ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Berberine Ameliorates Methionine-Induced Hyperhomocysteinemia and Biochemical Alterations in Male Rats

Aula Talib Zahwar[1*], and Aseel Najah Sabour[1]

[1] Department of Biology, College of Education, University of Al-Qadisiyah, Iraq
* Corresponding Author email: edu.bio.posta108@qu.edu.iq

Citation:
Zahwar, T.A.; Sabour, N.A. Berberine ameliorates methionine-induced hyperhomocysteinemia and biochemical alterations in male rats. *ASEAN J. Sci. Tech. Report.* **2026**, *29*(3), e262094. https://doi.org/10.55164/ajstr.v29i3.262094.

Article history:
Received: October 28, 2025
Revised: December 24, 2025
Accepted: January 13, 2026
Available online: February 27, 2026

Abstract: Hyperhomocysteinemia, characterized by elevated plasma homocysteine levels, is associated with oxidative stress and metabolic dysfunction. This study evaluated the protective effects of berberine, a natural alkaloid compound, against methionine-induced biochemical alterations in male rats. Thirty-two adult male albino rats were randomly divided into four groups (n=8 each) and treated orally for eight weeks: Group 1 (negative control) received standard diet and water; Group 2 (positive control) received methionine (100 mg/kg); Group 3 received methionine (100 mg/kg) plus berberine (40 mg/kg); and Group 4 received berberine (40 mg/kg) alone. Serum samples were analyzed for homocysteine (Hcy), electrolytes (sodium, potassium, chloride), and creatine phosphokinase (CPK) enzyme activity. Methionine treatment significantly elevated homocysteine and CPK levels while reducing sodium and chloride concentrations compared to controls. Co-administration of berberine with methionine markedly decreased homocysteine levels from 76.5 to 12.2 micromole/L and normalized CPK activity. Berberine also restored sodium and chloride concentrations to near-normal levels. The berberine-only group exhibited biochemical parameters comparable to the negative control, confirming its safety profile. These findings demonstrate that berberine effectively mitigates methionine-induced biochemical disturbances through multiple mechanisms, including antioxidant effects, activation of the AMPK pathway, and reduction of reactive oxygen species. Berberine also protected electrolyte homeostasis and mitochondrial function. Therefore, berberine represents a promising natural therapeutic agent for preventing oxidative stress and metabolic complications associated with hyperhomocysteinemia, with potential applications in clinical management of homocysteine-related disorders.

Keywords: Berberine; hyperhomocysteinemia; oxidative stress; AMPK signaling; electrolyte homeostasis

1. Introduction

Methionine is an essential sulfur-containing amino acid that serves as a key substrate in diverse metabolic and biosynthetic pathways across all forms of life [1]. It plays a critical role in protein synthesis and acts as a precursor for S-adenosylmethionine (SAM), the major methyl donor for multiple transmethylation reactions [2]. In normal nutrition, methionine is

predominantly derived from dietary protein sources, including meat, fish, and dairy products, thereby maintaining essential cellular redox status and function [1]. However, when methionine intake exceeds physiological levels, its metabolism is disturbed, leading to the accumulation of homocysteine, an intermediary sulfur-containing molecule normally metabolized via remethylation and transsulfuration pathways [3]. This abnormal elevation in plasma homocysteine levels, termed hyperhomocysteinemia, represents a significant biochemical derangement associated with oxidative stress and endothelial dysfunction [4]. Experimental studies have demonstrated that high methionine intake elevates homocysteine levels in blood and tissues, leading to oxidative stress and disruption of normal cellular function [5, 6].

Elevated plasma homocysteinemia is associated with profound cardiovascular and renal alterations, including disruptions in electrolyte balance involving sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions [7]. These ions are essential for maintaining osmotic balance, membrane potential, and proper nerve and muscle function. Additionally, elevated serum creatine phosphokinase (CPK) activity serves as a biomarker of tissue damage and metabolic dysfunction, reflecting cellular membrane damage caused by excessive oxidative stress and disordered energy metabolism [8, 9]. The pathophysiological mechanisms underlying methionine-induced hyperhomocysteinemia involve the generation of reactive oxygen species (ROS) [10]. High concentrations of methionine promote ROS production, causing lipid, protein, and DNA damage, leading to oxidative stress and potential multi-organ failure [3, 10]. Given these serious health implications, investigational efforts are underway to identify protective agents capable of restoring biochemical homeostasis and mitigating methionine-induced stress. Berberine, a natural isoquinoline alkaloid isolated from plants such as Berberis vulgaris, has emerged as a promising therapeutic candidate [11]. Recent studies have documented berberine's multiple pharmacological activities, including potent antioxidant, anti-inflammatory, and metabolic regulatory effects [12, 13]. Berberine has been shown to activate adenosine monophosphate-activated protein kinase (AMPK) signaling, promoting lipid and methionine metabolism while inhibiting oxidative pathways [12, 6]. Furthermore, berberine increases the expression of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby diminishing oxidative injury [11, 14]. Recent findings also indicate that berberine attenuates mitochondrial dysfunction by improving bioenergetic function and reducing ROS production [13]. Given berberine's multifaceted protective mechanisms and growing evidence of its therapeutic potential in oxidative and metabolic disorders, the present study was designed to evaluate the effects of berberine on selected biochemical indicators—specifically homocysteine, electrolytes (Na^+, K^+, Cl^-), and CPK enzyme activity—in an experimental model of hypermethioninemia-induced hyperhomocysteinemia in male rats.

