Research Article

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Nephroprotective Effects of Carvedilol and Sodium Copper Chlorophyllin in Glycerol-Induced Acute Kidney Injury in Female Rats

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Abstract

Oxidative stress and tubular damage are two major consequences of rhabdomyolysis-induced acute kidney injury (RIAKI). This study evaluated the nephroprotective effects of carvedilol and sodium copper chlorophyllin (SCC), administered separately and in combination, in rats with glycerol-induced RIAKI. Thirty-five female rats were randomly assigned to treatment groups receiving carvedilol (2.5 mg/kg), SCC (50 mg/kg), or a combination of both, as well as control and glycerol-induced groups. Electrolyte levels, oxidative stress markers, and renal function were assessed. Glycerol injection significantly impaired renal function ($p < 0.05$). Renal impairment was considerably reduced by treatment with carvedilol or SCC, while the combination of both drugs produced a particularly strong protective effect by normalising biochemical variables and reducing oxidative stress more effectively than either monotherapy. Through antioxidant mechanisms, carvedilol and SCC exert additive nephroprotective effects against RIAKI. However, careful interpretation is required because of variations in treatment duration and other study limitations.

Keywords: acute kidney injury; sodium copper chlorophyllin; carvedilol

1. INTRODUCTION

Acute kidney injury (AKI) caused by rhabdomyolysis, termed rhabdomyolysis-induced acute kidney injury (RIAKI), is the most common and potentially life-threatening complication of rhabdomyolysis and significantly increases the mortality associated with this condition. Generally, 13–50% of rhabdomyolysis cases lead to AKI, with a case-fatality rate ranging from 20% to 50% and reaching as high as 59% among severely affected patients. Nevertheless, specific therapies for RIAKI remain unavailable because of its complex pathogenesis. Currently, the primary clinical treatment for RIAKI is symptomatic and supportive, focusing on continuous renal replacement therapy, volume therapy, and the correction of water, electrolyte, and acid–base balance disorders, including the administration of sodium bicarbonate [1].

Rhabdomyolysis (RML), which involves the lysis of skeletal muscle cells, is a pathological syndrome with an acute or subacute onset in which a patient develops localised or generalised myalgia and weakness associated with a rapid increase in the serum creatine kinase (CK) level. The magnitude of CK elevation depends on the timing of the measurement relative to the onset of muscle injury. A systematic review concerning the definition of RML recommends using these clinical symptoms in combination with a CK cut-off value greater than 1000 IU/L or a CK level more than five times the upper limit of normal (ULN) to define mild RML [2].

Rhabdomyolysis is a serious condition in which damaged muscle cells break down and release their contents into the circulation. Various factors can cause rhabdomyolysis, including physical injuries associated with crush syndrome and earthquakes, as well as nontraumatic causes such as high body temperature, muscle oxygen deprivation, strenuous physical activity, and excessive alcohol consumption. Identifying the primary cause of rhabdomyolysis is important for providing appropriate treatment and preventing complications. Approximately 10–40% of people who develop rhabdomyolysis also experience acute kidney injury (AKI), but it remains unclear whether a specific treatment can directly target the underlying cause of the condition. Because of the severe complications associated with rhabdomyolysis, experimental models have been developed to investigate its pathophysiology. Animal models of glycerol-induced AKI are currently used frequently by researchers to investigate the condition because these models allow the controlled induction of the disease process without the confounding variables that may be present in human studies. The intramuscular injection of glycerol triggers the release of myoglobin and other muscle components into the bloodstream, ultimately leading to the development of AKI [3].

Following muscle damage, intracellular fluid leaks from the damaged cells and is subsequently sequestered within the extracellular spaces. Increased levels of myoglobin and its components exert destructive and cytotoxic effects on the nephrons. In particular, free iron reacts with peroxide compounds, thereby generating reactive oxygen species (ROS) [4].

Carvedilol, a nonspecific beta-adrenergic blocker, has been used safely in the management of congestive heart failure, hypertension, and coronary disease. Carvedilol has been found to possess characteristic antioxidant effects and ROS-suppressive properties. Furthermore, carvedilol has been reported to exert anti-inflammatory effects [5].

Chlorophyllin is a semisynthetic mixture of sodium copper complexes synthesised from chlorophyll. During its synthesis, the magnesium atom present at the centre of chlorophyll is replaced by copper, and the molecule loses its phytol tail. Chlorophyllin offers several advantages over chlorophyll, including greater hydrophilicity, improved colouring properties, and greater stability against acid and light. Chlorophyllin has considerable commercial importance as a food colourant and has also been found to be effective in the treatment of a broad spectrum of chronic diseases [6].

Sodium copper chlorophyllin (SCC), a derivative of chlorophyll, exhibits antioxidant, anti-inflammatory, and chemoprotective properties. It scavenges ROS and modulates the immune response [7].

Given the complex interplay between oxidative stress and inflammation in glycerol-induced RIAKI and the complementary pharmacodynamic profiles of carvedilol and SCC, we hypothesise that their combined administration may exert greater nephroprotective effects than either treatment administered individually. Therefore, this study investigates the individual and combined effects of carvedilol and SCC on renal function, oxidative biomarkers, and electrolyte outcomes in a rat model of glycerol-induced rhabdomyolysis-associated AKI.

2. MATERIALS AND METHODS

2.1. STUDY SETTING, ANIMAL HANDLING, AND ETHICAL APPROVAL

The research was conducted between October 2025 and January 2026 at the College of Pharmacy, University of Basrah. Thirty-five adult female rats weighing between 150 and 200 g were included in the experiment. The animals were housed in separate plastic cages, with three rats placed in each cage, containing wood-chip bedding in the animal house of the College of Pharmacy, University of Basrah. They were obtained from the College of Veterinary Medicine, University of Basrah, and were allowed to acclimatise for two weeks before the experiment began.

