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| RESEARCH ARTICLE

Protective Effect of Silymarin on Attenuating of Rhabdomyolysis -induced Acute Kidney Injury in Animal Models

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| ABSTRACT

Acute kidney injury is one of the most serious complications of rhabdomyolysis characterized by an acute loss of renal function. The study aims to investigate the renoprotective effect of silymarin against rhabdomyolysis-induced acute kidney injury in rats. Twenty-four healthy rats were equally divided into three groups: control, induction, and treatment groups. Rats in the control and induction groups received distilled water, while rats in the treatment group received silymarin (50 mg /kg/day p.o) for three consecutive days. On the first day, all rats (except rats of control group) received a single intramuscular injection of glycerol (10 mL/kg, 50% v/v in sterile saline) divided equally into each hind leg for induction of acute kidney injury. On the fourth day of experiment, blood and kidneys were collected from all rats for biochemical and histopathological assay. Silymarin efficiently attenuated acute kidney injury caused by rhabdomyolysis evidenced by a significant decrease in serum creatinine and blood urea nitrogen(BUN), along with a significant reduction in renal homogenate levels of TNF-α, IL-6, NF-κB, caspase-3, and MDA with a significant increase in GSH level, which was further confirmed by improving renal histological changes when compared to that of induction group. This study found that silymarin has a potential attenuating effect on acute kidney injury due to its antioxidant, anti-inflammatory and antiapoptotic actions.

| KEYWORDS

Rhabdomyolysis; Acute kidney injury; Silymarin; Glycerol; Renoprotective.

| ARTICLE INFORMATION

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1. Introduction

Rhabdomyolysis (RM) is a life-threatening medical condition characterized by severe skeletal muscle damage and the release of myoglobin into the bloodstream. This leads to oxidative kidney injury, which eventually manifests as acute renal failure (Ohtani et al., 2022). Rhabdomyolysis can occur from several causes, including medications, crush syndrome, infections, exhaustive exercise, trauma, and toxins (Wu et al. 2017).

Acute kidney injury (AKI), formerly termed acute renal failure (ARF), is considered one of the most severe complications of rhabdomyolysis characterized by an acute loss of renal function resulting in a rapid decrease in glomerular filtration rate, accumulation of waste products, and perturbation of body's fluid homeostasis (Adedapo, 2021). Acute kidney injury occurs in more than 40% of patients with rhabdomyolysis. This clinical problem can be acquired by using glycerol in animal models to search for effective therapies to prevent and recover AKI (Semenovich, 2022).

Glycerol is commonly used experimentally for the induction of rhabdomyolysis- myoglobinuric acute renal failure. When glycerol is injected intramuscularly into animals, it causes rhabdomyolysis, resulting in the release of muscle cell contents, especially myoglobin, into the circulation (Mousleh, 2018). Deposition of myoglobin in glomerular filtrate causes tubular obstruction, then induces oxidative stress and lipid peroxidation of the renal tubules and triggers the release of cytokines and chemokines, which ultimately leads to cell injury, inflammation, vasoconstriction, tubular necrosis, apoptosis, and renal failure (Asmari, 2017; Kwiatkowska, 2020).

Silymarin is a flavonolignans isolated from milk thistle and widely used as a hepatoprotective remedy. It contains several biologically active compounds, mainly flavonolignans, such as Silybin, silidianin, silychristin, and isosilybin (Guzel, 2020). It possesses various biological and pharmacological activities, including antioxidant, anticancer, antiinflammatory, antiapoptotic, antibacterial, antifungal, antiviral, antiulcer, antidiabetic, neuroprotective, hepatoprotective, cardioprotective and nephroprotective activities (Mohammed, 2019; Ghonaim, 2022; Ghodousi, 2023; Iqbal, 2022; Singh, 2023)**.**

2. Materials and Methods:

2.1 Drugs and chemicals:

Silymarin (70 mg) capsule and glycerol were obtained from Madaus Gmbh (Germany) and Sigma Chemical Co. (St. Louis, MO, USA) companies respectively. ELISA kits (MB -assay-kit; MyBioSource, Inc., San Diego, CA, USA) were used for the estimation of " serum creatinine, blood urea nitrogen (BUN), tumor necrosis factor alpha(TNF-α), interleukin-6(IL-6), nuclear Factor kappa-B (NF-κB), cysteine-aspartic acid protease (caspase-3), malondialdehyde (MDA) and reduced glutathione (GSH) activities". All the chemicals and reagents used were of analytical grade.

