Amoudi[50].

The results achieved in the existing study exposed a noteworthy elevation in MDA and a decreased GSH content in SVP group. SVP also multiply NO level. Extra NO reacts with superoxide anion to produce the hazardous peroxynitrite radical, which represents an additional source for cellular injury by oxidizing and nitrating molecules[51]. In addition, extra NO diminishes GSH, which is principal for increased liability to oxidative stress[52], and contribute to cellular damage and finally loss of the glomerular cell integrity and function. In this study, CARV and ROSU significantly decreased MDA and elevated GSH content. These results supported by other studies[49,53]. ROSU reduced MDA and increased GSH in better manner than CARV.

Moreover, administration of both drugs produced more profound effect on MDA and GSH contents.

Our result unveiled that SVP-prompted oxidative stress, up regulated NF- ***κ***B expression, which sequentially improved the transcription of TNF-α and iNOS leading to excessive NO production. Definitely, inflammation brightened up as one of the vital pathways through which SVP facilitates renal impairment[54]. Interestingly, ROSU is presented to have anti-inflammatory and antioxidant attributes in diabetic animals independent of its outcome on levels of plasma cholesterol[55,56].

Our work showed that administration of ROSU led to the reduction in iNOS content which is in contract with a former study[57]. Treatment with ROSU protected against SVP induced nephrotoxicity through down regulating NF- ***κ***B expression and TNF-α transcription[58]. This result is consistent with that of another report stating that statins grant prevention of cisplatin nephrotoxicity[59]. CARV also had the ability to protect against SVP-induced nephrotoxicity through the reduction of NF- ***κ***B expression, transcription of TNF-α and iNOS content. These findings of the present study are compatible with a previous study[60]. The effect of ROSU in the reduction of iNOS content is superior to CARV, but this effect is enhanced when ROSU and CARV are given together. Protective outcome of the combination between ROSU and CARV is better than using each as a single drug in reduction of NF-***κ***B protein expression.

In addition, Nrf2 pathway activation and NF-kB cascade suppression may tend to crosstalk with each other[61]. Nrf2, originally identified as a serious transcription factor in the Keap1-Nrf2 signaling pathway, functions to halt oxidative stress. The outcomes of the current work confirmed that SVP successfully down regulated the protein expression levels of Nrf2 signaling pathway. The destruction of the Nrf2 signaling pathway may result in imbalanced redox equilibrium in the cell, which may lead to elevation of ROS levels and initiation of apoptosis. However, the findings of the present study indicated that SVP significantly reduced Nrf2 expression and that of downstream effectors, including NQO-1 and HO-1. CARV has potent antioxidant properties which safeguard the tissues from toxicant[62]. ROSU also has ability to elevate Nrf2 pathway trying to protect organs[63].

Administration of CARV in the presence of SVP caused a significant increase in Nrf2, HO-1and NQO-1expression but this elevation was less than in the ROSU/SVP model. It is clear that the combination used in this study necessarily confers a great advantage over the use of each drug alone regarding Nrf2, HO-1and NQO-1 expression. The protection conferred by CARV, ROSU, or their combination against SVP-induced renal damage may probably be by way of up-regulation of Nrf2, and this may be considered as the corner stone of the antioxidant efficiency of CARV, ROSU, or their combination. Our observation is in agreement with previous studies that indicated the reduction of nuclear Nrf2 expressions and subsequent reduction in HO-1 and NQO-1 expressions represent a sign of nephrotoxicity[64-66]. Accumulating confirmation has discovered that Nrf2 plays a pivotal role as a defensive mechanism against renal damage in either acute or chronic renal impairement[67,68].

5. CONCLUSIONS

In conclusion, the fundamental results of the existing study illustrated that the combination of CARV and ROSU were able to mitigate nephrotoxicity caused by SVP through the ability to modulate different molecular targets involved in SVP-induced renal injury mainly NF-κB/Nrf2 signaling pathways (Figure 8).

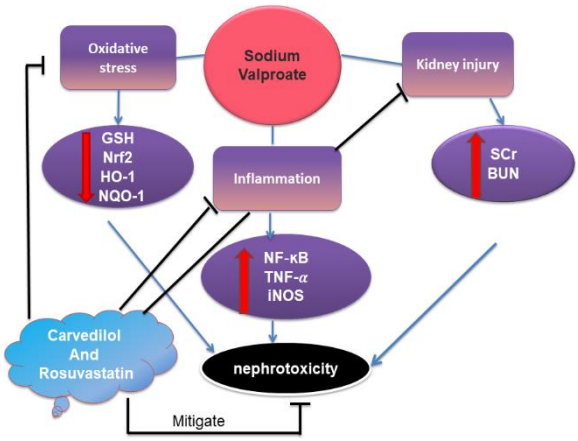

Figure 8. Schematic diagram showing the nephroprotective mechanistic pathways of Carvedilol and /or rosuvastatin against valproate-induced nephrotoxicity**.**

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Ethical approval statement: Everything in animals' techniques was done according to the Ethics Committee of the faculty of Pharmacy Al-Azhar University, Egypt (permit number: 203/2019). Unnecessary disturbance of animals, pressure and tough maneuver was avoided.

Author Contribution Statement: Hebatalla I. Ahmed, Heba S. Zaky and Ahmed H. Eid shared developing the research idea, designed the experiments, supervised the experiments performance, executed data analysis, wrote and revised the manuscript. Nourhan M. Abd El-Fattah performed the experiments, collected the data, carried out the graphical and statistical analysis and wrote the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Abbreviations: Blood urea nitrogen (BUN); Carvedilol (CARV); Glutathione (GSH); Hemoxygenease-1 (HO-1); Inducible nitric oxide synthase (iNOS); Malondialdehyde (MDA); Nuclear factor-kappa B (NF-κB); NAD(P)H quinine Oxidoreductase-1 (NQO-1); Nuclear related factor 2 (Nrf2); Reactive oxygen species (ROS); Rosuvastatin (ROSU); Serum creatinine (SCr); Sodium valproate (SVP); Tumor necrosis

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