All animals were housed under carefully regulated conditions. Sterilisation procedures, disposable plasticware, and sterilised reagents were used throughout the experiment. Food and water intake were assessed twice daily, while body

weight was measured at the beginning and end of the experimental period. The room temperature was maintained between 18 and 24 °C under a 12-hour light/12-hour dark cycle with typical ambient humidity. The rats were maintained on regular rodent food provided in pellet form. All experimental procedures were approved by the Animal Ethics Committee of the College of Pharmacy, University of Basrah (Approval No. EC 36, dated December 11, 2024).

2.2. SAMPLE SIZE CALCULATION, RANDOMISATION, AND BLINDING

For a one-way analysis of variance (ANOVA) involving five groups, with $\alpha = 0.05$, a statistical power of 0.80, and a large effect size of $f = 0.40$, the required sample size was calculated using G*Power software, version 3.1. The calculation indicated that six rats were required per group. The number was increased to seven rats per group to account for probable attrition during the experimental period.

A computer-generated randomisation procedure was used to assign the rats randomly to the experimental groups. Sequentially numbered and securely sealed envelopes were used to conceal group allocation. Group identities were concealed from the outcome assessors, including the histopathologists and biochemical analysts. The group codes were revealed only after the completion of the data analysis.

2.3. DRUG PREPARATION AND EXPERIMENTAL DESIGN

Sodium copper chlorophyllin (SCC) (Sigma-Aldrich, catalogue no. 262991, batch no. MKCL1677, purity $\geq 95\%$, copper content $\geq 4.5\%$, and sodium content $\geq 7.0\%$) was dissolved in distilled water to obtain a concentration suitable for oral gavage at a dose of 50 mg/kg/day. Carvedilol (Denk Pharma, Germany, batch no. 041837, purity 99.8%) was powdered and suspended in distilled water containing 0.5% carboxymethylcellulose sodium (CMC-Na) to improve its solubility. It was administered orally at a dose of 2.5 mg/kg/day. Fresh drug suspensions were prepared daily throughout the treatment period.

Rhabdomyolysis-induced AKI was induced in the rats using a hypertonic glycerol solution consisting of 50% v/v glycerol in normal saline following a 24-hour period of dehydration. The rats were administered 1 mL of normal saline daily for 14 days. To induce organ damage, the animals underwent a 24-hour fasting period, followed by the intramuscular administration of hypertonic glycerol solution at a dose of 8 mL/kg/day from day 10 to day 14, representing five consecutive days of injection. The total injected volume was divided equally between the two hind limbs.

The animals were randomly allocated to five groups, with each group containing seven rats:

1. **Group 1 (control group):** Rats were administered normal saline at a dose of 8 mL/kg body weight through intramuscular injection for five days.
2. **Group 2 (induced group):** Rats were administered glycerol at a dose of 8 mL/kg body weight/day through intramuscular injection, as described above [8].
3. **Group 3 (positive-control group):** Rats were administered carvedilol orally by gavage at a dose of 2.5 mg/kg body weight/day for 14 days [9].
4. **Group 4 (treatment group):** Rats were administered SCC orally by gavage at a dose of 50 mg/kg body weight/day for 14 days [7].
5. **Group 5 (combined-treatment group):** Rats were administered SCC at a dose of 50 mg/kg body weight/day and carvedilol at a dose of 2.5 mg/kg body weight/day orally by gavage for 14 days.

After the designated treatment period, the animals in Groups 3, 4, and 5 were deprived of water for 24 hours and subsequently administered the hypertonic glycerol solution intramuscularly at a dose of 8 mL/kg body weight/day [6].

2.4. BLOOD COLLECTION, BIOCHEMICAL ANALYSIS, AND HISTOPATHOLOGICAL INVESTIGATION

The animals were sacrificed at the end of the experiment, 24 hours after the administration of the final dose. They were anaesthetised using chloroform. Once each animal had entered a state of deep anaesthesia, the chest was opened, and blood was collected directly from the right ventricle of the heart using a syringe fitted with a 23G $\times$ 1-inch needle. The collected blood samples were transferred into gel serum-separator tubes and maintained at room temperature for 30 minutes. The samples were subsequently centrifuged at 1000 rpm for 20 minutes to separate the serum. The obtained serum was collected and stored at $-20\,^{\circ}\mathrm{C}$ until it was used for biochemical analysis.

Health checks were performed using the Vetube 30 chemistry analyser, which is intended for the *in vitro* quantitative determination of blood urea (BUN), creatinine (CREA), and electrolytes, including sodium (Na^{+}), potassium (K^{+}), and

chloride (Cl^-). The blood samples were allowed to clot at room temperature and were subsequently centrifuged to obtain the serum. The serum samples were analysed using the Mindray Animal Care Comprehensive Diagnosis Panel on the Vet Xpert analyser system (Mindray Animal Medical Technology, China).

Approximately 100 μL of serum was carefully dispensed into the sample port of the disposable reagent disc in accordance with the manufacturer's instructions. The loaded reagent disc was then inserted into the analyser, where all biochemical reactions and measurements were performed automatically. The analyser uses dry-chemistry and integrated optical-detection technologies to quantify multiple biochemical and electrolyte parameters simultaneously.

Standardised enzyme-linked immunosorbent assay (ELISA) kits were used to measure the serum levels of glutathione (GSH), malondialdehyde (MDA), and NADPH oxidase (NOX). The kits described below were used in accordance with their intended applications:

- **GSH:** ELISA kit (catalogue no. E-EL-0026, Elabscience, Wuhan, China), approved for rat specimens, with a sensitivity of 0.38 ng/mL, an intra-assay coefficient of variation (CV) of $< 8\%$, and an inter-assay CV of $< 10\%$.
- **MDA:** ELISA kit (catalogue no. E-EL-0060, Elabscience, Wuhan, China), approved for rat specimens, with a sensitivity of 0.94 ng/mL, an intra-assay CV of $< 8\%$, and an inter-assay CV of $< 10\%$.