2.2 Animals and experimental design:

Healthy albino rats of both sexes weighing (200 to 300) g were housed and acclimated in cages under a controlled environment with free access to food and water. "This study has been approved by the Animal Ethical Committee at Al-Ameed University". After 24 hours of water deprivation, rats were randomly divided into three groups of eight animals each and were given a single orally daily dose of the following at 9:00 a.m. for three consecutive days.

Group 1 (Control group): received (3ml) of distilled water.
Group 2 (Induction group): received (3ml) of distilled water.
Group 3(Treatment group): received silymarin (50 mg /kg) in (3 ml distilled water) [(14)].

At 9:30 a.m. of the first day, all rats of group 1 received a single intramuscular injection of normal saline (10 ml/kg) divided equally into each hind leg, while rats of groups 2 and 3 received a single intramuscular injection of glycerol (10 mL/kg, 50% v/v in sterile saline) divided equally into each hind leg for induction of acute kidney injury (Suna, 2020).

2.3 Serum and kidney homogenate analysis:

Blood samples were taken from rat hearts on the fourth day of the experiment, then centrifuged for 15 minutes at 3000 rpm to obtain serum for the determination of" creatinine and blood urea nitrogen(BUN) using ELISA kits" according to the manufacturer's instructions (Hussein, 2022).

Subsequently, all rats were sacrificed, and their kidneys were harvested. The right kidney was weighed, homogenized and centrifuged to get supernatants for measuring "tumor necrosis factor alpha(TNF-α), interleukin-6(IL-6), nuclear Factor kappa-B (NF-κB), cysteine-aspartic acid protease (caspase-3), malondialdehyde (MDA) and reduced glutathione (GSH)using ELISA kits according to the manufacturer's instructions" (Hussein, 2023).

2.4 Histological examination:

The left kidney of each rat was immersed in 10% formalin and embedded in paraffin, then cut into 5 µm sections and stained with hematoxylin and eosin for histological examination (Altındağ, 2022).

2.5 Statistical analysis:

Results were statistically analyzed by SPSS 20 using a one-way analysis of variance (ANOVA). Statistical significance was set at $P \leq 0.05$.

3. Results:

In this study, injection of glycerol to rats (induction group) led to acute kidney injury, which manifested by a significant increase($P \leq 0.05$) in serum creatinine and BUN levels, with a significant increase in tissue levels of TNF-α, IL-6, NF-κB caspase-3, and MDA accompanied with a significant decrease in GSH level when compared to that of the control group (Table 1).

Silymarin, when administered to rats (treatment groups), exerted a renoprotective effect and attenuated kidney injury induced by glycerol, as evidenced by producing a significant decrease in the levels of creatinine, BUN, TNF-α, IL-6, NF-κB, caspase-3 and MDA, with a significant increase in GSH level when compared to that of induction group. Besides, the biochemical changes were almost nearly restored to normal values compared to the control group (Table 1).

Renal histological examination showed that the control group had normal renal tissues (Figure 1A). Whereas the glycerol in the induction group produced sever renal lesions manifested by glomerular congestion, sever vacuolation of tubular epithelial cells with accumulation of proteinacious material in the tubular lumen and necrosis (Figure 1B & 1C). While in the treatment group, silymarin significantly reversed the histopathological changes induced by glycerol, which showed normal glomeruli with deposition of scanty amounts of proteinacious material in the tubular lumen (Figures 1 D & E).

Table 1: Effect of silymarin on serum and renal tissue homogenate biomarkers.

Groups (n=8)	Serum Mean ± S.E.M		Tissue homogenate Mean ± S.E.M					
	Creatinine (mg/dl)	BUN (mg/dl)	TNF-α (pg/g)	IL-6 (ng/g)	NF-κB (ng/g)	Caspase-3 (ng/g)	MDA (nmol/g)	GSH (nmol/g)
Control group	0.78±0.19	20±0.14	45±0.29	23±0.20	4±0.24	15±0.13	34±0.40	3.5±0.04
Induction group	3.6±0.32 a*	65±0.25 a*	111±0.47 a*	60±0.17 a*	8.2±0.34 a*	40.2±0.10 a*	120±0.32 a*	1.7±0.10 a*
Treatment group	1.1±0.21 b*	25±0.19 b*	59±0.33 a*, b*	30±0.12 a*, b*	5.5±0.42 b*	19.5±0.30 b*	52±0.15 a*, b*	2.8±0.13 b*