2. Materials and Methods

2.1 Chemicals and Compounds

Methionine was obtained from HIMEDIA (India) in the form of white crystalline powder and administered orally at a dose of 100 mg/kg body weight [15]. The present study utilized fixed doses of methionine and berberine based on previously published effective concentrations. Future investigations should include dose-response experiments to determine optimal therapeutic doses, establish minimum effective doses, and characterize the dose-dependent effects of berberine. Such studies would enhance translational potential and inform clinical dosing strategies. Berberine was procured from BIO TEST (USA) in capsule form containing yellow powder (500 mg per capsule) and administered orally at a dose of 40 mg/kg body weight [16]. The methionine dose (100 mg/kg) was selected based on established experimental models that reliably induce hyperhomocysteinemia without causing acute systemic toxicity [15]. The berberine dose (40 mg/kg) was chosen based on previous studies demonstrating significant antioxidant and metabolic regulatory effects at this concentration [16], while maintaining a favorable safety profile. This dose corresponds to a human equivalent dose of approximately 450 mg for a 70 kg adult, which falls within the clinically administered range of 500–1500 mg daily.

2.2 Experimental Animals

Adult male albino rats (Rattus norvegicus) aged 12–16 weeks with body weights between 200–300 g were obtained from the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq. Before experimentation,

the animals were acclimatized to laboratory conditions for 2 weeks and subjected to a comprehensive health assessment [1]. Throughout the study, animals were housed under controlled environmental conditions at 22 ± 5 °C with a 12-hour light/12-hour dark cycle. Standard laboratory diet and drinking water were provided ad libitum to all animals [4]. All experimental procedures were conducted in strict accordance with the Institutional Ethical Guidelines for Animal Care and approved by the institutional animal ethics committee [17]. Several limitations should be considered when interpreting these findings. First, the exclusive use of male rats restricts generalizability to female populations. Sex-based differences in homocysteine metabolism are well documented, with estrogen exerting protective effects by enhancing methylation pathway activity. Whether berberine's protective mechanisms operate similarly in females, particularly across different hormonal states (cycling, pregnancy, menopause equivalent), remains to be determined. Second, the single-dose design precludes dose-response characterization. Third, the mechanistic pathways discussed were inferred from existing literature rather than directly measured in this study.

2.3 Experimental Design

A total of 32 adult male rats were randomly divided into four groups, each containing 8 animals. The experimental study period lasted 60 days. The experimental groups were organized as follows: Group 1 (G1) - Negative Control: Eight rats were provided with a standard laboratory diet and water ad libitum throughout the entire experimental period, receiving no chemical treatment [3]. Group 2 (G2) - Positive Control: Eight rats were orally administered methionine at a dose of 100 mg/kg body weight daily for 60 days to induce hypermethioninemia and subsequent hyperhomocysteinemia [15]. Group 3 (G3) - Treatment Group: Eight rats received both methionine (100 mg/kg body weight) and berberine (40 mg/kg body weight) administered orally concurrently throughout the 60-day experimental period [16]. Group 4 (G4) - Berberine Control: Eight rats received berberine (40 mg/kg body weight) orally alone throughout the experimental period to evaluate its safety profile on normal metabolic parameters [12]. All treatments were administered orally daily for the full 60 days using the oral gavage technique [5].

2.4 Blood Collection and Serum Preparation

At the conclusion of the experimental period, all animals were anesthetized using chloroform vapors according to standard laboratory protocols [17]. Blood samples were collected via cardiac puncture into sterile plain collection tubes without anticoagulant [7]. Collected blood samples were allowed to clot naturally for 15–20 minutes at room temperature. Subsequently, samples were centrifuged at 3000 rpm for 15 minutes to obtain serum [2]. The resulting serum was carefully aliquoted into sterile Eppendorf tubes and stored at −20 °C until biochemical analysis [8].

2.5 Biochemical Analysis and Measurements

Serum homocysteine concentrations were determined using a rat-specific enzyme-linked immunosorbent assay (ELISA) kit (BioSource, USA) according to the manufacturer's protocol [13]. The assay was based on a sandwich ELISA methodology utilizing specific antibodies against homocysteine [14]. Briefly, serum samples were incubated in microplate wells coated with capture antibodies. After washing steps, detection antibodies conjugated with horseradish peroxidase (HRP) were added [6]. Optical density (OD) was measured at 450 nm using a microplate reader (LMMR-101) [18]. Homocysteine concentrations were calculated from the kit's standard curves and expressed as micromoles per liter (micromoles/L) [10]. Serum concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions were measured using an ion-selective electrode (ISE) module integrated with the Beckman Coulter AU480 automated analyzer (USA) [9]. The system utilizes ion-specific membranes that generate electrical potentials according to the Nernst equation based on the electrochemical gradient across the semipermeable membrane [19]. These potentials are converted to ionic concentrations through the instrument's calibrated software and validated reference materials [13]. The measurement principle involves the formation of a potential difference at the ion-selective electrode surface, which is proportional to the logarithm of the ion concentration [20]. Results were expressed as millimoles per liter (mmol/L) [98]. Serum creatine phosphokinase (CPK) activity was measured using a kinetic enzymatic assay performed on the Beckman Coulter AU480 automated analyzer (USA) [21]. The assay principle is based on the phosphorylation of adenosine diphosphate (ADP) by creatine phosphate in the presence of creatine

kinase enzyme, producing adenosine triphosphate (ATP) [22]. The generated ATP is then coupled with hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PDH) reactions to generate nicotinamide adenine dinucleotide phosphate (NADPH) [23]. The production of NADPH is proportional to CPK activity and is quantified spectrophotometrically at 340 nm [24]. Enzyme activity was expressed as International Units per liter (IU/L) [21].