For the histopathological investigation, kidney tissues were preserved in 10% formalin for approximately four days to achieve optimal fixation. The specimens were subsequently dehydrated using graded concentrations of ethanol, cleared with xylene, and embedded in paraffin wax. The paraffin-embedded tissues were sectioned using a microtome to obtain slices approximately 4–5 μm thick. Serum was collected as described above, and the tissue sections mounted on glass slides were stained with haematoxylin and eosin (H&E) for histological evaluation. Microscopic examination of the stained sections was performed using a Genex light microscope at $20\times$ magnification.

2.5. STATISTICAL ANALYSIS

IBM SPSS Statistics, version 26.0, and GraphPad Prism, version 7.0 (USA), were used for the statistical analyses. The Shapiro–Wilk test was used to determine whether the data were normally distributed, while Levene's test was used to assess the homogeneity of variances. Non-normally distributed data, including the histopathological scores, are presented as the median and interquartile range (IQR), whereas normally distributed data are presented as the mean $\pm$ standard deviation (SD).

One-way ANOVA was performed to compare the mean values among the five experimental groups for the biochemical indicators, including serum creatinine, BUN, GSH, MDA, NADPH oxidase, and electrolyte levels. Fisher's least significant difference (LSD) test was conducted for post hoc pairwise comparisons when the overall ANOVA indicated a statistically significant difference ($p < 0.05$). For the prespecified analyses, which involved comparing all treatment groups with the glycerol-induced group and comparing the combined-treatment group with each monotherapy group, the LSD test was selected because of its balance between statistical power and Type I error control. To illustrate the minimum difference required to achieve statistical significance at $\alpha = 0.05$, the tables present the corresponding least significant difference values.

The Kruskal–Wallis H test was used to investigate differences among the groups in the histopathological scoring data, which were ordinal and nonparametric. Dunn's post hoc test with Bonferroni correction for multiple comparisons was performed for pairwise group analyses when a statistically significant difference ($p < 0.05$) was identified.

Exact p-values are reported for the principal comparisons, together with 95% confidence intervals where appropriate. Effect sizes for the major outcomes were calculated using Cohen's f for parametric data and eta-squared (η^2) for nonparametric data. For each type of statistical analysis, the threshold for statistical significance was established at $p < 0.05$.

3. RESULTS

3.1. EFFECT OF TREATMENT ON RENAL FUNCTION MARKERS

The induction of renal injury using glycerol (G2) resulted in profound elevations in serum creatinine (CREA) and blood urea nitrogen (BUN) levels compared with the control group (G1), indicating severe renal impairment ($p < 0.05$).

Treatment with carvedilol (G3) or sodium copper chlorophyllin (SCC) (G4) significantly attenuated these elevations compared with the glycerol-treated group. Notably, the combination therapy (G5: carvedilol + SCC) demonstrated a superior renoprotective effect, reducing CREA and BUN levels to near-baseline values. Statistical analysis revealed that G5 was significantly more effective than carvedilol monotherapy (G3) and SCC monotherapy (G4) in restoring BUN levels ($p < 0.05$), highlighting the therapeutic potential of the combined treatment. These results are presented in Table 1 and Figure 1.

Table 1. *Effects of treatment with sodium copper chlorophyllin (SCC; 50 mg/kg) and carvedilol (2.5 mg/kg/day) on serum creatinine and BUN levels*

Group	CREA Mean ± SD (μmol/L)	BUN Mean ± SD (mmol/L)
G1 (control)	33.42 ± 8.802102	6.632 ± 0.61739
G2 (glycerol)	503.2 ± 210.1959^{a}	68.314 ± 9.942735^{a}
G3 (carvedilol + glycerol)	224.46 ± 120.8794^{ab}	40.438 ± 25.6239^{ab}
G4 (SCC + glycerol)	63.26 ± 24.66532^{bc}	29.42 ± 19.4805^{abc}
G5 (carvedilol + SCC + glycerol)	46.74 ± 9.791221^{bc}	$8.938 \pm 6.211463^{abcd}$
LSD value (0.05)	150.3	20.48

$^{a}p < 0.05$ versus G1 (control); $^{b}p < 0.05$ versus G2 (glycerol); $^{c}p < 0.05$ versus G3 (carvedilol + glycerol); $^{d}p < 0.05$ versus G4 (SCC + glycerol).

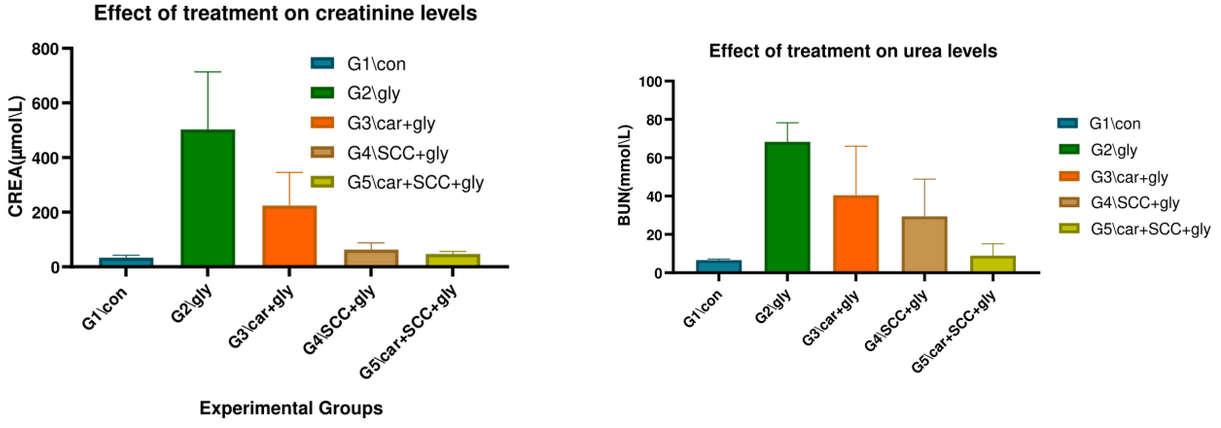

Figure 1. *Effects of treatment with sodium copper chlorophyllin (SCC; 50 mg/kg) and carvedilol (2.5 mg/kg/day) on serum creatinine and BUN levels*

3.2. IMPACT ON OXIDATIVE STRESS AND ANTIOXIDANT STATUS

Glycerol administration (G2) induced substantial oxidative stress, as evidenced by a significant decrease in glutathione (GSH) levels and concomitant increases in NADPH oxidase activity and malondialdehyde (MDA) levels compared with the control group ($p < 0.05$). These results are presented in Table 2 and Figure 2.