***= Significant (P≤0.05), a = as compared to control group, b= as compared to induction group.**

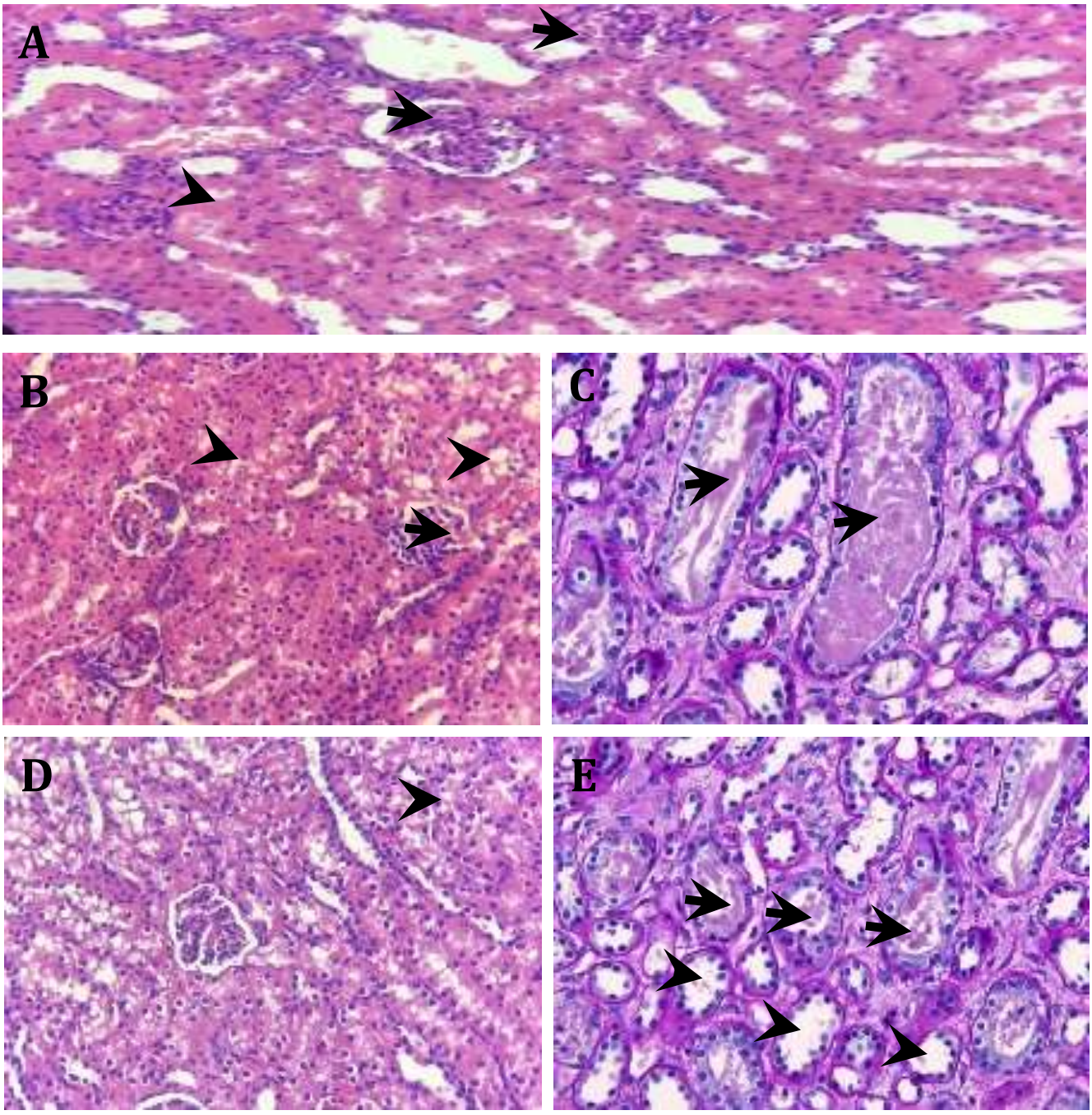

Figure 1: Photomicrographs of rat kidney sections

(A) Control group shows normal structures in the cortex – normal glomeruli (arrow) and normal renal tubular epithelium, (arrowhead) 10X, (B) Induction group shows vacuolation of renal tubular epithelial cells (arrowhead) and glomerular congestion (arrow) 10X (C) Induction group shows plugging of the renal tubular lumen by proteinaceous cast (arrow) 40X (D) Silymarin treated group shows mild cortical epithelial hyaline degeneration, (arrowhead) 10X (E) Silymarin treated group shows normal renal tubules (arrowhead) and deposition of scanty amounts of proteinacious material in the tubular lumen (arrow) 40X .