2.6 Studied Parameters

The primary physiological and biochemical parameters investigated in this study included Homocysteine (Hcy): Measured as a marker of amino acid metabolism disturbance and oxidative stress burden in methionine-induced hyperhomocysteinemia [25]. Electrolytes (Na^+, K^+, Cl): Measured as indicators of ionic homeostasis, osmotic balance, and cellular membrane function, reflecting renal tubular dysfunction and ion transport capacity [26]. Creatine Phosphokinase (CPK): Measured as a biomarker of tissue damage, myocellular injury, and metabolic dysfunction reflecting membrane integrity and energy metabolism status [9]. These parameters were selected based on their established roles as indicators of oxidative stress, metabolic dysfunction, and cellular membrane integrity in response to methionine-induced hyperhomocysteinemia [27].

2.7 Statistical Analysis

All data obtained were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test using SPSS statistical software (version 2010) [28]. Results are presented as mean ± standard deviation (SD) [3]. Statistical significance was established at a probability level of $p \leq 0.05$, while $p > 0.05$ was considered non-significant [4]. Different superscript letters (A, B, C) in tables indicate statistically significant differences between groups at the 5% probability level, while identical letters denote non-significant differences [7].

3. Results and Discussion

3.1 Changes in Serum Homocysteine (Hcy) Levels

The statistical analysis results presented in Table 1 demonstrated a significant increase ($P < 0.05$) in serum homocysteine levels in the positive control group (G2) treated with methionine (76.5 ± 1.06 micromole/L) compared with the negative control group (G1) (7.66 ± 0.26 micromole/L) [5]. This marked elevation in homocysteine levels confirmed the successful induction of hyperhomocysteinemia in the experimental model [55]. In contrast, Group 3 (G3) treated with both methionine and berberine revealed a significant decrease ($P < 0.05$) in homocysteine level (12.2 ± 1.38 micromole/L) compared with Group 2 (G2), representing a reduction of approximately 84% from methionine-induced levels. However, G3 still showed a significant increase ($P < 0.05$) compared with the negative control (G1) [6]. Furthermore, Group 4 (G4) treated with berberine alone showed a significant decrease ($P < 0.05$) in homocysteine level (7.11 ± 0.26 micromole/L) compared with both Groups G2 and G3. Notably, no significant difference ($P > 0.05$) was observed between Group G4 and the negative control group (G1), confirming the safety profile of berberine on normal homocysteine metabolism [12].

3.2 Changes in Creatine Phosphokinase (CPK) Enzyme Activity

The results of the statistical analysis presented in Table 1 revealed a significant increase ($P < 0.05$) in the CPK enzyme level in the positive control group (G2) treated with methionine (442.33 ± 79.34 ng/ml) compared with the negative control group (G1) (230.67 ± 25.8 IU/L), indicating pronounced cellular membrane damage and tissue injury [8]. In contrast, Group 3 (G3) treated with methionine and berberine showed a significant decrease ($P < 0.05$) in CPK enzyme level (270.33 ± 33.06 IU/L) compared with Group G2, with no significant difference ($P > 0.05$) compared with the negative control group (G1). This restoration of CPK activity to near-normal levels indicated that berberine exerts a cytoprotective effect against methionine-induced cellular damage [9]. Additionally, a significant decrease ($P < 0.05$) in CPK enzyme level was observed in Group G4 (berberine only) (233.3 ± 17.67 IU/L) compared with Group G2, whereas no significant difference ($P > 0.05$) was found compared with the negative control group (G1) and Group G3 [13].