Table 2. *Effects of treatment with sodium copper chlorophyllin (SCC; 50 mg/kg) and carvedilol (2.5 mg/kg/day) on oxidative stress parameters*

Group	GSH (μg/mL) Mean ± SD	NADPH oxidase (U/L) Mean ± SD	MDA (ng/mL) Mean ± SD
G1 (control)	55.98 ± 2.48	24.82 ± 3.64	3.37 ± 1.38
G2 (glycerol)	34.89 ± 3.59^{a}	85.73 ± 3.86^{a}	19.65 ± 4.69^{a}
G3 (carvedilol + glycerol)	49.93 ± 1.73^{ab}	52.51 ± 2.34^{ab}	16.67 ± 5.78^{a}
G4 (SCC + glycerol)	73.318 ± 0.52^{abc}	44.21 ± 2.37^{abc}	12.19 ± 1.41^{ab}
G5 (carvedilol + SCC + glycerol)	68.49 ± 1.48^{abc}	34.13 ± 1.61^{abcd}	8.96 ± 1.96^{abc}
LSD value (0.05)	6.01	8.3	4.24

$^{a}p < 0.05$ versus G1 (control); $^{b}p < 0.05$ versus G2 (glycerol); $^{c}p < 0.05$ versus G3 (carvedilol + glycerol); $^{d}p < 0.05$ versus G4 (SCC + glycerol).

Antioxidant recovery: GSH levels were significantly preserved in all treatment groups (G3, G4, and G5) compared with the glycerol-treated group (G2). Interestingly, SCC monotherapy (G4) and the combined treatment (G5) resulted in GSH levels that were significantly higher than those observed in the carvedilol monotherapy group (G3).

Oxidative inhibition: NADPH oxidase activity, a key driver of superoxide production, was suppressed most effectively in the combined-treatment group (G5), showing significant differences from all other glycerol-treated groups (G2, G3, and G4).

Lipid peroxidation: MDA levels followed a similar downward trend, with G5 achieving the lowest concentration among the treated groups, indicating a robust reduction in lipid peroxidation.

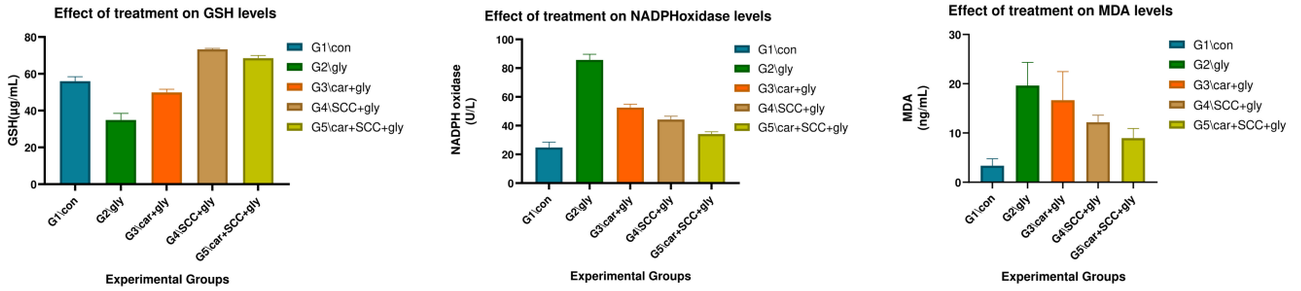

Figure 2. *Effects of treatment with sodium copper chlorophyllin (SCC; 50 mg/kg) and carvedilol (2.5 mg/kg/day) on oxidative stress parameters*

3.3. EFFECT ON SERUM ELECTROLYTE BALANCE

Glycerol-induced renal injury was associated with significant electrolyte imbalances characterised by hyponatraemia, hypochloraemia, and hyperkalaemia ($p < 0.05$), as presented in Table 3 and Figure 3.

Table 3. *Effects of treatment with sodium copper chlorophyllin (SCC; 50 mg/kg) and carvedilol (2.5 mg/kg/day) on serum electrolyte balance*

Group	Electrolytes		
	Na^+ Mean ± SD (mmol/L)	K^+ Mean ± SD (mmol/L)	Cl^- Mean ± SD (mmol/L)
G1 (control)	146.76 ± 2.580	7.24 ± 0.8203	106.3 ± 4.4
G2 (glycerol)	136.76 ± 3.114^a	8.36 ± 0.7155^a	95.26 ± 6.061^a
G3 (carvedilol + glycerol)	140.66 ± 4.482^a	8.3 ± 1.292^a	92.68 ± 8.803^a
G4 (SCC + glycerol)	139.7 ± 3.120^a	6.58 ± 2.893^{bc}	98 ± 9.006^a
G5 (carvedilol + SCC + glycerol)	143.34 ± 4.234^b	7.6 ± 1.292^{bcd}	108.6 ± 2.281^{bcd}
LSD value	4.724493	0.490285	8.749516

Data were analysed using one-way ANOVA followed by the LSD post hoc test. $^a p < 0.05$ versus G1 (control); $^b p < 0.05$ versus G2 (glycerol); $^c p < 0.05$ versus G3 (carvedilol + glycerol); $^d p < 0.05$ versus G4 (SCC + glycerol).