4. Discussion:

Acute kidney injury is one of the most serious complications of rhabdomyolysis, characterized by an acute loss of renal function.

In the current study, injection of glycerol-induced rhabdomyolysis resulting in functional (elevated serum creatinine and BUN levels) and structural (tubular necrosis) changes in kidneys, with concomitant increases in oxidative stress (increased renal MDA and decreased GSH levels), pro-inflammatory (TNF-α, IL-6 and NF-κB) and apoptotic factors production (Caspase-3). These findings are consistent with previous studies indicating that rhabdomyolysis induced by glycerol causes myoglobinuric acute kidney injury through several mechanisms, mainly oxidative stress, inflammation and apoptosis (Kwiatkowska, 2022; Yun-feng, 2019).

Silymarin significantly attenuated the myoglobinuric acute kidney injury, as shown by decreasing the biochemical and histopathological changes induced by glycerol and maintaining renal integrity. These effects could be due to antioxidant activity of silymarin, which provides a renoprotective effect mediated by preventing oxidative stress, scavenging free radicals and inhibition of fatty acid peroxidation (Dumludag, 2022), which can be proven in our study by decreasing the MDA and increasing GSH levels in the treatment group. This is agreed with other studies documented that antioxidants play an important role in the prevention of AKI-induced by glycerol (Adedapo, 2021; Semenovich, 2022).

Moreover, the renoprotective effect of silymarin can be attributed to its antiinflammatory activity by inhibiting the release of pro-inflammatory cytokines[14,20], which can be seen in this study by attenuation the renal damage and improving renal function along with reducing inflammatory biomarker levels in renal tissue. These results are compatible with other studies displayed that inflammatory mediators such as TNF-α, IL-1B, IL-6, and NF-κB play a major role in the pathogenesis of rhabdomyolysis-induced AKI after glycerol administration in animals (Suna, 2020; Wang, 2023).

Beyond its antiinflammatory effects, this study showed that silymarin significaltly ameliorating necrotic renal damage and reduces caspase-3 levels in renal tissue homogeneity. This could be credited to its anti-apoptotic activity mediated by the downregulation of the expression of the apoptotic marker caspase-3, thereby improving the survival of renal cells (Kandemir, 2017; Mokhtari Sangdehi, 2022). This agreed with another study, which showed that caspase-dependent apoptotic signaling plays a crucial role in the pathogenesis of apoptotic renal damages induced by glycerol (Adedapo, 2023; Chang, 2022).

The renoprotective effect of silymarin may also be mediated by preserved vasodilator nitric oxide (NO) through scavenging of superoxide anions, leading to an increase in NO content, which plays an important protective role against renal damage by improving renal blood flow and exertion anti-proliferative effects on vascular smooth muscle (Kabel, 2013; Pourová, 2019). This is consistent with previous studies showing that myoglobin scavenges nitric oxide, which contributes to renal hypoperfusion and tissue injury in the setting of rhabdomyolysis (Suna, 2020; Valdivielso, 2000; Blomberg, 2004).

5. Conclusion

The purpose of this study is to investigate the renoprotective effect of silymarin on acute kidney injury caused by rhabdomyolysis in rats. Silymarin efficiently attenuated acute kidney injury caused by rhabdomyolysis, evidenced by a significant decrease in serum creatinine and blood urea nitrogen(BUN), besides a significant reduction in renal homogenate levels of TNF-α, IL-6, NF-κB, caspase-3, and MDA with a significant increase in GSH level, which was further confirmed by improving renal histological changes when compared to that of induction group. According to this study, silymarin has a potential attenuating effect on acute kidney injury due to its antioxidant, anti-inflammatory, and antiapoptotic activities. The use of glycerol to cause acute kidney failure in rats for a short period of time is an important limitation of this study. Therefore, additional studies are needed to investigate the role of silymarin against different substances that cause acute kidney failure and for a longer period of time.

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