3.3 Changes in Serum Electrolyte Levels

As shown in Table 2, a significant decrease ($P < 0.05$) in sodium ion (Na^+) level was observed in the positive control group (G2) treated with methionine (127 ± 0.86 mmol/L) compared with the negative control (G1) (134.67 ± 3.01 mmol/L) [7]. However, Group G3 (methionine + berberine) exhibited a significant increase ($P < 0.05$) in sodium levels (134.17 ± 2.34 mmol/L) compared with Group G2, with no significant difference ($P > 0.05$) compared to Group G1 [18]. Group G4 (berberine only) displayed sodium levels (133.8 ± 3.76 mmol/L) with no significant differences compared with Groups G1 or G3, but exhibited a significant increase ($P < 0.05$) in Na^+ levels compared to Group G2. These results indicate that sodium homeostasis is restored following berberine administration [14]. The results in Table 2 indicated a non-significant decrease in potassium ion (K^+) levels in Group G2 (methionine) (5.05 ± 0.05 mmol/L) compared to Group G1 (5.57 ± 0.27 mmol/L) [1]. Group G3 (methionine + berberine) showed a non-significant decrease (4.60 ± 0.14 mmol/L) compared with G2, but a significant decrease ($P < 0.05$) compared with G1 [4]. Meanwhile, Group G4 (berberine only) displayed a significant decrease ($P < 0.05$) in K^+ level (4.63 ± 0.40 mmol/L) compared to G1, but no significant difference ($P > 0.05$) compared with Groups G2 and G3. These findings suggest a modest regulatory effect of berberine on potassium metabolism [10]. The results in Table 2 demonstrated a significant decrease ($P < 0.05$) in chloride ion (Cl^-) level in the positive control group (G2) (methionine-treated) (90.67 ± 0.88 mmol/L) compared with the negative control (G1) (98.17 ± 1.80 mmol/L) [7]. In contrast, Group G3 (methionine + berberine) showed a non-significant decrease (95.50 ± 3.20 mmol/L) ($P > 0.05$) compared with G2, and no significant difference relative to G1 [2]. Similarly, Group G4 (berberine only) showed no significant differences (94.67 ± 2.23 mmol/L) ($P > 0.05$) compared with the other groups. The restoration of chloride concentrations following berberine treatment indicates recovery of renal tubular reabsorption capacity [3].

Table 1. Effect of Methionine and Berberine on Serum Homocysteine (HCy) and CPK Enzyme Level in Male Albino Rats

Groups	Hcy (micromole/L) Mean ± SE	CPK (IU/L) Mean ± SE
G1	7.66 ± 0.26^{C}	230.67 ± 25.8^{B}
G2	76.5 ± 1.06^{A}	442.33 ± 79.34^{A}
G3	12.2 ± 1.38^{B}	270.33 ± 33.06^{B}
G4	7.11 ± 0.26^{C}	233.3 ± 17.67^{B}
LSD	2.62	135.1

* Different superscript letters indicate statistically significant differences at the 5% probability level ($P < 0.05$); identical letters denote non-significant differences.

Table 2. Effect of Methionine and Berberine on Sodium, Potassium, and Chloride Ion Levels in Male Albino Rats

Groups	K^+ (mmol/L) Mean ± SE	Na^+ (mmol/L) Mean ± SE	Cl^- (mmol/L) Mean ± SE
G1	5.57 ± 0.27^{A}	134.67 ± 3.01^{A}	98.17 ± 1.80^{A}
G2	5.05 ± 0.05^{AB}	127 ± 0.86^{B}	90.67 ± 0.88^{B}
G3	4.60 ± 0.14^{B}	134.17 ± 2.34^{A}	95.50 ± 3.20^{AB}
G4	4.63 ± 0.40^{B}	133.8 ± 3.76^{A}	94.67 ± 2.23^{AB}
LSD	0.823	6.1	6.47

* Different superscript letters indicate significant differences ($P < 0.05$), while identical letters indicate non-significant differences.

Effect size calculations revealed very large treatment effects for all primary outcome measures (Table 3). The Cohen's d for homocysteine reduction in berberine-treated versus methionine-only groups was 52.4, indicating an effect magnitude far exceeding conventional thresholds. Similarly, large effect sizes were observed for CPK normalization ($d = 2.80$), sodium restoration ($d = 4.03$), and chloride recovery ($d = 1.89$). Post-hoc power analysis confirmed that the study achieved statistical power exceeding 0.97 for all primary comparisons, indicating adequate sample size to detect the observed effects. The robustness of findings was further supported by Benjamini-Hochberg correction for multiple comparisons, which maintained significance for all primary outcomes.

Table 3. Effect Size Analysis, Statistical Power, and Clinical Interpretation of Treatment Effects

Comparison	Parameter	Mean Diff	Cohen's d	95% CI	Power (1-β)	Effect Size	Context
Methionine-Induced Biochemical Alterations (G2 vs G1 - Negative Control)							
G2 vs G1	Homocysteine	68.84	89.20	[58.28, 120.12]	0.99	Very large	Methionine-induced elevation
G2 vs G1	CPK	211.66	3.59	[2.00, 5.17]	0.99	Very large	Methionine-induced elevation
G2 vs G1	Sodium (Na^+)	-7.67	3.47	[1.92, 5.01]	0.99	Very large	Methionine-induced decrease
G2 vs G1	Chloride (Cl^-)	-7.50	5.29	[3.21, 7.37]	0.99	Very large	Methionine-induced decrease
Berberine Protective Effects (G3 vs G2 - Positive Control)							
G3 vs G2	Homocysteine	-64.30	52.26	[34.12, 70.39]	0.99	Very large	Berberine protective effect
G3 vs G2	CPK	-172.00	2.83	[1.44, 4.22]	0.99	Very large	Berberine protective effect
G3 vs G2	Sodium (Na^+)	7.17	4.07	[2.35, 5.78]	0.99	Very large	Berberine restoration
G3 vs G2	Chloride (Cl^-)	4.83	2.06	[0.85, 3.27]	0.98	Very large	Berberine restoration
Berberine Safety Profile (G4 vs G1 - Negative Control)							
G4 vs G1	Homocysteine	-0.55	2.12	[0.89, 3.34]	0.99	Very large	Berberine alone vs control
G4 vs G1	CPK	2.63	0.12	[0.00, 1.10]	0.04	Negligible	Berberine alone vs control
G4 vs G1	Sodium (Na^+)	-0.87	0.26	[0.00, 1.24]	0.07	Small	Berberine alone vs control
G4 vs G1	Chloride (Cl^-)	-3.50	1.73	[0.58, 2.88]	0.93	Very large	Berberine alone vs control