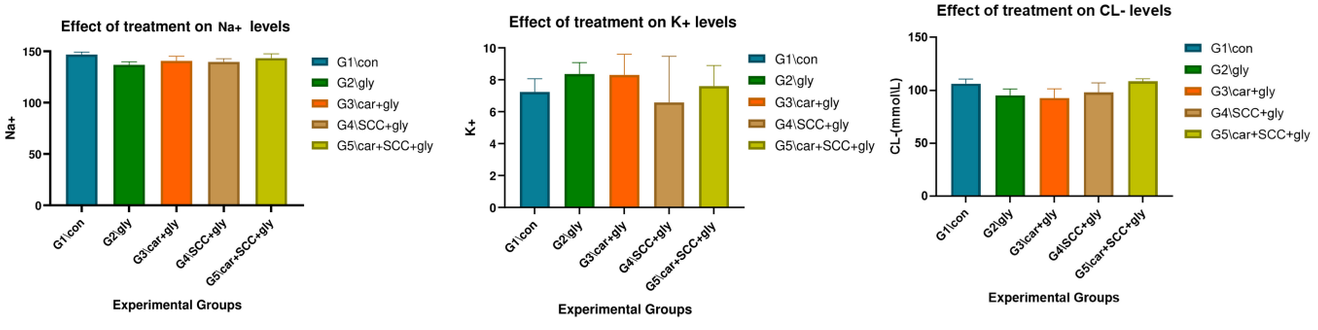

Figure 3. *Effects of treatment with sodium copper chlorophyllin (SCC; 50 mg/kg) and carvedilol (2.5 mg/kg/day) on serum electrolyte balance*

Sodium and chloride (Na^+ and Cl^-): While the monotherapy groups (G3 and G4) showed limited recovery of sodium and chloride levels, the combination of carvedilol and SCC (G5) significantly restored these electrolyte levels compared with the glycerol-treated group (G2).

Potassium (K^+): The elevation in serum potassium observed in G2 and G3 was significantly mitigated in the SCC-treated groups (G4 and G5). Specifically, G5 showed significant differences compared with G2, G3, and G4, suggesting that the combined treatment optimises the homeostatic regulation of potassium during renal stress.

3.4. HISTOPATHOLOGICAL INVESTIGATION OF THE KIDNEY

The control rats (G1) exhibited minimal interstitial cellularity, an absence of tubular necrosis, and normal glomerular and tubular architecture with intact brush borders. The median overall histopathological score of 0 (IQR: 0–0.5) indicated no evidence of renal damage. In contrast, the rats in the AKI-induced group (G2) exhibited signs of severe renal damage, particularly extensive acute tubular necrosis, considerable loss of tubular epithelial cells accompanied by degeneration of the basement membranes, multiple pigmented casts within the tubular lumina, significant interstitial oedema, and evident inflammatory cell infiltration. The median overall histopathological score was 11 (IQR: 9.5–12), which was significantly

higher than that of the control group ($p < 0.001$).

Rats treated with carvedilol at 2.5 mg/kg/day (G3) exhibited a modest improvement in renal histology. Tubular necrosis, cast formation, interstitial oedema, and inflammatory infiltration were all reduced. A partial renoprotective effect was demonstrated by the median total score of 7 (IQR: 4–8), which was significantly lower than that of the glycerol-treated group ($p < 0.05$).

Rats treated with sodium copper chlorophyllin at 50 mg/kg (G4) exhibited a significantly greater histopathological improvement than rats treated with carvedilol alone. The renal sections showed minimal oedema, infrequent casts, and minor focal tubular damage. Improved renoprotection was indicated by the median total score of 4 (IQR: 2–5), which was significantly lower than the scores recorded in both the glycerol-treated group ($p < 0.001$) and the carvedilol-treated group ($p < 0.05$).

The greatest histological improvement was observed in rats treated with a combination of carvedilol at 2.5 mg/kg/day and sodium copper chlorophyllin at 50 mg/kg (G5). Renal architecture was largely preserved, with intact tubular epithelium, minimal or absent cast formation, and a low level of interstitial inflammation. The median overall histopathological score was 0.5 (IQR: 0–2), a value comparable to that of the control group ($p = 0.742$) and significantly lower than those of the other glycerol-treated groups (G2, G3, and G4; $p < 0.05$ for each comparison). These histopathological findings are illustrated in Figure 4.

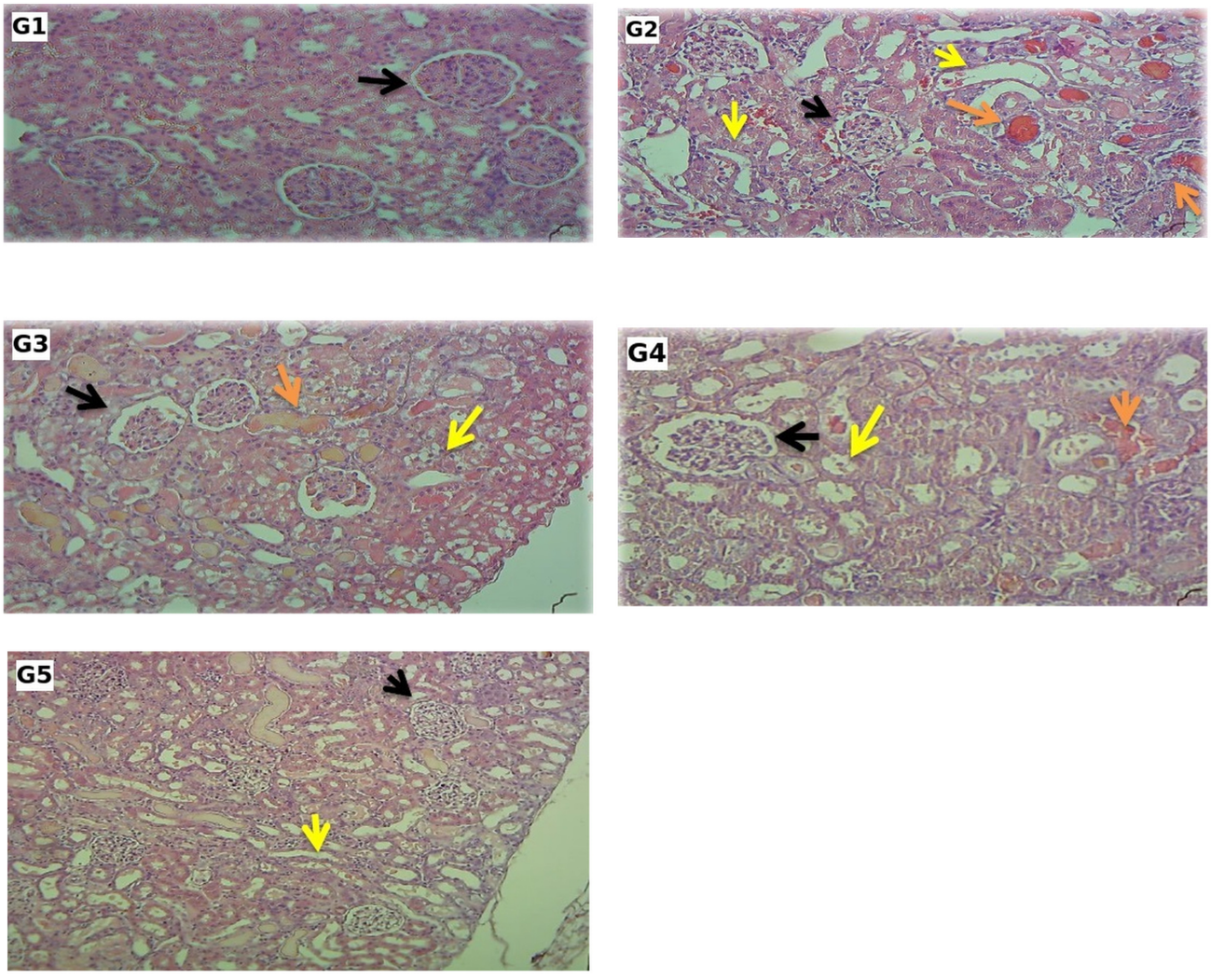

Figure 4. *Photomicrographs of renal sections stained with haematoxylin and eosin (H&E) at* $10\times$ *magnification. Glomeruli are indicated by black arrows, renal tubules by yellow arrows, and congestion by red arrows. G1: control; G2: glycerol; G3: carvedilol + glycerol; G4: SCC + glycerol; G5: carvedilol + SCC + glycerol*

For each histopathological parameter and the overall histopathological score, the Kruskal–Wallis analysis revealed significant differences among the groups ($p < 0.001$ for all parameters). Consistent with the biochemical findings, Dunn's post hoc analysis confirmed that the combined-treatment group (G5) demonstrated significantly better histopathological

outcomes than both monotherapy groups.

Table 4. *Semiquantitative histopathological scoring of renal injury parameters*

Parameter	G1 (Control)	G2 (Glycerol)	G3 (Carvedilol + Glycerol)	G4 (SCC + Glycerol)	G5 (Carvedilol + SCC + Glycerol)	p-value
Tubular necrosis	0 (0–0)	$3\,(3--3)^{a}$	$2\,(1--2)^{ab}$	$1\,(1--1.5)^{abc}$	$0.5\,(0--1)^{abcd}$	< 0.001
Cast formation	0 (0–0)	$3\,(2.5--3)^{a}$	$2\,(1--2.5)^{ab}$	$1\,(0.5--1)^{abc}$	$0\,(0--0.5)^{abcd}$	< 0.001
Interstitial oedema	0 (0–0)	$2.5\,(2--3)^{a}$	$1.5\,(1--2)^{ab}$	$1\,(0.5--1.5)^{abc}$	$0\,(0--1)^{abcd}$	< 0.001
Inflammatory infiltration	0 (0–0.5)	$2.5\,(2--3)^{a}$	$1.5\,(1--2)^{ab}$	$1\,(0.5--1)^{abc}$	$0\,(0--0.5)^{abcd}$	< 0.001
Total score	0 (0–0.5)	$11\,(9.5--12)^{a}$	$7\,(4--8)^{ab}$	$4\,(2--5)^{abc}$	$0.5\,(0--2)^{abcd}$	< 0.001

Data are presented as the median (interquartile range, IQR), with $n = 7$ animals per group. The Kruskal–Wallis H test was followed by Dunn's post hoc test. $^{a}p < 0.05$ versus G1 (control); $^{b}p < 0.05$ versus G2 (glycerol); $^{c}p < 0.05$ versus G3 (carvedilol + glycerol); $^{d}p < 0.05$ versus G4 (SCC + glycerol).

4. DISCUSSION

The accumulation of nitrogenous waste products and a marked reduction in the glomerular filtration rate are typical manifestations of acute kidney injury (AKI). One of the most widely used experimental methods for evaluating potential therapies for AKI is the glycerol-induced rhabdomyolysis model.

Identifying medicines that can reverse or mitigate AKI remains a primary objective because AKI is considered an important clinical consequence of rhabdomyolysis [10]. In the present study, serum creatinine (approximately 503 μmol/L) and BUN (approximately 68 mmol/L) were substantially higher in the glycerol-treated group than in the control group, demonstrating tubular dysfunction accompanied by a significant reduction in the glomerular filtration rate (GFR). This finding is consistent with observations from models of glycerol-induced rhabdomyolysis in which acute renal failure develops as a consequence of oxidative tubular damage [8].