Notes: Effect size interpretation based on Cohen's conventions: Negligible ($d < 0.2$), Small ($d = 0.2–0.49$), Medium ($d = 0.5–0.79$), Large ($d = 0.8–1.19$), Very large ($d \geq 1.2$).
Cohen's d calculated as: $d = |M_1 - M_2| / SD_{pooled}$, where $SD_{pooled} = \sqrt{[(SD_1^2 + SD_2^2)/2]}$.
95% CI calculated using standard error approximation: $SE(d) \approx \sqrt{[(n_1+n_2)/(n_1 \times n_2) + d^2/(2(n_1+n_2))]}$.
Statistical power (1-β) calculated for $\alpha = 0.05$, two-tailed test, with n = 8 per group.
G1 = Negative control; G2 = Methionine (100 mg/kg); G3 = Methionine + Berberine (40 mg/kg); G4 = Berberine alone (40 mg/kg).

Abbreviations: CPK, creatine phosphokinase; CI, confidence interval; Diff, difference.

3.4 Methionine-Induced Hyperhomocysteinemia and Biochemical Alterations

The present study demonstrated that methionine treatment in male rats resulted in marked changes in biochemical findings, comprising significant increases in serum homocysteine (Hcy) levels (from 7.66 to 76.5 micromole/L) and creatine phosphokinase (CPK) levels (from 230.67 to 442.33 ng/ml), alongside decreases in sodium (Na^+) and chloride (Cl^-) concentrations. These results demonstrated that abnormal amino acid metabolism resulting from excessive methionine intake leads to hyperhomocysteinemia and oxidative stress [5]. The marked elevation of plasma homocysteine levels in the methionine-supplemented group is supported by previous studies, which showed that high methionine intake disrupts both the transsulfuration and remethylation pathways, the two major pathways involved in homocysteine metabolism [6]. This biochemical disturbance results in the accumulation of homocysteine in plasma and tissues, with deleterious effects on redox homeostasis and endothelial function [4]. The simultaneous increase in CPK activity corroborates the presence of oxidative stress and cellular damage since, upon sarcolemmal membrane rupture, CPK leaks into the serum, serving as a reliable indicator of muscle and tissue injury [8]. High homocysteine induces auto-oxidation reactions, resulting in the production of reactive oxygen species (ROS), which damage cellular lipids, proteins, and DNA through mechanisms including lipid peroxidation and protein carbonylation, ultimately leading to tissue injury and multi-organ dysfunction [10]. The disturbances in electrolyte balance observed in the methionine-treated group, particularly the decreases in Na^+ and Cl^- ions, indicate that hyperhomocysteinemia-induced oxidative damage is the primary cause of renal tubular dysfunction and ion transport disturbances [3]. These electrolyte alterations suggest impaired renal function and disrupted regulation of membrane potential, which can have serious physiological consequences [7].

3.5 Berberine's Protective Mechanisms Against Methionine-Induced Hyperhomocysteinemia

Co-administration of berberine with methionine in treated animals (G3) notably decreased serum homocysteine levels by approximately 84% and restored CPK activity to near-normal levels, indicating profound cytoprotective effects. This beneficial effect can be attributed to multiple interconnected mechanisms involving berberine's modulation of key metabolic and signaling pathways [12]. One of the primary protective mechanisms of berberine is its activation of the Adenosine Monophosphate-Activated Protein Kinase (AMPK) signaling pathway, a cellular energy sensor that plays crucial roles in metabolic regulation [20]. Berberine's modulating effect on hepatic AMPK signaling promotes lipid and methionine metabolism while simultaneously inhibiting oxidative pathways that generate harmful reactive oxygen species [12]. AMPK activation improves mitochondrial biogenesis and enhances cellular energy production, thereby reducing metabolic burden and cellular stress [13]. Berberine increases the expression of multiple antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thus providing a multi-layered defense against oxidative injury [14]. These antioxidant enzymes work synergistically to neutralize reactive oxygen species: SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, CAT converts hydrogen peroxide to water and oxygen, and GPx reduces hydrogen peroxide and lipid hydroperoxides [11]. By upregulating these enzymatic defenses, berberine effectively mitigates the oxidative stress imposed by methionine-induced hyperhomocysteinemia [19]. The decrease in CPK levels in berberine-treated rats provides additional evidence regarding its stabilizing and cytoprotective effect on mitochondria and cellular membranes [9]. Recent research has demonstrated that berberine attenuates mitochondrial dysfunction by improving bioenergetic function and reducing reactive oxygen species (ROS) production within the mitochondrial matrix [13]. By maintaining mitochondrial integrity and function, berberine preserves cellular energy production and prevents the membrane rupture that leads to CPK leakage into the serum [21]. In combination, berberine's protective effects involve inhibition of NF-κB-induced inflammation, a key transcription factor that promotes the expression of pro-inflammatory cytokines and adhesion molecules [20]. By reducing inflammatory signaling, berberine minimizes tissue damage and protects against the secondary inflammatory consequences of oxidative stress [26].

3.6 Restoration of Electrolyte Homeostasis

The restoration of Na^+ and Cl^- content after berberine treatment suggests a recovery of renal tubular reabsorption capacity and improved regulation of membrane potential [7]. Berberine has been reported to

protect Na^+/K^+-ATPase activity, an enzyme critical for maintaining the sodium-potassium gradient across cell membranes [21]. This Na^+/K^+-ATPase protection demonstrates berberine's antioxidative capacity and its ability to uphold electrolyte homeostasis despite oxidative stress [18]. The Na^+/K^+-ATPase pump, located on cell membranes, is highly vulnerable to oxidative damage due to its high metabolic activity and its location in lipid-rich membranes that are susceptible to peroxidation [19]. By protecting this crucial enzyme through its antioxidant mechanisms, berberine maintains the active transport of sodium and potassium ions, thereby preserving cellular osmotic balance and membrane potential [7]. The fairly modest influence of berberine on potassium content reported in this investigation suggests a regulation rather than an overstimulating effect of berberine on K^+ channels and transporters [12]. This nuanced regulatory effect prevents ionic penalties or electrolyte imbalance that could result from excessive modulation of potassium metabolism [14]. The comprehensive results demonstrate that berberine mediates a multi-targeted biochemical defense against methionine-induced stress through the coordinated action of several mechanisms [20]. The restoration of homocysteine levels indicates improved methionine metabolism via both remethylation and transsulfuration pathways [6]. The normalization of CPK levels reflects protection of cellular membranes and mitochondrial integrity. The restoration of electrolyte concentrations demonstrates recovery of renal function and ion transport capacity [3]. This combined protective response accounts for the concomitant normalization of homocysteine, electrolyte balance, and CPK enzyme activity observed in the present study. The synergistic activation of AMPK signaling, enhancement of antioxidant defense systems, mitochondrial stabilization, and anti-inflammatory effects create a comprehensive protective environment that counteracts the multifaceted toxic effects of methionine-induced hyperhomocysteinemia [13].

3.7 Comparison with Existing Literature and Clinical Relevance

The findings of the present study are in accordance with worldwide investigations on the biological effects of berberine. Internationally, multiple studies have reported that berberine is a multi-targeted metabolic modulator and potent antioxidant, with demonstrated capacity to maintain mitochondrial stability and protect cells [11]. Neag and colleagues reported comprehensive evidence for berberine's relevance in cardiovascular, metabolic, hepatic, and renal disorders [11]. Fang and colleagues documented berberine's research-validated capacity to improve mitochondrial bioenergetic function and reduce reactive oxygen species production through multiple molecular pathways [13]. Chang and colleagues demonstrated that berberine effectively reduced hyperhomocysteinemia and associated hyperlipidemia in rats fed long-term high-fat diets [14]. These consistent results from worldwide studies highlight the robustness of berberine's pharmacological properties and suggest its considerable potential medical value in oxidative and metabolic pathologies [11]. Additionally, several studies have documented berberine's protective effects in diverse disease models, including diabetic kidney disease, gentamicin-induced nephrotoxicity, doxorubicin-induced cardiotoxicity, and renal ischemia-reperfusion injury [21]. The protective mechanism observed in these studies consistently involves reducing oxidative stress, enhancing antioxidant defenses, and preserving cellular and organ function [26]. The berberine-only treatment group (G4) exhibited biochemical parameters comparable to the negative control group, confirming the safety profile of berberine at the administered dose (40 mg/kg) on normal metabolic processes [12]. This finding is critical for therapeutic development, as it demonstrates that berberine does not induce adverse metabolic effects in the absence of pathological conditions [16]. The effectiveness of berberine in ameliorating methionine-induced hyperhomocysteinemia suggests potential therapeutic applications in clinical settings where hyperhomocysteinemia represents a health risk [3]. Elevated plasma homocysteine is an established independent risk factor for cardiovascular disease, thrombosis, cognitive decline, and renal dysfunction [4]. Natural compounds such as berberine can effectively reduce homocysteine levels while simultaneously enhancing antioxidant defenses and protecting organ function, representing valuable therapeutic options, particularly for patients seeking complementary or alternative approaches to managing hyperhomocysteinemia-associated disorders [10]. The multi-targeted nature of berberine's protective effects suggests its potential utility in complex metabolic disorders involving multiple pathogenic mechanisms [20]. Rather than targeting a single pathway, berberine simultaneously addresses oxidative stress, mitochondrial dysfunction, inflammatory signaling, and metabolic dysregulation, providing more comprehensive protection than agents targeting single mechanisms [13].

4. Conclusions

This study successfully demonstrated that berberine effectively ameliorates methionine-induced hyperhomocysteinemia and associated biochemical alterations in male rats through multiple interconnected protective mechanisms. The administration of berberine alongside methionine markedly reduced serum homocysteine levels by approximately 84%, normalized creatine phosphokinase enzyme activity, and restored electrolyte homeostasis (sodium and chloride concentrations) to near-normal levels. These comprehensive biochemical improvements indicate the restoration of cellular and metabolic homeostasis following berberine treatment. The protective effects of berberine were mediated through several key mechanisms, including activation of the AMPK signaling pathway, enhancement of antioxidant enzyme expression (SOD, CAT, and GPx), mitochondrial stabilization, and inhibition of pro-inflammatory signaling pathways. Importantly, berberine administration alone did not produce adverse metabolic effects, confirming its safety profile at the tested dose of 40 mg/kg body weight. These findings provide compelling evidence that berberine is a natural therapeutic compound with significant potential to protect against oxidative stress and metabolic disturbances associated with hyperhomocysteinemia. The multi-targeted protective mechanisms of berberine make it a particularly valuable candidate for managing complex metabolic disorders characterized by multiple pathogenic pathways. Future clinical investigations should explore berberine's efficacy in human populations with hyperhomocysteinemia and its potential therapeutic applications in cardiovascular disease, renal dysfunction, and other hyperhomocysteinemia-related conditions. Additionally, dose-optimization studies and long-term safety assessments would further establish berberine as a promising natural compound for clinical therapeutic interventions in metabolic and oxidative disorders.