The carvedilol-treated group (G3) exhibited reductions in serum creatinine to approximately 224 μmol/L and BUN to approximately 40 mmol/L, which may indicate attenuation of renal damage. Previous experimental evidence has shown that carvedilol, a nonselective β-blocker with recognised antioxidant activity, preserves kidney function by minimising oxidative stress and lipid peroxidation, thereby reducing blood creatinine and urea levels in models of AKI [5]. Similarly, compared with carvedilol alone, SCC treatment (G4) produced a more pronounced reduction in BUN to approximately 29 mmol/L and creatinine to approximately 63 μmol/L, suggesting improved preservation of renal function. Previous studies conducted using chronic kidney disease models have revealed that SCC substantially improves kidney function and reduces oxidative stress and inflammation, suggesting a renoprotective benefit. However, specific evidence concerning the effects of SCC in rhabdomyolysis models remains limited [11]. Interestingly, creatinine and BUN reached approximately 47 μmol/L and 9 mmol/L, respectively, and approached the corresponding control values when carvedilol and SCC were administered together in G5, resulting in nearly complete recovery of renal functional capacity. This enhanced effect may be explained by the complementary antioxidant activities of the two agents: SCC scavenges reactive oxygen species and modulates inflammatory regulators, whereas carvedilol inhibits ROS formation. The interaction of these activities may therefore provide improved renal protection.

Oxidative stress has a substantial influence on the mechanism underlying glycerol-induced AKI, and renal viability requires an appropriate balance between pro-oxidant and antioxidant systems [12]. Glutathione (GSH) is a key component of intracellular antioxidant protection, and its depletion may trigger the iron-dependent formation of reactive oxygen species (ROS), particularly lipid ROS, which can damage cells and kidney tissue [13]. In the present study, the glycerol-treated group (G2) exhibited significantly lower GSH levels than the control group (G1), a finding consistent with the reduced antioxidant potential observed in models of oxidative renal damage. Treatment with carvedilol (G3) and SCC (G4) substantially increased GSH levels, reflecting the restoration of antioxidant defences, whereas the combination group (G5) showed the greatest degree of recovery. This outcome is consistent with earlier research demonstrating that carvedilol maintains normal GSH levels by activating the Nrf2 pathway [5], whereas SCC enhances GSH levels primarily by scavenging free radicals [7]. The concurrent effect observed in G5 suggests that combined antioxidant therapies can improve the restoration of intracellular GSH concentrations while also strengthening the natural renal defence systems against oxidative damage.

In several clinical conditions, including rhabdomyolysis and AKI, NADPH oxidase (NOX) is an important enzymatic generator of superoxide and other reactive oxygen species [13]. The increased NOX activity observed in the glycerol-treated group was associated with increased ROS generation, thereby contributing to lipid peroxidation, enhanced oxidative injury, and cellular destruction. NADPH oxidase activity decreased significantly following treatment with carvedilol (G3) or SCC (G4), reflecting a reduction in ROS generation. The combination group (G5) exhibited the most pronounced effect. The enhanced renoprotection observed in G5 may be attributed to additive or complementary suppression of ROS-generating pathways, as indicated by the significant reduction in NOX activity following the combined treatment. These outcomes agree with studies showing that SCC modulates redox-sensitive signalling mechanisms [11] and that carvedilol reduces NOX4 expression [1]. The simultaneous suppression of NOX activity may therefore represent an important mechanism

through which the combined treatment reduces oxidative damage and promotes improved kidney function.

Malondialdehyde (MDA), a product of lipid peroxidation, is a commonly used marker of oxidative membrane damage [14]. Consistent with other findings obtained using rhabdomyolysis models, the increased MDA levels in the glycerol-treated group confirmed the presence of severe lipid peroxidation caused by increased ROS production [8]. The reduction in MDA levels in both the SCC-treated group (G4) and the carvedilol-treated group (G3) demonstrated a decline in lipid peroxidation, which was consistent with an increase in antioxidant capacity. Among the treated groups, the combination group (G5) showed significantly reduced MDA levels and had the lowest recorded values, suggesting strong protection against oxidative membrane damage. This evidence provides further support for the proposition that treatment with carvedilol and SCC can provide antioxidant protection by effectively neutralising ROS and preventing cellular damage associated with lipid peroxidation. The improved management of electrolytes and enhanced tubular efficiency observed in the combination group are also probably attributable, at least in part, to the preservation of membrane integrity.

Normal renal physiology depends on electrolyte equilibrium, and AKI is frequently associated with abnormalities in sodium (Na^+), potassium (K^+), and chloride (Cl^-) concentrations [15]. In the present investigation, glycerol administration produced considerable electrolyte disturbances, including hyponatraemia, hypochloraemia, and hyperkalaemia, reflecting impaired renal tubular function. The results presented here are consistent with clinical evidence from patients with rhabdomyolysis, in whom AKI is frequently accompanied by electrolyte imbalances [16]. Hyperkalaemia is possibly caused by impaired glomerular filtration and reduced potassium excretion, which are further aggravated by the release of cellular potassium from injured muscle tissue. The reported reduction in sodium levels probably reflects diminished tubular reabsorption capacity and inadequate renal concentrating function [8].

Compared with the glycerol-treated group, administration of carvedilol (G3) resulted in modest increases in both sodium and chloride concentrations. However, these values remained considerably lower than those recorded in the control group. In addition to its antioxidant and anti-inflammatory properties, the nonselective β-adrenergic blocking action of carvedilol may reduce oxidative stress and renal tissue injury, thereby improving electrolyte regulation while preserving tubular function [19]. Nevertheless, potassium levels in the carvedilol-treated group remained comparatively high, indicating that the protective role of carvedilol might not be sufficient to restore renal electrolyte balance completely in this experimental model. According to earlier research, the α_1-adrenergic blocking effect of carvedilol might promote intracellular potassium retention, which may contribute to hypokalaemia under certain circumstances [9]. The residual hyperkalaemia identified in G3 may reflect the severity of kidney impairment and the limitations of monotherapy in fully restoring potassium homeostasis.

In contrast to the glycerol-treated group, the SCC-treated group (G4) exhibited a different pattern of electrolyte response, with lower potassium concentrations. This result could be attributed to the potent antioxidant properties of SCC, which are believed to promote cellular redox equilibrium and facilitate the scavenging of reactive oxygen species [23]. By minimising oxidative stress within renal tissues, SCC may help preserve tubular architecture and improve the ability of the kidneys to regulate potassium homeostasis [11]. The observation that SCC restored normal potassium levels more successfully than carvedilol alone indicates that antioxidant-mediated tubular protection may be particularly important for potassium homeostasis. However, the sodium and chloride concentrations in G4 remained close to the control levels, indicating that tubular function was only partially restored following SCC monotherapy.

The combination treatment (G5) produced the most complete restoration of electrolyte balance, with sodium, potassium, and chloride concentrations becoming relatively close to the corresponding control values. This improved effect is probably explained by the combined benefits of the haemodynamic and antioxidant activities of carvedilol and the potent anti-inflammatory and free radical-scavenging properties of SCC [9, 23]. The restoration of electrolyte balance in G5 indicates that the combined treatment effectively preserved tubular integrity and function, thereby facilitating normal electrolyte reabsorption and excretion. This outcome supports the concept that complementary mechanisms underlie the more pronounced renoprotective benefits of the combined treatment, as also reflected by the observed improvements in oxidative stress measurements and renal function markers in the combination group.

The biochemical findings were supported by the morphological evidence obtained from the histopathological evaluation. Extensive interstitial oedema, widespread cast formation, severe acute tubular necrosis, and evident inflammatory cell infiltration were observed in the glycerol-treated group (G2), indicating severe renal damage. These histological changes reflect the cytotoxic effects of myoglobin and free iron on renal tubular epithelial cells, which are further aggravated by oxidative stress and the inflammatory response [4]. Although the carvedilol regimen (G3) produced a modest improvement, with reductions in tubular necrosis and cast formation, SCC treatment (G4) resulted in more evident histological repair, characterised by minor focal tubular degeneration and mild inflammation. The combined treatment in G5 produced nearly normal renal histology relative to that of the control group, including intact tubular epithelium, minimal cast formation, and mild interstitial inflammation. Consistent with the biochemical evidence of improved renoprotection, the semiquantitative scoring confirmed that the combination group had significantly lower histopathological scores than both monotherapy groups.

The complementary modes of action of carvedilol and SCC may be responsible for the beneficial nephroprotective effects identified in this investigation. While SCC neutralises existing free radicals and modulates immune responses, carvedilol reduces ROS generation and inhibits inflammatory signalling through its intrinsic antioxidant activity and β-adrenergic blocking action [5, 7]. By using carvedilol to suppress ROS production and SCC to neutralise the reactive species that are generated, the combination of these agents targets oxidative stress at multiple levels and produces broader suppression of oxidative damage. In addition, the anti-inflammatory action of SCC may prevent leukocyte infiltration and tissue damage, while the vasodilatory mechanisms of carvedilol may improve renal blood flow. Consequently, the combination strategy provides greater renal protection by addressing both the production of ROS and the subsequent consequences of oxidative stress.

The observations of the present study may have implications for the management of AKI caused by rhabdomyolysis. Currently, no approved pharmacological treatments are available specifically for RIAKI, and nearly all available treatments are supportive [1]. In this preclinical model, combined therapy with carvedilol and SCC effectively reduced renal damage, indicating that this combination may represent a potential therapeutic approach. Carvedilol is a well-established therapeutic agent with a favourable safety profile, while SCC is currently used as a food additive and nutraceutical and has an established record of safety in humans [6]. Because the safety profiles and pharmacokinetic properties of these agents have already been extensively investigated, adapting them for the treatment of AKI could facilitate and accelerate their clinical translation.

5. LIMITATIONS

Several limitations of this study should be considered. The absence of assays for myoglobin and serum creatine kinase limited the direct confirmation and assessment of rhabdomyolysis. Urinary biomarkers and urine output were not evaluated. In addition, a relatively small sample size ($n = 7$ per group) was used, and the histopathological examination was conducted without confirmatory staining methods, such as Perls' Prussian blue staining or myoglobin immunohistochemistry. Because only female rats were included in the study, the broader applicability and generalisability of the findings are limited. Furthermore, the acute experimental model does not reflect the chronic manifestations of kidney injury. No dose–response studies were conducted to determine whether different doses of carvedilol or SCC would produce different degrees of nephroprotection. More extensive and sex-balanced investigations using comprehensive biomarkers and confirmatory histopathological methods are therefore required to validate these findings.

6. CONCLUSION

This study demonstrated that glycerol-induced AKI in female rats was associated with severe oxidative stress, electrolyte imbalance, and renal impairment. Carvedilol and SCC, particularly when administered in combination, demonstrated potential nephroprotective effects by reducing oxidative stress markers, including MDA and NADPH oxidase; preserving antioxidant capacity through GSH; restoring renal function, as indicated by creatinine and BUN levels; and improving electrolyte balance. These biochemical findings were further supported by the histopathological investigation.

The complementary antioxidant and anti-inflammatory mechanisms of carvedilol and SCC—with carvedilol regulating ROS generation and SCC scavenging free radicals—probably account for the observed beneficial effects. Despite the limitations of the study, these findings indicate that combined antioxidant treatment warrants further investigation using standardised experimental models, comprehensive biomarkers, dose–response assessments, and larger, sex-balanced samples to determine its therapeutic efficacy in reducing acute kidney damage associated with rhabdomyolysis.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to the conception and design of the study, experimental work, data analysis and interpretation, and preparation of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for the integrity of the work.

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DATA AVAILABILITY

The data generated and analysed during this study are available from the corresponding author upon reasonable request.

ETHICAL STATEMENT

All animal experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and Council Directive 86/609/EEC. The experimental protocol was reviewed and approved by the Animal Ethics Committee of the College of Pharmacy, University of Basrah (Approval No. EC 36, dated 11 December 2024).

INFORMED CONSENT

Not applicable, as this study did not involve human participants.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this manuscript, the authors used ChatGPT to improve the linguistic quality and readability of the content. After using this AI tool, the authors comprehensively reviewed and revised the manuscript to ensure its accuracy, integrity, and scientific rigour. All authors bear full responsibility for the final content of the published work, including any errors or inconsistencies that may remain.

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