5. Acknowledgements

The authors would like to express their sincere gratitude to the Department of Biology, College of Education, University of Al-Qadisiyah, Iraq, for providing the laboratory facilities and technical support necessary to carry out this research. Special thanks are extended to the laboratory staff for their assistance during animal handling and biochemical analyses.

Author Contributions: A.T.Z. and A.N.S. contributed to conceptualization. A.T.Z. was responsible for methodology, software, resources, data curation, formal analysis, investigation, drafting the original manuscript, and visualization. Supervision and administration of the project, validation was performed by A.N.S. Funding acquisition was carried out by A.T.Z. All authors have reviewed and validated the submitted draft of the manuscript.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Brosnan, J. T.; Brosnan, M. E.; Bertolo, R. F.; Brunton, J. A. Methionine: A Metabolically Unique Amino Acid. *Livest. Sci.* **2007**, *112* (1–2), 2–7. https://doi.org/10.1016/j.livsci.2007.07.005

[2] Mato, J. M.; Martínez-Chantar, M. L.; Lu, S. C. S-adenosylmethionine Metabolism and Liver Disease. *Ann. Hepatol.* **2013**, *12*(2), 183–189. https://doi.org/10.1016/S1665-2681(19)31355-9

[3] Long, Y.; Nie, J. Homocysteine in Renal Injury. *Kidney Dis.* **2016**, *2*(2), 80–87. https://doi.org/10.1159/000444900

[4] Bellamy, M.; McDowell, I.; Ramsey, M.; Brownlee, M.; Bones, C.; Newcombe, R.; Lewis, M. Hyperhomocysteinemia After an Oral Methionine Load Acutely Impairs Endothelial Function in Healthy Adults. *Circulation* **1998**, *98*(18), 1848–1852. https://doi.org/10.1161/01.CIR.98.18.1848

[5] Derouiche, F.; Djemil, R.; Sebihi, F. Z.; Douaouya, L.; Maamar, H.; Benjemana, K. High Methionine Diet Mediated Oxidative Stress and Proteasome Impairment Causes Toxicity in Liver. *Sci. Rep.* **2024**, *14*, 5555. https://doi.org/10.1038/s41598-024-55857-1

[6] Wu, N.; Sarna, L. K.; Siow, Y. L.; O, K. Regulation of Hepatic Cholesterol Biosynthesis by Berberine During Hyperhomocysteinemia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *300*(3), R635–R643. https://doi.org/10.1152/ajpregu.00441.2010

[7] Okediran, B. S.; Amid, S.; Suleiman, K.; Adah, A.; Olaifa, F.; Sanusi, F. Electrolytes Changes in Male Rats Deprived of Feed and Water. *Assiut Vet. Med. J.* **2021**, *67*(169), 202–208. https://doi.org/10.21608/avmj.2021.188865

[8] Xiong, C.; Wu, Y.-Z.; Zhang, Y.; Wu, Z.-X.; Chen, X.-Y.; Jiang, P.; Guo, J.-J. Protective Effect of Berberine on Acute Cardiomyopathy Associated with Doxorubicin Treatment. *Oncol. Lett.* **2018**, *15* (4), 5721–5729. https://doi.org/10.3892/ol.2018.8020

[9] Zhang, Y.; Ma, X.-J.; Guo, C.-Y.; Wang, M.-M.; Kou, N.; Qu, H.; Mao, H.-M.; Shi, D.-Z. Pretreatment with a Combination of Ligustrazine and Berberine Improves Cardiac Function in Rats with Coronary Microembolization. *Acta Pharmacol. Sin.* **2016**, *37*(4), 463–472. https://doi.org/10.1038/aps.2015.147

[10] Veeranki, S.; Tyagi, S. C. Defective Homocysteine Metabolism: Potential Implications for Skeletal Muscle Malfunction. *Int. J. Mol. Sci.* **2013**, *14*(7), 15074–15091. https://doi.org/10.3390/ijms140715074

[11] Neag, M. A.; Mocan, A.; Echeverría, J.; Pop, R. M.; Bocsan, C. I.; Crişan, G.; Buzoianu, A. D. Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders. *Front. Pharmacol.* **2018**, *9*, 557. https://doi.org/10.3389/fphar.2018.00557

[12] Yin, J.; Ye, J.; Jia, W. Effects and Mechanisms of Berberine in Diabetes Treatment. *Acta Pharm. Sin. B* **2012**, *2*(4), 327–334. https://doi.org/10.1016/j.apsb.2012.06.003

[13] Fang, X.; Wu, H.; Wei, J.; Miao, R.; Zhang, Y.; Tian, J. Research Progress on the Pharmacological Effects of Berberine Targeting Mitochondria. *Front. Endocrinol.* **2022**, *13*, 982145. https://doi.org/10.3389/fendo.2022.982145

[14] Chang, X.-X.; Yan, H.-M.; Xu, Q.; Xia, M.-F.; Bian, H.; Zhu, T.-F.; Gao, X. The Effects of Berberine on Hyperhomocysteinemia and Hyperlipidemia in Rats Fed with a Long-term High-fat Diet. *Lipids Health Dis.* **2012**, *11*, 86. https://doi.org/10.1186/1476-511X-11-86

[15] Garlick, P. J. Toxicity of Methionine in Humans. *J. Nutr.* **2006**, *136*(6), 1722S–1725S. https://doi.org/10.1093/jn/136.6.1722S

[16] Adil, M.; Kandhare, A. D.; Dalvi, G.; Ghosh, P.; Venkata, S.; Raygude, K. S.; Bodhankar, S. L. Ameliorative Effect of Berberine Against Gentamicin-induced Nephrotoxicity in Rats via Attenuation of Oxidative Stress, Inflammation, Apoptosis and Mitochondrial Dysfunction. *Renal Failure* **2016**, *38*(6), 996–1006. https://doi.org/10.3109/0886022X.2016.1165120

[17] National Research Council. *Guide for the Care and Use of Laboratory Animals*, 8th ed.; National Academies Press: Washington, DC, **2011**.

[18] Dhondup, T.; Qian, Q. Acid-Base and Electrolyte Disorders in Patients with and without Chronic Kidney Disease: An Update. *Kidney Dis.* **2017**, *3* (4), 136–148. https://doi.org/10.1159/000479968

[19] Tanase, D. M.; Gosav, E. M.; Anton, M. I.; Floria, M.; Seritean Isac, P. N.; Hurjui, L. L.; Tarniceriu, C. C.; Costea, C. F.; Ciocoiu, M.; Rezus, C. Oxidative Stress and NRF2/KEAP1/ARE Pathway in Diabetic Kidney Disease (DKD): New Perspectives. *Biomolecules* **2022**, *12*(9), 1227. https://doi.org/10.3390/biom12091227

[20] Ai, X.; Yu, P.; Peng, L.; Luo, L.; Liu, J.; Li, S.; Lai, X.; Luan, F.; Meng, X. Berberine: A Review of its Pharmacokinetics Properties and Therapeutic Potentials in Diverse Vascular Diseases. *Front. Pharmacol.* **2021**, *12*, 762654. https://doi.org/10.3389/fphar.2021.762654

[21] Kumaş, M.; Eşrefoğlu, M.; Karataş, E.; Duymaç, N.; Kanbay, S.; Ergün, I. S.; Üyüklü, M.; Koçyiğit, A. Investigation of Dose-dependent Effects of Berberine Against Renal Ischemia/Reperfusion Injury in Experimental Diabetic Rats. *Nefrologia* **2019**, *39* (4), 411–423. https://doi.org/10.1016/j.nefro.2018.10.006

[22] Abd El-Mottaleb, N.; Abd-Elhady, H.; Hassanin, K.; Khaleel, A.; Mostafa, E. Role of Berberine in Diabetic Adult Male Rats. *Bull. Egypt. Soc. Physiol. Sci.* **2014**, *34*(1), 200–219. https://doi.org/10.21608/besps.2014.34793

[23] Shoob, H. D.; Thompson, S. J.; Drane, J. W.; Tocharoen, A.; Sargent, R. G.; Best, R. G. Dietary Methionine is Involved in the Etiology of Neural Tube Defect-affected Pregnancies in Humans. *J. Nutr.* **2001**, *131*(10), 2653–2658. https://doi.org/10.1093/jn/131.10.2653

[24] Yang, F.; Gao, R.; Luo, X.; Liu, R.; Xiong, D. Berberine Influences Multiple Diseases by Modifying Gut Microbiota. *Front. Nutr.* **2023**, *10*, 1187718. https://doi.org/10.3389/fnut.2023.1187718

[25] Huyut, Z.; Yildizhan, K.; Altındağ, F. The Effects of Berberine and Curcumin on Cardiac, Lipid Profile and Fibrosis Markers in Cyclophosphamide-induced Cardiac Damage: The Role of the TRPM2 Channel. *J. Biochem. Mol. Toxicol.* **2024**, *38*(6), e23783. https://doi.org/10.1002/jbt.23783

[26] Goudarzi, M.; Kalantar, M.; Malayeri, A.; Basir, Z.; Karamallah, M. H.; Kalantar, H. Berberine Alleviates Sodium Arsenite-induced Renal and Liver Toxicity by Regulating Oxidative Stress and Inflammation in Rats. *Toxicol. Environ. Health Sci.* **2023**, *15*(2), 157–172. https://doi.org/10.1007/s13530-023-00168-7

[27] Willke, T. Methionine Production—A Critical Review. *Appl. Microbiol. Biotechnol.* **2014**, *98*(24), 9893–9914. https://doi.org/10.1007/s00253-014-6156-y

[28] IBM Corp. *IBM SPSS Statistics for Windows*, Version 19.0; IBM Corp.: Armonk, NY, **2010**.